# BIOLOGICAL ACTIVITY OF EXTRACTS FROM *COMMIPHORA SWYNNERTONII* AGAINST MICROBES OF VETERINARY IMPORTANCE IN CHICKENS

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## ABSTRACT

Studies were carried out to establish ethno-botanical information and biological activities of crude extracts from *Commiphora swynnertonii* against selected microbes of veterinary importance in chickens. Initially, a questionnaire survey was conducted to gather information on practices and knowledge on ethno-botanical uses of *C. swynnertonii* in the study area. Then extracts from different morphological parts of the study plant were tested against selected bacteria and fungi in vitro using agar well diffusion assay. Resin and root bark extracts showed significant activities ($P < 0.001$) against *S. pyogenes, E. coli* and *B. subtilis* compared to other extracts. The fungi, *C. albicans* and *A. niger*, were moderately inhibited. Antiviral activity of the resin and root bark extract was tested in ovo using embryonated chicken eggs inoculated with Newcastle disease virus (NDV). Both extracts significantly ($P < 0.001$) and effectively reduced virus titres. An animal trial was carried out using the resin and chickens experimentally infected with NDV. Results revealed significant reduction ($P < 0.05$) in clinical signs and mortality rates following administration of the resin before and after the infection. Prophylactic administration of the extract was found to be more effective than the therapeutic approach. HI titres decreased significantly ($P < 0.001$) in resin and root bark treated groups and in all chickens treated with resin irrespective of dose given and on whether the extract was administered before or after infection suggesting that the plant materials were capable of destroying the NDV before stimulating the developing chick's immunity. Another animal trial investigated the effect of the resin against

experimental coccidiosis in chickens. Results showed that oral administration of the resin significantly ($P < 0.001$) reduced mortality rate. Safety margin of the resin was also investigated by determining its effects on selected physiological and biochemical parameters in chickens. The results revealed a good margin of safety provided that the dosage ranges between 200 to 800 mg resin/kg body weights. A phytochemical study was also carried to determine major bioactive compounds in the resin and root bark extracts. With these studies, it is concluded that extracts from *C. swynnertonii* especially resin, has significant antibacterial, antifungal, antiviral and anticoccidial effect against the selected microbes. Further research is required to test and validate the extract against other pathogens of medical and veterinary importance.

## DECLARATION

I**, Gaymary George Bakari** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

_____ _____

**Gaymary George Bakari (PhD. Candidate)** **Date**

The above declaration is confirmed

_____ _____

**Prof. M.M.A Mtambo (Supervisor)** **Date**

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**Prof. . R.A. Max (Supervisor)** **Date**

_____ _____

**Prof. E.C.J. Phiri (Supervisor)** **Date**

## COPYRIGHT

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I would like to dedicate this work to my beloved husband (Daudi P.N. Siarra), children (Aaron and Mary-glory) and mostly, to my parents, Dr. George and Mrs. Mariam Bakari who laid the foundation of my education.

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## ABBREVIATIONS

| | |
|---|---|
| ABTS | 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) |
| AIDS | Aquired Immunodefficient Virus |
| ALT | Alanine transaminase enzyme |
| AST | Aspartate transaminase enzyme |
| BC | Before Christ |
| BST | Brine Shrimp Letality Test |
| CDCA | Chenodeoxycholic acid |
| DMSO | Dimethyl sulphoxide |
| DPPH | 1,1-diphenyl-2-picrylhydrazyl |
| ECE | Embryonated Chicken Egg |
| ELISA | Enzyme-Linked Immunosorbent Assays |
| FAO | Food and Agriculture Organization |
| FOG | Faecal Oocysts Count |
| FXR | Farnesoid X receptor |
| GDP | Gross Domestic Product |
| $H_2O_2$ | Hydrogen Peroxide |
| $H_2SO_4$ | Sulphuric acid. |
| HA | Haemaglutination Assay |
| Hb | Haemoglobin |
| HCl | Hydrochloric acid |
| HDL-c | High Density Lipoprotein Cholesterol |
| HI | Haemaglutination Inhibition |
| HIV | Human Immunodeficiency Virus |
| HPRT | Hypoxanthine Guanine Phosphoribosyl Transferase |
| IBD | Infectious Bursa Disease |
| $IC_{50}$ | Inhibitory Concentration |
| INT | Iodo-Nitro Tetrazolium |

| | |
|---|---|
| IOE | World Organization for Animal Health (formerly the Office International des Epizooties (OIE) |
| $LC_{50}$ | Lethal Concentration |
| LDL-c | Low Density Lipoprotein Cholesterol |
| MAFF | Ministry of Agriculture Forestry and Fisheries |
| MCH | Mean Corpuscular Haemoglobin |
| MCHC | Mean Corpuscular Haemoglobin Concentration |
| MH | Muller Hinton |
| MIC | Minimum Inhibitory Concentration |
| MOA | Ministry of Agriculture |
| MRSA | Methicillin Resistant Strain *Staphylococcus aureus* |
| ND | Newcastle Disease |
| OPG | Percentage reduction in oocysts per gram |
| PBS | Phospate Buffered Saline |
| PCR | Polymerase Chain Reaction |
| PCV | Packed Cell Volume |
| PMNs | Polymorphonuclear Cells |
| RBC | Red Blood Cell Count |
| rpm | revolutions per minute |
| SD | Standard Deviation |
| SDA | Saboraud's Dextrose Agar |
| SUA | Sokoine University of Agriculture |
| $T_3$ | Triiodothyronine |
| $T_4$ | Thyroxine |
| USD | United State Dollar |
| VLDL-c | Very Low Density Lipoprotein Cholesterol |
| WBC | White Blood Cell Count |
| WHO | World Health Organization |

# CHAPTER ONE

## 1. INTRODUCTION

### 1.1 Importance of medicinal plants

Plants with medicinal properties have been used against various ailments inflicting human and animals since time immemorial (Ruffo *et al.,* 2002; Saimo *et al.,* 2003). The use of medicinal plants and search for new drugs and dietary supplements from plants has been accelerated in recent years particularly in developing countries for treatment of human and animal diseases (WHO, 1999; Elujoba *et al.,* 2005). A number of factors have fuelled increased dependency on plants as an alternative for the treatment of various diseases in humans and animals by some communities. Occurrence and spread of microbes resistant to conventional drugs, unaffordable cost and unavailability of the conventional drugs as well as consumers concerns over chemical residues in foodstuff and the environment are among main factors behind this trend (Cowan, 1999, Kone, *et al.,* 2004; Sibanda and Okoh, 2008).

Exploitation of naturally occurring products has been advocated as an alternative way of addressing the problems associated with the use of conventional drugs. In the recent past, the pharmaceutical industry has turned its interests to medicinal plants as a reliable source of new drugs and food supplements (Patwardhan *et al.,* 2004). Examples of important drugs and food supplements derived from plants include atropine (*Atropa beladona*), digitoxin (*Digitalis purpurea*), quinine (*Cinchona ledgeriana*), gugulipid

(*Commiphora mukul*), aloe vera gel and other nutritive products (Aloe spp.), etc (Fabricant and Fansworth 2001). Other reports indicate that *Commiphora* spp. have been used against respiratory, fungal infections and considered as one of the potential antibacterial and antiviral agents against uterine and vaginal infections in human (El-Sherbiny and El- Sherbiny, 2011).

Bacterial and fungal infections are the cause of a large burden of diseases worldwide. Bacteria and fungi are listed in the first position among the common microorganisms responsible for opportunistic diseases in animals and humans (Musa, 2008). They increase vulnerability to other non-infectious agents and pathogenic infections. *Escherichia coli, Bacillus subtilis, Streptococcus pyogenes* and *Staphylococcus aureus* and *Candida albicans* are commonly considered as normal flora in human and animals. But they can be infectious when body defences are compromised or when the organisms are able to penetrate the constitutive defences. All these infectious agents are of great importance in animals and humans because of high morbidity and mortality they cause in immunocompromised patients suffering from devastating diseases such as cancer, viral diseases like Newcastle disease in chickens, mastitis, HIV/AIDS in humans and other skin conditions whereby the mentioned bacterial and fungal agents serves as the opportunistic pathogens. Management of these infections is a serious challenge due to emergence of resistant or multi-resistant strains to numerous antibiotics and antifungal agents, drug toxicity, high costs of antibiotics and antifungal agents and relapse of

infections especially for fungal infections. Thus, there is increased exploitation of medicinal plants as alternative for treatment of such infections.

Several herbal preparations are frequently used in the management of diseases of chickens in rural areas (Minja, 1989; Bizimana, 1994; ITDG and IIRR, 1996: Musa *et al.,* 2008). In Tanzania, stems of *Euphorbia candelabrum*, (Euphorbiaceae) fruits of *Capsicum annum* (Solanaceae) and *Iboza multiflora* (Liliaceae) have been used against Newcastle Disease (ND) (Minja, 1989; Mtambo *et al*., 1999). Newcastle disease (ND) is the major constraint to productivity of local chickens in rural areas (Yongolo, 1996). The disease is mainly controlled by vaccination and good husbandry that are significantly practiced in commercial poultry. The costs for vaccines are not readily afforded by the resource poor farmers in most of the developing countries and hence rely on indigenous knowledge to control various poultry diseases (Bizimana, 1994; Guèye, 1997). In ethno veterinary medicine (EVM), natural products, especially those of plant origin are generally used for treatment and/or, in some cases, prevention of poultry diseases (Waihenya *et al.,* 2002).

The recent increase in the use of plants and other naturally occurring products to treat diseases and modify health in humans and animals has been attributed by a number of factors. Emergence and spread of microbes resistant to conventional drugs, concerns over chemical residues in foodstuff and the environment, as well as ever increasing cost of proprietary drugs are among factors which have contributed to this tendency (van

Wyk *et al.,* 1997; Waller, 1999; Ayaz, 2003). Studies using other *Commiphora* spp have reported antiparasitic effects against gut parasites (Fathy, 2005, 2011; Baghdadi and Al-Mathal, 2010). Coccidiosis is among diseases caused by gut parasites with poor response to commercial anticoccidial agents (Ayaz, 2003; Nwosu, 2011). However farmers are constrained from controlling the disease by the inhibitory high costs of drugs and the fragmented veterinary service provision (Kusina *et al*., 2001). Even if farmers could afford buying expensive antibacterial drugs such as $ESB_3$, Anticox ® research shows that they have residual effects on human beings who may consume the meat products of such animals dosed with synthetic drugs (Kusina *et al*., 2001). The use of *Commiphora* is believed to be cheaper and reliable though there is no documented evidence to substantiate such a claim. Therefore, there is a need to explore on local remedies that are readily available and socially acceptable.

With the ever-increasing use of natural products worldwide and the rapid expansion of the global market for these products, their safety and quality of their materials and products have become a major concern for health authorities, pharmaceutical industries and the public (WHO, 2007). According to WHO, 2007 approximately 80% of the world's inhabitants rely mainly on traditional medicines particularly medicinal plants for their primary health care. Plants have been recognized as indispensable sources of both preventive and curative traditional medicine preparations, for human beings and livestock (Adnan *et al.,* 2010).

*Commiphora (Burceraceae)* is among plants claimed to have broad spectrum of activities. In Tanzania the plant species is claimed to be used for treating wounds, intestinal parasites, and diarrhea, cough and chest aliments and also has been used as anti-parasitic (Kaoneka *et al.*, 2002). In comparison with synthetic drug, there is lack of reliable information's on their safety and efficacy on the important haematological and biochemical parameters. Relatively little research has been investigated in clinical trials on medicinal plants therefore, information on side effects largely depends on spontaneous or non scientific reports.

## 1.2 Research problem and justification of study

Traditionally, control and treatment of poultry diseases is based on the use of synthetic products but the increased trend of antimicrobial resistance call for the need of having alternative approaches to management of poultry diseases. Antimicrobial resistance, cost and availability of the conventional medicines and residues in animal products and the environment are the major challenges in the poultry industry in the developing countries. In addition, drug misuse, including adulteration and improper dosage arising from lack of knowledge adds to the problem of drug resistance. As the consequences of these problems, people in rural communities have continued to use indigenous plants as a convenient alternative for control and treatment of diseases. In most cases, plants are used for treatment in their crude forms without scientifically known concentration, established dosages and known side effects to treated subjects (Mills and Bone, 2005).

Although *Commiphora swynnertonii* is widely used in many pastoral communities for treatment of various diseases, as mentioned in the general introduction section, there is no scientific evidence that justifies the claimed uses. Moreover, information on the use of *C. swynnertonii* as regards to chicken diseases is not available. This work was therefore undertaken to establish the ethno-botanical use of *C. swynnertonii* in a selected pastoral community and to investigate the biological activities of the plant against selected microbes of veterinary importance in chickens.

## 1.3 Objectives

### *1.3.1 General objective*

To establish ethno-botanical information and investigate biological activities of crude extracts from various morphological parts of *Commiphora swynnertonii* against protozoan, bacteria, fungi and viruses of veterinary importance in chickens.

### *1.3.2 Specific objectives*

i. To establish ethno-botanical use of *C. swynnertonii* in Simanjiro District, Manyara Region, Tanzania

*ii.* To investigate the effect of crude extracts from different morphological parts of *C. swynnertonii* against selected virus, bacteria and fungi isolates *in vitro*

iii. To investigate the effect of resinous crude extract from *C. swynnertonii* against Newcastle disease virus in chickens

iv. To determine the effect of resinous crude extract from *C. swynnertonii* against coccidial infection in chickens

v. To assess the effect of the resinous extract on cells, biochemical and physiological parameters in chickens

vi. To carry out phytochemical screening of bioactive constituents from the most active crude extracts of *C. swynnertonii*

### 1.4 Significance of this research

It is anticipated that, this work will reveal information about the extent and form in which the selected pastoral community uses the *C. swynnertonii*. This information will be helpful in devising experiments aimed at validating the effective use of the plant. The *in vitro* and *in vivo* studies will give information on the most potent, safe and environmentally friendly part of the plant to be used. Results against tested diseases provided an avenue for further studies to validate the use of the plant against the disease. Studies on the effect of the plant on cells, biochemical and physiological parameters will shade more light on safety of the plant extracts works in chickens. Finally, results of the current work will also save as a template for further research on the use of *C. swynnertonii* in other animal species including humans.

# CHAPTER TWO

## 2. LITERATURE REVIEW

### 2.1 The genus *Commiphora*

The Burseraceae family consists of approximately 700 species from18 genera including *Commiphora*. The genus *Commiphora* dominates over 1.6 million km$^2$ of *Acacia-Commiphora* woodland in tropical and subtropical Africa (Paraskeva, 2008). More than 200 species of *Commiphora* are in Africa, Arabia and India and about 40 species occur in southern Africa (Steyn, 2003). The name *Commiphora* originates from the Greek words *kommi* (meaning 'gum') and *phero* (meaning 'to bear'). In Tanzania, *Commiphora* species have different synonyms: mbambara, mponda, mturituri, mtwitwi, (Swahili); oltemwai (Maasai); mguta (Sukuma); dumbechanda (Taturu); mzilanzi (Gogo) (Minja 1999; Sambuta and Masola, 2008).

#### *2.1.1 Features of* **Commiphora** *species*

Characteristic features of *Commiphora* species are very diverse; therefore for proper identification a combination of morphological characters is required. *Commiphora* plants range from small to medium-sized thorny shrub tree 3.5 – 4.0 m tall (Paraskeva *et al.*, 2008). The bark of most *Commiphora* species is smooth, deep green in colour and covered with grey brown papery flakes which can peel off easily. When damaged, the bark exudate watery to milky sap which later becomes resin. Leaves are generally

compound (divided into several units), shiny copper green and normally come out at or before the beginning of wet season and most lose leaves at the beginning of the dry seasons (Paraskeva *et al.*, 2008). The fruit of *Commiphora* greatly enhances the identification of the species; fruiting and flowering are irregular and do not occur every year (Steyn, 2003). When ripe, the fruit splits into halves revealing a brightly coloured pseudo-aril (Paraskeva, 2008). Most of *Commiphora* species are dioecious; flowers are very small and usually yellow or white. In most species flowers are produced in the first half of the dry season and are followed by leaves and fruits if any. The flowers may be uni or bi-sexual, with the uni-sexual flowers only being semi developed with non-functional stamens (Steyn, 2003). Most of seeds produced by *Commiphora* species are hard and dispersed by various means mainly animals, birds and winds. The stem colour may vary from grayish, green, yellowish pale to pinkish (Steyn, 2003).

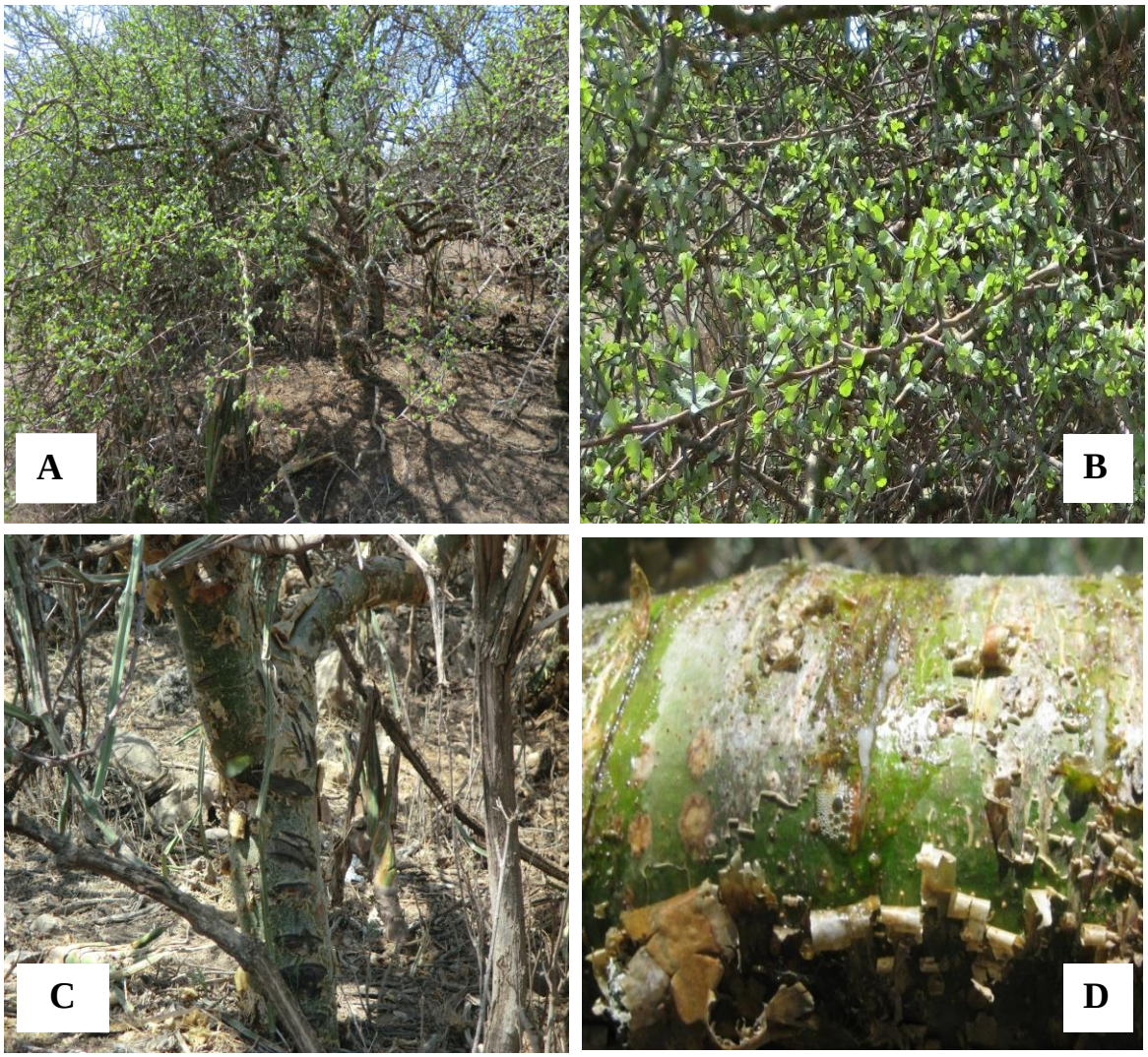


**Plate 1:** *C swynnertonii* tree [A] showing closer view of leaves [B] and stem [C] the plant stem showing resin [D].

### *2.1.3 History of Commiphora spp*

*C. swynnertonii* was first collected and verified by Burtt, B.D. in Dodoma District, Tanzania in 1932 and kept at National Museums of Kenya, East Africa Herbarium, Nairobi Kenya with voucher specimen no 3827. For many years products from *Commiphora* plants, have been used for its healing qualities during injuries (Hanus *et al.,* 2005). Product from *Commiphora* plants (myrrh) have been used since biblical times. Prophet Moses used myrrh during sacred Jewish ceremonies as anointing oil, and it also was one of the three gifts given to the infant Jesus ("they presented unto Him gifts: with gold and frankincense") and was used to anoint the body of Christ after the crucifixion (Dharmananda, 2003). The myrrh was an important ingredient in the Egyptian embalming process of 2000 B.C., and insect repellent to rid their homes of fleas. It was also used as a medicine and wound dressing agent and has been used as ritual oil for purification rites of women, for treatment of menstrual pain thus considered as an emminogorgue, an agent that promotes the onset of menstruation and regulates its flow (Hanus *et al.,* 2005). Myrrh is used to stimulate blood circulation and stagnant blood, especially in the case of women's delayed or scanty menses and is considered one of the finest antibacterial and antiviral agents, fighting against uterine and vaginal infections (Hanus *et al*., 2005).

### *2.1.4 Phytochemistry of* **Commiphora** *plants*

Plant-derived substances have recently become of great interest owing to their importance and broad applications. Medicinal plants are the richest bio-resource of

drugs in medicine, food supplements, and chemical entities for synthetic drugs. This is due to plants ability to synthesize secondary metabolites, most of which are phenols or their oxygen-substituted derivatives (Cowan, 1999). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. For instance, terpenoids give plants their odors; others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavor (e.g., the terpenoid capsaicin from chili peppers). Most of these bioactive metabolites have been found in different *Commiphora* spp and their concentrations vary widely depending on species, season and geographical location. Different biological activities have been associated with these metabolites as summarized in Table 1.

**Table 1:** Mechanism of action of some phytochemicals (adopted from Tiwari *et al.*, 2011)

| **Phytochemical** | **Activity** | **Mechanism of action** |
|---|---|---|
| Quinones | Antimicrobial | Binds to adhesins, complex with cell wall, inactivates enzymes in bacteria |
| Flavonoids | Antimicrobial | Complex with cell wall, binds to adhesins |
| | Antidiarrhoeal | Inhibits release of autocoids and prostaglandins<br>Inhibits contractions caused by spasmogens<br>Stimulates normalization of the deranged water transport across the mucosal cells<br>Inhibits GI release of acetylcholine |
| Polyphenols and tannins | Antimicrobial | Binds to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption, and metal ion complexation. |
| | Antidiarrhoeal | Makes intestinal mucosa more resistant and reduces secretion, stimulates normalization of deranged water transport across the mucosal cells and reduction of the intestinal transit |
| | Anthelmintic | Improves proteins by metabolism in rumen, interferes with oxidative phosphorylation. |
| Coumarins | Antiviral | Interaction with eucaryotic DNA |
| Terpenoid/essential oils | Antimicrobial | Membrane disruption |
| | Antidiarrhoeal | Inhibits release of autocoids and prostaglandins |
| Alkaloids | Antimicrobial | Intercalates into cell wall and DNA of parasites |
| | Antidiarrhoeal | Inhibits release of autocoids and prostaglandins |
| | Anthelmintic | Possess anti-oxidating effects, thus reduces nitrate useful for protein synthesis<br>Acts on CNS causing paralysis |
| Lectins/ Polypeptides | Antiviral | Blocks viral fusion or adsorption, forms disulfide bridges |
| Glycosides | Antidiarrhoeal | Inhibits release of autacoids and prostaglandins |
| Saponins | Antidiarrhoeal | Inhibits histamine release in vitro |
| | Anticancer | Imprioves membrane permeability |
| | Anthelmintic | Leads to vacuolization and disintegration of teguments |
| Steroids | Antidiarrhoeal | Enhance intestinal absorption of Na+ and water |

Generally, *Commiphora* spp resin consists of water-soluble gum (40 - 60%), alcohol-soluble resins (23 - 40%), volatile oils (2 - 8%) and a bitter principle (10 - 25%), and has a characteristic odour ascribed to the presence of furanosesquiterpenes (El-Ashry *et al*., 2003). Other reported constituents includes: dammarene triterpenes, ferulates (Zhu *et al.*, 2001), furanosesquiterpenes, guggultetrols, guggulsterones, lignans, flavanones (Fatope *et al.,* 2003), sesquiterpenes, esters cumunic aldehyde, eugenol, steroids, resin acids and proteins (Al-Harbi *et al.,* 1997). Ethanolic extract of *Commiphora africana* was shown to contain phenolic compounds and tannins, whereas the hexane fraction contained alkaloids triterpenes and sterols (Hanus *et al*., 2005; Aliyu *et al.*, 2007). Kaoneka *et al.*, (2007) analyzed essential oils from leaves of *C. swynnertonii* using gas chromatographic technique and identified five sesquiterpenoid and four sesquiterpenoid derivatives.

*Commiphora swynnertonii* (Burtt) leaves contain essential oils (Kaoneka *et al.,* 2007). The identified essential oils included the five sesquiterpenoids and four sesquiterpenoid derivatives, copaene and isocaryophyllene. Other *Commiphora* species resins have been found to contain terpenes, sesquiterpenes, esters cumunic aldehyde, eugenol, steroids, resin acids and proteins (Al-Harbi *et al.,* 1994). Ethanolic extracts from other species such as *Commiphora africana* contain phenolic compounds and tannins, whereas the hexane fraction contained alkaloids triterpenes and sterols (Hanus *et al.,* 2005; Aliyu *et al*., 2007).

### *2.1.5 Uses of* **Commiphora** *plants*

*Commiphora swynnertonii* has been known for wide range of utilization, but little has been documented. Therefore most of the literature review is based on other species of *Commiphora*.

#### 2.1.5.1 Common uses of *Commiphora* plants

Locally the wood of *Commiphora* is used for construction purposes for houses and animal enclosures because it is termite resistant. Also, the wood is used for making fence posts, tool handles, beehives, spoons, water troughs, musical instruments and furniture (Schmidt and Mbora, 2008). Some species are even cultivated and propagated as a quick growing live fence for boundary marking and for yam supports (Hines and Eckman, 1993; Schmidt and Mbora, 2008). Dried sap and bark are used as incense (Steyn, 2003). Extracted oils are used in perfumes and religious ceremonies (Paraskeva *et al.*, 2008). Leaves (8 to 14% crude protein) are browsed by goats especially at the end of the dry season when young leaves appear (Schmidt and Mbora, 2008). Despite availability of different *Commiphora* species which are widely distributed with wide spectra of activities, there is no any documentation on the use of *C. swynnertonii* as medicinal plant for animals and human beings. The wider availability of *Commiphora* plants in rural areas of Tanzania increases its utilization potential particularly in resource-poor communities where there is shortage of synthetic drugs. This study will promote the use of *Commiphora* products in a user friendly forms that will be affordable and available with little residual effects in the environment.

2.1.5.2 Medicinal uses of *Commiphora* spp

- Anti-lipidemic, anti-cholesterolaemic and anti-atherosclerotic potential of *Commiphora* spp - cardiovascular diseases are the leading cause of death in human population. These diseases have been associated with increased in levels of blood cholesterol particularly the low density lipoprotein cholesterol. Several *Commiphora* species have been studied on their activities as antilipidemic, anticholesterolaemic and anti atherosclerotic, thus reducing serum cholesterol concentrations without causing any detrimental side effects (Adebayo *et al.,* 2006). In various studies *C. mukul* has been claimed to decrease atherosclerosis and lower serum cholesterol by 27% and triglycerides by 31%. Guggulipid, a product from *C. mukul*, was shown to cause an increase in high density lipoprotein cholesterol (HDL) (Singh *et al.,* 1994). Guggulipid exert its activity by lowering the level of cholesterol by reducing total cholesterol, low density lipoprotein cholesterol (LDL-c), and very low density lipoprotein (VLDL-c) cholesterol at the same time elevating the high density lipoprotein cholesterol (HDL-c) (Adebayo *et al.,* 2006). *C. mukul* contains guggulsterone, a compound which act by antagonizing the effect of the nuclear farnesoid X receptor (FXR) (Huang *et al.*, 2003; Adebayo *et al.,* 2006). This receptor is identified as a bile acid receptor and biological sensor for the regulation of bile acid biosynthesis (Huang *et al*., 2003). FXR has shown to regulate cholesterol metabolism in two ways: (i) chenodeoxycholic acid (CDCA), a primary bile acid, binds directly to and activates FXR, which then mediates the feedback suppression by bile acids of cholesterol 7 alpha-hydroxylase (CYP7A1), the rate-limiting enzyme

in bile acid biosynthesis from cholesterol. (ii) FXR participates in the activation of intestinal bile acid binding protein (IBABP), which is involved in the enterohepatic circulation of bile acids. Thus FXR constitutes a potential therapeutic target that can be modulated to enhance the removal of cholesterol from the body (Tu *et al.,* 2000). The other mechanism reported by (Wang *et al.,* 2004), is through the presence of ketosteroid , an active compound of *C. mukul* which acts by stimulating the thyroid gland and has also found to reverse the decrease of catecholamine and dopamine –p-decarboxylase activity that is involved with anticholesterolaemia (Wang *et al.,* 2004). This is done by improving the liver's ability to process, metabolize and excrete cholesterol and improving thyroid function by increasing $T_3$ and $T_4$ conversion (Wang *et al.,* 2004). Ethanolic leaf extract of *C. africana* and *C. myrrha* were also shown to exhibit hypolipidaemic activity in experimental rats (Adebayo *et al.,* 2006).

- Antihypoglycemic potential - globally, the number of people that have been diagnosed with diabetes has been increasing significantly over the last three decades (Bellakonda *et al.,* 2011). Management of diabetes is based on the use of insulin and other oral hypoglyceaemic agents (Bellakonda *et al.,* 2011). Side effects' arising from the use of these conversional drugs is still a challenge to the medical system. Therefore, this has led to an increased demand on natural products with anti-diabetic activity. Several medicinal plants have been reported to treat *Diabetes mellitus* (Babu *et al.,* 2006). These plants are considered to be a good source of new drugs or a lead

to make new drugs. An ethanolic stem bark extract of *Commiphora africana* was shown to have anti-hyperglycemic potential in alloxan induced-diabetic rats at dose of 400 mg/kg body weight (Goji *et al.*, 2009). The anti-hyperglycemic effect was associated with stimulation of the pancreatic beta-cells and or due to its insulin like activity. Another study done by Bellakonda *et al.*, (2011) revealed the use of *Commiphora mukul* to have beneficial effect in the treatment of diabetes by decreasing the effect of blood glucose and cause increased levels of plasma insulin in Streptozotocin induced diabetic rats. Streptozotocin causes destruction of β- cells of the islets of Langerhan of the pancreas.

- The antihypertensive effects - aqueous extracts from *Commiphora opobalsamum* (L.) Engl. (Burseraceae) 'Balessan' tree were investigated by intravenous administration at a dose of 4 mg/kg (Abdul-Ghani and Amin, 1997). Results showed that there was depression of systemic arterial blood pressure by 20% ($P < 0.01$) and reduction of heart rate of anaesthetised rats by 14% ($P < 0.05$). The hypotensive and the bradycardiac effects were immediate and in a dose related manner. The hypotensive effect of *C. opobalsamum* was inhibited by the pre-treatment with atropine sulphate (1 – 4 mg/kg). These results suggest that the hypotensive effect of *C. opobalsamum* is due to the activation of muscarinic cholinergic receptors (Abdul-Ghani and Amin, 1997). Also *C. mukul* was reported to have fibrinolytic activity (i.e., a decrease or no change in platelet aggregation, and an increase in the prothombin time, clotting time, and the coagulation time).

- Anti-ulcerative effect - *Commiphora opobalsamum* (L.) Engl. (Burseraceae) also known as Balessan possesses anti ulcerative effect (Al-Howiriny *et al.,* 2005). The latter was assessed against different acute gastric ulcer models in rats induced by necrotizing agents. Results showed that the extract had a dose-dependent protection against ethanol-induced depletion of stomach wall mucus reduction in nonprotein sulfhydryl concentration. Pre-treatment with Balessan extract provided a complete protection of gastric mucosa through supporting both the offensive and defensive factors with a large margin of safety without any apparent adverse effects in rats (Al-Howiriny *et al.,* 2005). A similar study done by Al- Harbi *et al.,* (1997) reported that the aqueous suspension of *C. molmol* (oleo-gum resin) had protective effect which was attributed to its effect on mucus production, increased nucleic acid and non-protein sulfhydryl concentration. This appeared to be mediated through its free radical-scavenging, thyroid-stimulating and prostaglandin-inducing properties (Al-Harbi *et al.,* 1997). Also Kannan (2009) demonstrated ulcerogenic activity of *C. caudata* bark extract against ethanol-induced gastric ulcer in rats.

- Antibacterial and antifungal effect - different studies have revealed potential antimicrobial activities against gram-positive, gram-negative bacteria and fungi. All, *et al.,* (2007) reported antibacterial activity of toluene-methanol-extracts from leaves and roots of *C. quadricincta* against *Yersinia enterocolitica, Staphylococcus aureus, Escherichia coli* and *Staphylococcus epidermidis.* Akor and Anjorin (2009) reported the effect of root ethanolic extract from *Commiphora africana* to be active against

*Staphylococcus aureus, Escherichia coli* and *Candida albicans*. Musa, (2008) showed that *C. kerstingii* stem bark contains antimicrobial activities against *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Pseudomonas fluorescens* and *Bacillus subtilis*. Study done by (Paraskeva *et al.,* 2008) showed that *Commiphora africana* has been used against *Salmonella typhi* (typhoid) in South Africa. Some of *Commiphora* species have shown a potential activity against some resistant strains of bacteria. This included *Commiphora molmol* and *Boswellia papyrifera*; the two have shown activity against methicillin resistant strain *Staphylococcus aureus* (MRSA).

- Antimycobacterial activity - *Commiphora* plants are reported to be used traditionally for treatment of tuberculosis. Newton *et al.,* (2002) showed that methanolic extract from resin of *Commiphora mukul* had significant antimycobacterial activity, with a minimum inhibitory concentration (MIC) of 62.5 μg/ml against *Mycobacterium aurum*.

- Anticancer effect – cytotoxic and antitumor activity of resin from several *Commiphora* spp was demonstrated by You and Woo, (2004). The ability of medicinal herbs, one of which being *C. molmol*, to suppress $H_2O_2$-induced mutant frequency in treated human fibroblast cells (GM00637) at the hypoxanthine guanine phosphoribosyl transferase (HPRT) locus was shown to have inhibition greater than 60% (You and Woo, 2004). Also methanolic extracts of C. *berryi* and *C. caudata*

showed moderate cytotoxic activity against a human mammary carcinoma cell line (MCF-7), with values $IC_{50}$ of 82.6 and 88.4 µg/mL, respectively (Kumari *et al.,* 2009). *In vitro* studies done by Paraskeva *et al.,* (2008) revealed that *C. glandulosa,* C. *marlothii* (leaf). *C. edulis* (leaf and stem), *C. glandulosa* (leaf and stem), *C. marlothii* (leaf), *C. pyracanthoides* (leaf and stem), *C. schimperi* (stem), and *C. viminea* (stem) had antincancer activity using all possessing a percentage inhibition greater than 80% at 100 µg/ml. *C. glandulosa* (leaf and stem) and *C. pyracanthoides* (leaf and stem) had anticancer activity against the SF-268 cells using SRB anticancer assay with $IC_{50}$ values ranging between 68.6 ± 2.0 and 71.5 ±1.2 µg/ml.

- Antioxidant effect - antioxidant agents are a possible mediators in the protection against cancers, myocardial necrosis as well as increased fibrinolysis. The ability of different *Commiphora* species to scavenge free radicals radical was documented by Paraskeva *et al*., (2008). The *in vitro* antioxidant activities of extracts obtained from *C. tenuipetiolata, C. neglecta* and *C. mollis C. berryi* and *C. caudata* (Burseraceae) was assessed using 1,1-diphenyl-2-picryl hydrazyl (DPPH) and nitric oxide assays (Kumari *et al.,* 2011). The methanolic extracts of *C. berryi* and *C. caudata* showed significant DPPH radical scavenging activity, with $IC_{50}$ values of 26.9 and 21.2µg/mL respectively.

- Anti-protozoal effects - myrrh from *C. molmol* was shown to have effect against hepatic coccidiosis induced by the parasite *Eimeria stiedae* in domestic rabbits

(Baghdadi and Al-Mathal, 2010). Another study reported effect of *C. molmol* (myrrh) against *Trichomonas vaginali* which is now an important worldwide health problem. Treatment of patients with metronidazole refractory vaginal trichomoniasis constitutes a major therapeutic challenge and treatment options are extremely limited. The anti-trichomoniasis activity of *C. molmol* (*in-vivo*) extracts gave promising results (El-Sherbiny *et al.,* 2009*:* El-Sherbiny and El- Sherbiny, 2011). Also *C. molmol* has been recently reported to be effective against protozoa like *Giardia* and *Cryptosporidium* in humans (Fathy, 2011). Results proved the efficacy of *C. molmol*, as indicated by a 100% reduction in parasite-load of both intestinal and faecal parasitic counts, a direct toxic effect on *Giardia* trophozoite (Fathy , 2011).

- Anthelmintic effect - myrrh from *Commiphora molmol* has been used in the treatment of human schistosomiasis and fasciolosis (Massoud *et al.,* 2001, Haridy *et al*., 2004). Myrrh was found to be effective in the treatment of schistosomiasis with a high cure rate and without side effects (Massoud *et al.,* 2001). *C. molmol* therapy at the dose of 12 mg/kg proved to be effective, with pronounced improvement of the general condition and amelioration of all symptoms and signs in patients infested with *Fasciola* spp. The efficacy of *Commiphora molmol* was also evaluated in treating sheep naturally infected with *Moniezia expansa* at a dose of 4,800 mg and a cure rate of 100.0% with no clinical side effects was observed (Haridy *et al.,* 2004; Al-Mathal and Fouad, 2004). The mirazid proved to be safe and very effective in sheep

*Monieziasis expansa* and *Dicrocoeliasis dendriticum*. Massoud *et al.,* (2006) reported the use of *C. molmol* in treating *Strongyloides stercoralis* in human.

- Anti ectoparasitic and repellent effects - *C. swynnertonii*, *C. holtziana* and *C. erythraea* have repellent activity against ticks, lice, fleas and mites (Carroll *et al.,* 1989, Minja, 1999; Sambuta and Masola, 2006; Kaoneka *et al.,* 2007; Birkett *et al*., 2008). Myrrh is used as repellent against termites and mosquito when blended as incense sticks. Hexane extract of the gum of *C. erythraea* (Engler) has larvicidal and repellent activity against the ticks of different species such as *Amblyomma americanum* and *Dermacentor variabilis* (Carroll *et al.,* 1989). A resin produced by *C. holtziana* (Burseraceae), was investigated against cattle tick, *Boophilus microplus* and showed activity which lasted up to 5 hours (Birkett *et al.*, 2008).

- Anti-inflammatory and wound healing effect - anti-inflammatory effects of several *Commiphora* spp have been documented. Ethanolic extracts from stem bark *C. africana* was shown to have anti-inflammatory effect in adult Wistar rats using paw edema model (Ezekiel *et al.,* 2010). The anti-inflammatory effect was explained to be due to presence of flavonoids metabolites which acts by inhibiting production of prostaglandin (signaling molecule) and phosphosdiesterases involved in cell activation, the effect which predominantly depend upon biosynthesis of protein cytokines that mediate migration and diapedesis of circulating leucocytes to site of injury (Manthey *et al.,* 2001). Study by Paraskeva *et al.,* (2008) has shown that *C.*

*pyracanthoides, C. schimperi* and *C. glandulosa* possessed anti inflammatory effect *in vitro*. Some of *Commiphoras* are known to facilitate wound healing. This was demonstrated by Haffor, (2010) who showed complete epithelialization, new granulation tissues formation and new blood vessels development, leucocytes infiltration with increased polymorphonuclear cells (PMNs) in wounded rats treated with myrrh from *C. molmol*.

## 2.2 Local chickens in Tanzania

Livestock production is one of the major agricultural activities in Tanzania. Agriculture contributes to national food supply, converts rangelands resources into products suitable for human consumption and is a source of cash incomes. Livestock industry provides about 30% of the Agricultural GDP, out of this 40% originates from beef production, 30% percent from milk production and another 30% percent from poultry and other small stock production (MOA, 2002/2003).

Local scavenging poultry is the dominant form of poultry keeping in Tanzania and other developing countries (Minga *et al.,* 1989). According to MOA census of 2002/2003, Tanzania has approximately 35 million poultry population whereby about 33 million were local village chicken kept predominantly in the rural areas. Commercial birds included 590,000 broiler and 1.2 million layer chickens kept by smallholder farmers and 457,000 birds (both broilers and layers) on large-scale farms. There were 1.4 million

ducks and 214,000 turkeys. Other types of poultry kept include guinea fowls and pigeons (FAO, 2008).

Management and diseases are among major constraints of local chicken productivity (Minga *et al.,* 1989). Their management is considered as low input low output husbandry system that is characterised by poor nutrition and housing. Local chickens scavenge mainly on household scraps, green forages, insects and worms, occasionally supplemented with residues from harvest such as maize bran, millet and sorghum. Housing facilities involve poorly constructed sheds most of the time they are raised in the kitchen or store with other facilities. Although local chickens are known to be resistant and/or resilient to diseases, Newcastle disease (ND) is responsible for significant mortality of local chickens (Minga *et al.,* 1989;Yongolo, 1996).

**2.3 Newcastle disease**

Newcastle disease (ND) is a major problem in the development of village chickens in Tanzania (Waihenya *et al.,* 2002). This disease was first reported in Java in 1926. Its history, origin and spread to Tanzania have not been explained. However, it is wide spread in the country and it has numerous names depending on the locality. The common names are “kideri, mdonde, mdondo” and “sotoka ya kuku” in Swahili (Yongolo *et al.,* 1996).

### *2.3.1 Aetiology*

ND is caused by avian paramyxovirus serotype 1 (APMV) NDV of the genus Rubulavirus belonging to the subfamily Paramyxoviridae, order Mononegavirales (Spadbrow, 1993; Alexander and Senne, 2008; IOE, 2009). Isolates of NDV are classified depending on the degree of virulence, this includes virulent (velogenic), moderately virulent (mesogenic), or of low virulence (lentogenic). Lentogenic strains are used in production of live vaccines in healthy chickens (IOE, 1996).

### *2.3.2 Host range and transmission*

Majority of birds are susceptible to infection with NDV with disease severity varying from one species to another. Chickens are highly susceptible, but other poultry species such as ducks, geese, turkeys, doves, and guinea fowl are less susceptible and may play a role in the spread of NDV. Such birds can become infected with NDV without overt clinical signs hence acting as a source of infection to chickens. Waterfowls are the least susceptible of all domestic poultry (Martin, 1992; Spadbrow, 1993).

NDV is transmitted through aerosol. However, oral route of infection seems to be more common for the transmission of NDV in scavenging local chickens (Gueye`, 2000). Movement of live birds, people, equipment, poultry products, contaminated feeds and improper use of vaccines are considered to be responsible for the spread of NDV from infected to susceptible flocks in extensively managed rural poultry.

### *2.3.4 Clinical symptoms*

The incubation period of ND after natural exposure has been reported to vary from 2-15 days or longer, with an average of 5 - 6 days (Spadbrow, 1993; Alexander, 1997). Clinical signs and the speed of which they appear depend on the strain of virus, host age, condition and species of the infected birds (Martin, 1992; Alexander, 1997). Observed signs depend on whether the infecting virus has a predilection for respiratory, digestive, or nervous systems and the phenotype involved (Alexander, 1997). Viscerotropic velogenic Newcastle disease, mortality rate can be up to 100 per cent in severe (virulent) forms of the disease (Martin, 1992). More often death occurs within 4-8 days preceded by weakness and prostration. Varying degrees of depression and inappetence are observed. A partial or complete cessation of egg production may occur. Eggs may be abnormal in colour, shape, or surface, and have watery albumen. Oedema of head, wattles and tissues around eyes, increased respiration. Watery greenish diarrhoea, sometimes blood stained. Respiratory signs of gasping, coughing, sneezing, and rales predominate in low virulence infections. Nervous signs of tremors, paralyzed wings and legs, twisted necks, circling and complete paralysis of legs and occasionally of the wing may occur (Jordan, 1990). In neurotropic velogenic, mortality of 0 to 100% and of 90% in young immature chicken may occur. In adult birds, mortality rate may range from 10-50% with respiratory distress, coughing and gasping. Decline or cessation of egg production, nervous signs may appear within a day or two, such as paralysis of legs or wings and torticollis. In the mesogenic, mortality of up to 50% with acute respiratory disease (coughing but rarely gasping), decline in egg production for about 1-3 weeks

with decreased egg quality (Martin, 1992). In this condition some chickens failed to return to normal production occasionally with appearance of nervous signs (Martin, 1992).

### *2.3.5 Gross and pathological lesions*

Gross lesions are usually observed only with viscerogenic velogenic new castle disease Dark red, purplish hemorrhagic and necrotic lesions, especially the digestive tract and intestinal wall (jejunum, ileum, and posterior half of duodenum). Lesions vary in length from a few mm to 15 mm or more. Petechiae may be seen on the serous membranes; hemorrhages of the proventricular mucosa and intestinal serosa are accompanied by multifocal, necrotic hemorrhagic areas on the mucosal surface of the intestine, especially at lymphoid foci such as caecal tonsils. Splenic necrosis and hemorrhage and edema around the thymus may also be observed. In contrast, the lesions in birds infected with lower virulence NDV strains may be limited to congestion and mucoid exudates seen in the respiratory tract with opacity and thickening of the air sacs. Secondary bacterial infections will increase the severity of the respiratory lesions.

### *2.3.6 Control and prevention measures*

To date there is no reported drugs for treatment of ND. Control of ND is based on vaccination using thermostable, dead and live attenuated vaccines (Sally, 2002). Live lentogenic vaccines, mainly $B_1$ and La sota strains, are widely used and are administered as mass application in drinking water. Alternatively, individual administration is via the

nares or conjunctival sac. Healthy chicks are vaccinated as early as day 1 - 4 of life. However, delaying vaccination until the second or third week is recommended to avoid maternal antibody interference with an active immune response. The frequency of revaccination to protect chickens throughout life largely depends on the risk of exposure and virulence of the field virus challenge. But the use of vaccines has been restricted by their storage requirements of cold chain facilities whereby many Newcastle disease vaccines deteriorate after storage for one or two hours at room temperature (Sally, 2002). This makes them unsuitable for use in remote rural areas where the vaccine may need to be transported for hours or in some cases days at ambient temperature. Also in some areas, the vaccines are not readily available. Where vaccines are available, cost becomes another constraint in resource poor communities under rural settings. Vaccines are usually packed in large quantities of 1,000 birds per vial, while the flock size of local chicken in rural areas range between 15 to 20 birds. Therefore, people in rural setup use traditional way of managing poultry diseases including ND. Traditionally, several plant species have been used for treatment of chicken diseases particularly in rural areas. Stems of *Euphorbia candelabrum* (Euphorbiaceae), pepper fruits of *Capsicum frutescens* and *Capsicum annum* (Solanaceae) and *Iboza multiflora* (Liliaceae) root barks of *Khaya senegalensis*, wild garden egg *Solanum nodiflorum* and bitter leaf *Vernonia amygdalina* have been used against new castle disease (Minja, 1989; Gueyè, 1999; Musa *et al.,* 2008). Study done by Waihenya *et al*., 2002 demonstrated the reduced severity of New castle disease symptoms in chicken treated with *Aloe secundiflora* as opposed to untreated group of chicken. Various chemotherapeutical

trials using plant extracts against NDV have been performed with little success (Mtambo *et al.,* 1999; Waihenya *et al.,* 2002).

## 2.4 Poultry coccidiosis

Avian coccidiosis is another important disease affecting local chicken mainly as a result of poor management. The disease is most prevalent under conditions of poor nutrition, poor sanitation, or overcrowding, or after the stresses of weaning, shipping, sudden changes of feed or severe weather.

### *2.4.1 Aetiology and host range*

Coccidiosis is caused by a protozoan from phylum *Apicomplexa* (MAFF, 1986), subclass *Coccidia* parasite and the genus *Eimeria* (MAFF, 1986). Coccidiosis is an intestinal disease that affects different animal species including domestic animals, humans and birds. *Eimeria* species are found in the domestic fowl, turkeys, waterfowl (geese and ducks) and pigeons. In chickens nine *Eimeria* spp. have been described: i.e. *E. acervulina E. brunetti, E. maxima, E. mitis, E. mivati, E. necatrix, E. praecox, E. tenella and E. hagani* (Thebo, *et al.,* 1988; Shirley; 1995 Helm, 1999; McDougald, 1982; Peek, 2010). The most important pathogenic species in turkeys are *E. meleagrimitis, E. adenoeides, E. gallopavonis* and *E. dispers. E. truncata*, which causes renal coccidiosis, and *E. anseris* which causes intestinal coccidiosis are known to be the most prevailing and harmful in commercial flocks of geese (McDougald, 1982; Peek, 2010). *E. truncata* occurs in the kidney of young goslings and may cause high mortality

due to a blockage of the kidney function (Peek, 2010). Two species of coccidia have been reported in pigeons worldwide, these include *E. labbeana* and *E. columbarum.*

### *2.4.3 Transmission*

Local chickens pick up the infection from contaminated premises (soil, houses, utensils, beddings etc.). These premises may have been contaminated previously by other infected birds that have recovered from the condition. Dampy environment facilitate sporulation of oocysts and infection (Kennedy, 2001). Other predisposing factors for the infection include the number of oocysts eaten, strain of coccidia, predilection site in the host, age of the bird and nutritional status (Saif *et al.*, 2003).

### *2.4.4 General life cycle of* **Eimeria** *spp.*

Developmental stages of coccidian protozoa take place in the environment and within the host. Microscopic eggs (oocysts) develop in the chicken and are passed out in droppings. Under proper conditions of temperature and moisture the oocysts develop within one to two days to form sporulated oocysts which are capable of infecting other chickens. At this stage the oocysts contains eight bodies (sporozoites), each of which is capable of entering a cell in the chicken's intestine after the oocyst is eaten. When sporozoites enter the cells in the intestinal mucosa, they divide many times producing off springs called merozoites. Each merozoite in turn may enter another intestinal cell. This cycle may be repeated several times; because of this cyclic multiplication, large numbers of intestinal cells are destroyed. Eventually, the cycle stops and sex cells

microgametocytes and macrogametocytes (male and female) are produced. The male fertilizes the female to produce and oocyst which ruptures from the intestinal cell and passes in the droppings (Kennedy, 2001). Oocysts can remain alive in poultry sheds for more than a year and they are very resistant to most disinfectants.

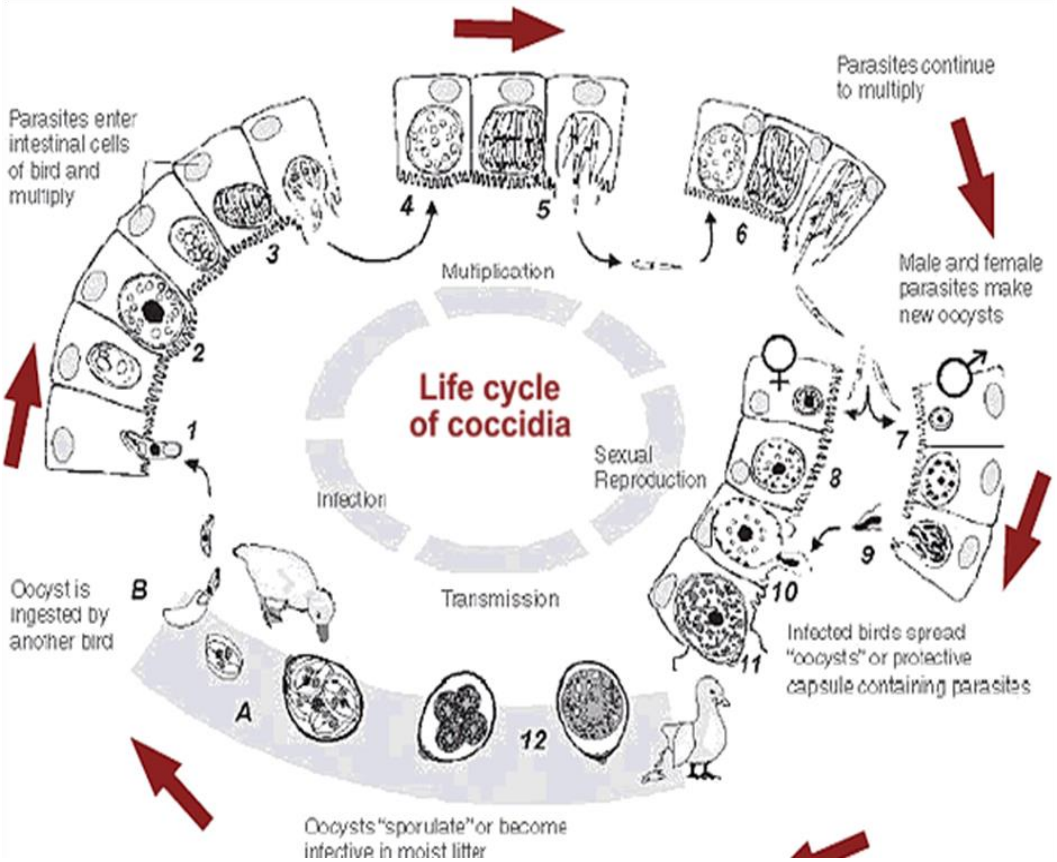


**Figure 1:** Life cycle of *Eimeria* spp. adapted from Dinev (2000).

### *2.4.5 Clinical symptoms*

Birds of any age may be affected; although birds above 3 weeks of age are at higher risk of infection due to maternal immunity. Affected chickens show signs depression, loss of body weight, paleness, ruffled feathers, drooping wings, pale and dry flanks, slight whitish soiling around the vent (only occasionally), diarrhoea and sometimes fresh blood in the droppings/ bloody diarrhoea (Biu *et al.,* 2006; Nwosu *et al.,* 2011). Often, a large percentage of the chickens are sick and may die suddenly before the above symptoms are obvious or the performance of birds may be affected without the disease causing obvious signs (Saif *et al.,* 2003; Biu, 2006; Nwosu *et al.,* 2011). Mortality can range from mild to severe, depending on the species of coccidia involved (Helm, 1999; Peek, 2010). The bird develops reduced ability to absorb nutrients, which results in weight loss and eventually death. Sub clinically, it is manifested by poor performance, impaired feed conversion, poor flock uniformity and poor growth (Conway *et al.,* 1993; Shirley *et al.,* 2005). Coccidial infection can also damage the immune system and leave poultry more vulnerable to pathogens like *Clostridium, Salmonella* and *E. coli* (Conway *et al.,* 1993; Shirley *et al.,* 2005).

### *2.4.6 Gross and pathological lesions*

Post-mortem findings vary depending on the type of coccidia involved. In caecal coccidiosis, which is caused by the species *Eimeria tenella*, the blind gut (caeca) are swollen and filled with blood and cheesy plugs. In intestinal coccidiosis, white streaks or spots in the upper part of the intestine, ballooned and blood-filled intestine, or reddish

spots, inflammation and dead tissue in the lower part of the small intestine. Microscopically small focal hemorrhagic areas and necrosis near blood vessels are with heterophils infiltration of sub mucosa is a common finding (Saif *et al.,* 2003). Lesions produced by *E. tenella* are usually found in the caecum, occasionally some strains of *E. tenella* causes lesions in the rectal area. *E. acervulina* and *E. mivati* occur primarily in the duodenal loop and the upper part of the jejunum. *E. maxima* and *E. necatrix* produce their most severe lesions in the mid intestinal area (Conway and McKenzie, 2007).

### *2.4.7 Management and treatment of avian coccidiosis*

Modern intensive poultry production is largely dependent upon chemoprophylaxis by adding coccidiostats as feed additives and good hygienic practice (Sousby, 1982). In developed countries, management of coccidiosis is based on the use of vaccines, which contain all the important species because of the species specificity of immunity (Mc Dougald, 1982; 2003). Vaccines have also been used for some time to provide immunity particularly for commercial egg layers, but there is less use in broilers and other local species of chickens. The use of vaccine has been challenged due to *Eimeria* species specification. In rural settings, infection from contaminated litter is relied on to induce natural immunity. In Tanzania, the use of coccidiostats such as sulphonamides, quinoline, polyether ionophorous and clopidol is more common. Nevertheless, no drug and/or drug combination that has been found to be effective in eliminating coccidiosis in chickens. In addition, *Eimeria* strains resista nt to most coccidiostats have been reported (Tipu *et al.,* 2006). The emergence of resistance has therefore prompted for search of

medicinal plants as an alternative approach (Tipu *et al.,* 2006). Several herbal and medicinal plants have been used as coccidiostat; in Nigeria several plant species have been claimed to have medicinal value for treatment of coccidiosis. These plants include *Anona senegalensis, Khaya senegalensis* and *Butyrospermum paradoxum* showed to posses' anticoccidial effect in chickens (Nwosu *et al.,* 2011). Other plants reported to have anticoccidial effect are *Azadirachta indica* and *Artemisia annua* (Allen *et al.,* 1997; Arab *et al.,* 2006; Brisibe *et al.,* 2008). Study done by (Kitandu and Juranová, 2006; Ogbe *et al.,* 2010) reported the use of mushrooms such as *Pleurotus ostreatus, Ganoderma lucidum, Lentinus edodes* and *Tremella fuciformis* as probiotic agents and food supplements in the control of *Eimeria tenella* infection. *Commiphora molmol* was also studied for the anticoccidial activity against *Eimeria stiedae* has shown potential source anticoccidial compounds were as infected rabbits treated recovered from all signs and symptoms of infection (Baghdadi and Al- Mathal, 2010). In Zimbabwe several medicinal herbs are reported to be used against coccidiosis, these other spp include ; *Aloe vera, Aloe spicata, Lycopersicon esculentum, Myrothamnus flabellifoilius, Lannea stullmannii, Ficus burkei, Sarcostemma viminale, Capsicum annum, Parinaria curatellifolia, Albizia gummisera* and *Albizia adianthifolia*.

# CHAPTER THREE

## 3. MATERIALS AND METHODS

**Summary**

This chapter outlines all the materials, methods and techniques used in this study. These include the study area, ethno botanical survey done to describe where *Commiphora swynnertonii* was obtained, and collection of all information concerning this plant from the field. Also the chapter describes the methods for collection of the plant materials from the field; standard extraction and processing used. Methodologies used to investigate the *in vitro* techniques to assess the antiviral effect of *Commiphora swynnertonii* against New castle diseases virus (NDV) using in *ovo* assay are described. Also this chapter describes the methodology used for determining the effect of the study plant on selected bacteria, fungi, NDV and coccidian of veterinary importance. Finally, the methods used to evaluate phytochemical analysis of bioactive compounds from the most active morphological part (root bark and resin extract) described.

### 3.1 Study area

The survey was conducted at Mererani Ward in Simanjiro District of Manyara Region in northern Tanzania ($4^{o}0'0$ S, $36^{o}$ 30'0 E; 1360 m above sea level). Simanjiro is one of the five districts of the Manyara Region; it is bordered to the north by Arusha Region, to the north east by Kilimanjaro Region, to the south east by Tanga Region, to the south by

Kiteto District, to the south west by Dodoma Region and to the west by Babati District (Figure 2). Generally, the district is semi arid with annual rainfall of 500 mm. The topography varies from black sandy soil to mountainous fragile soil (Minja, 2006). The area is endowed with closed thick forests around mountains and wooded grasslands on the lower flatlands, the latter is commonly known as "savannah woodlands" (*Acacia commiphora* woodland).

### 3.2 Questionnaire design and administration

A questionnaire with 27 open-ended structured questions was designed with the aim to gathering information on participants, knowledge on plant identification and general uses of the plant (Appendix 1). Bio-data information of each respondent was recorded and questions on plant identification were asked. Respondents were also asked questions on the use of *C. swynnertonii* in human and other animal species; the questions included plant part used, preparations, doses indicated, route of administration and type of diseases treated. A cross-sectional purposive study was carried out involving 106 respondents from four randomly chosen villages of Simanjiro District. At least 26 respondents were randomly chosen from each village. An inclusion and exclusion criterion was based on age (above 18 years) of respondent and willingness (consent) to be involved in the study.

### 3.3 Sample collection

The plant was identified by a botanist (Mr. J. Kayombo, 2009) as *Commiphora Swynnertonii* from the family Burseraceae. A voucher specimen (reference number CK 6489) was prepared and preserved at Tanzania National Herbarium, in Arusha (Kayombo, J. personal communication, 2009). Different morphological parts namely, leaves, stem barks, root barks and resin of the plant were freshly collected from the area and transported to Sokoine University of Agriculture for preparation, extraction and testing.

#### *3.3.1 Preparation and extraction of plant materials*

In the laboratory each morphological part of *C. swynnertonii* was handled separately. The materials were cleaned of debris using running tap water; barks were first peeled from stem/root stumps and chopped into small pieces before sun drying. The dried materials were then ground to pass through 0.1 mm sieve size using a laboratory mill (Christy Hunt Engineering Ltd, England) and then stored in airtight bags in a cool dry room until used. Solvent extraction was carried out according to a method described by Parekh and Chanda (2006) with some modifications.

Exactly 500 g of ground plant material were soaked in 1,000 ml of ethanol (99.8% v/v) in a conical flask, plugged with aluminium foil and kept for 72 h in a dark place at a room temperature. After soaking, the suspensions were filtered using Whatmann® filter paper No. 1 and the filtrate was concentrated on water bath at 50°C using a rotary

evaporator (BUCHI, Switzerland) until all the ethanol was cleared. The resin material was treated differently in that after soaking it was immediately concentrated using the rotary evaporator. The resulting crude extracts were then stored at 4 $^{o}$C in airtight bottles until used. Then stock solutions were prepared by dissolving 0.5 g of the crude ethanolic extracts in 5 mL of dimethyl sulphoxide (DMSO) to make 10% *w/v* working solutions. A serial dilution method was used to prepare the working solution of different concentrations which were used in different bioassays.

### *3.3.2 Preparation resin extract for clinical trials*

The resin extract was weighed and diluted with distilled water at different doses and given orally in chickens.

## 3.4. Investigating the effect *C. swynnertonii* on selected bacteria and fungi

### *3.4.1. Agar well diffusion method*

The agar well diffusion method was used to test the antimicrobial activity of the study plant extracts for different bacterial and fungal strains. The nutrient agar plates was prepared by pouring 15 ml of warm molten media into sterile Petri dishes and allowed to cool. The bacterial and fungal isolates were then inoculated into Muller Hinton and Saboraud's Dextrose Agar respectively, then incubated at 37ºC for about 6 hours after which the nutrient agar plates were seeded with the test microorganisms by the spread plate technique, and were left for about 30 minutes to dry (Ayo *et al.*, 2007). After

allowing the plates to dry, wells with 6 mm diameter was punched on each agar plate. The wells were numbered accordingly to match with the code number of test extract concentrations; this was done in duplicate. Then, the extracts were poured into wells, while matching the well number with the corresponding code number of the extract concentration. After allowing for about 15 minutes, the plates were incubated at 37°C for 24 hrs. The assessment of antimicrobial activity was based on the measurement of the diameter of the inhibition zone formed around the wells. A 50 µg/ml of the antibiotic Gentamycin 10% and 200 mg of antifungal agent (Ketoconazole) were prepared and impregnated into ditches in agar medium.

### *3.4.2 Minimum inhibitory concentration (MIC)*

The minimum inhibitory concentration (MIC) was evaluated on plant extracts which showed some antimicrobial activity. MIC, the lowest concentration of a compound that inhibits growth of a microorganism, was determined by the standard two-fold dilution technique using microdilution technique with nutrient broth medium (Obi *et al.*, 2007). A serial dilution of 100 µl of the extracts was made in microtitre wells with 50 µl nutrient broth to obtain a concentration ranging from 500 to 1.95 mg/ml. A 0.5 McFarland standard suspension of test bacteria was made in nutrient broth, from which100 µl of the final inoculums containing approximately 1.0 $x10^8$ colonies forming units (cfu) was added to the appropriate wells to make a final volume of 200 µl in each well. Inoculated plates were incubated at 37°C for 24 h. One hour before the end of incubation 40 µl of a 0.2% solution of Iodo-Nitro Tetrazolium (INT) (Merck, Germany)

was added to the wells and the plates were then incubated for another hour. Since the colorless tetrazolium salt is reduced to a red coloured product by biologically active organisms, the inhibition of growth can be detected when the solution in the well remains clear after incubation with INT (Samie *et al.*, 2005; *Obi et al.*, 2007). The test was done in duplicates. The mean of the lowest concentrations of each extract showing no visible growth was as recorded as the minimum inhibitory concentration (MIC). Gentamycin 10% and Ketoconazole 200 mg was used as the positive control while the negative control comprised the test bacteria with only DMSO.

### 3.5 Investigating the cytotoxicity effect of C. *swynnertonii* on brine shrimps

#### *3.5.1 Hatching the brine shrimp*

Brine shrimp eggs (*Artemia salina*, Sanders$^{TM}$ Great Salt Lake, Brine Shrimp Company L.C., U.S.A.) were hatched in simulated seawater prepared from sea salt, which was prepared by boiling seawater to evaporation. The simulated sea water was prepared by dissolving 3.8 g sea salt in one litre of distilled water. Rectangular glass chamber divided into two unequal compartments with holes on the divider was used for hatching. The eggs were sprinkled into the larger compartment that is darkened, while the smaller compartment was illuminated. After 24 hours of incubation at room temperature, *nauplii* (larvae) were collected by Pasteur pipette from the lighted chamber, whereas their shells were left in the darkened chamber.

### *3.5.2 The Bioassay*

The brine shrimp lethality test was carried out using the standard procedure as described by Meyer *et al.* (1982) and McLaughlin *et al.* (1991), with slight modifications. The stock solution of the study plants extracts were prepared by dissolving 160 mg of the dry extract in 4 ml of DMSO, to get a concentration of 40 mg/ml. Taking 30, 15, 10, 5, 3, and 1µl of the stock solutions, the final concentrations of 240, 120, 80, 40, 24 and 8 $\mu g ml^{-1}$ were obtained by dilution with the 5 ml of the sea salt solution in the vials. Each concentration was tested in triplicate, 18 vials per test extract and one set of 18 vials were prepared using DMSO as a negative control (Moshi and Mbwambo, 2004; Moshi *et al.,* 2006). Ten larvae of brine shrimps were transferred into each vial containing test extract with sea salt solution using Pasteur pipettes, followed immediately with adjusting the volume of the sea salt solution to 5 ml mark. The vials were maintained at room temperature on a laboratory bench. Survivors were counted after 24 h and from these the percentage death at each concentration was determined (Meyer *et al.,* 1982; McLaughlin *et al.,* 1991).

## 3.6 Investigating the effect *C. swynnertonii* on Newcastle disease virus

### *3.6.1 Preparation of the inoculums and in-ovo antiviral assay*

The local strain of NDV was used in the preparation of the inocula for *in ovo* assay. The assay was carried out using local strain of Newcastle disease virus on infection-free nine day old chicken embryonated eggs (ECE) according to a method described by Senne, (1998) and Sally, (2002) with some modifications. The ECE were randomized into

seven groups (n = 10) as shown the Table 2 below. Stock solutions were prepared by dissolving 0.5 g of the crude ethanolic extracts in 5 mL of dimethyl sulphoxide (DMSO) to make 10% w/v working solutions. A serial dilution method was used to prepare the working solution of seven different concentrations of 50, 150, 200, 250, 300, 400 and 500µg/mL. A volume of 0.1 mL solution of the virus suspension containing 32 HA units of NDV and 0.9 mL crude extracts from the stock solution at different concentrations was mixed and inoculated vertically into the allantoic cavity of viable ten days old ECE following the procedure outlined in IOE (2008, 2012) manual. The day ten ECE was checked for their viability by the candling process, air sac marked and the surface disinfected with 70% ethyl alcohol. Then the inoculum was infused using the insulin syringe with a 25 gauge X 0.5 inch needle and the inoculated site was sealed with paraffin wax and the inoculated eggs were incubated at $37^{o}$C for four days with the air sac in an upright position. Un-inoculated eggs served as negative controls while eggs with virus suspension without extract served as positive controls. Survivals of the embryos were monitored daily for four days through candling of eggs. Observations were made on the embryo movements, blood vessels, weight of the embryo and time of embryo death. Dead embryos were removed chilled for one hour and allantoic fluid harvested for estimation of viral titres using the HA methods described by IOE (2008). At day four post inoculation, the allantoic fluid was harvested from the surviving embryos in a sterile plastic bottle, and analyzed for virus titres using a standard haemaglutination (HA) test.

**Table 2:** Grouping and treatment allocation for the *in ovo* assay

| Group (n = 10) | Treatment | Observational time (hours) |
|---|---|---|
| G1 | Untreated egg (no NDV; no extract) | 96 |
| G2 | NDV alone | 96 |
| G3 | DMSO + NDV | 96 |
| G4 | *Leaf extract + DMSO + NDV | 96 |
| G5 | *Stem bark extract + DMSO + NDV | 96 |
| G6 | *Root bark extract + DMSO + NDV | 96 |
| G7 | *Resin extract + DMSO + NDV | 96 |

*Concentrations used for each extracts were 50, 250 and 500µg/mL.

Another batch of forty five eggs (15 eggs for root bark, stem bark and resin treatment) at the concentration of 250 µg/ mL and 15 eggs from the negative control group were left to hatch 12 days post infection. Blood samples were obtained from the hatched chicks and tested for their antibody titres using a standard haemaglutination inhibition test (HI) on day 0, 14 and 28 post hatching. Body conditions of the chicks were assessed using a score scale of 3 points with body weight, activeness and feather condition as scoring criteria.

### *3.6.2 Estimation of virus in allantoic fluid using haemaglutination assay (HA) titre*

This method is convenient when testing allantoic fluid from a large number of embryonated eggs for the presence or absence of haemaglutinin (IOE, 1996, 2008, 2012; Sally, 2002). At day four post inoculation, the allantoic fluid which was harvested from the eggs of the surviving embryos was collected in a sterile plastic bottle. Then, 25 µL of the harvested fluid was serially diluted with 25 µL of PBS (at pH 7.2) in a 96 wells V

bottom shaped microtitre plate. Then 50 µL of 1% red blood cells (fresh chicken erythrocytes) was added to each well mixed and allowed to stand for 45 minutes at room temperature. After incubation, virus titre was read as the reciprocal of the highest dilution that caused agglutination of the chicken red blood cells.

### *3.6.3 Haemaglutination Inhibition (HI) titres against NDV in hatched chicks*

This was done as described by Sally (2002). Blood samples were collected for testing for the presence of antibodies to Newcastle disease virus in a plain vacutainer tube. The blood samples were immediately centrifuged at 3000 rpm for 10 minutes. The serum was separated from red blood cells. Then, 25 µL of the harvested serum was serially diluted with 25 µL of PBS (at pH 7.2) in a 96 wells V bottom shaped microtitre plate. Then 25 µL of the 4HA dilution of antigen was added to each well. Then 50 µL of 1% red blood cells (fresh chicken erythrocytes) was added to each well mixed and allowed to stand for 45 minutes at room temperature. After incubation, virus titre was read as the reciprocal of the highest dilution that caused agglutination of the chicken red blood cells.

### *3.6.4 Preparation of NDV inoculums for in vivo trial*

ND viruses were isolated from livers and proventriculi of naturally infected chickens. The organs were cut into small pieces and washed with phosphate buffer saline (PBS) before being ground into homogenious mixture using a mortar and pestle with aid of sterile sand. The resultant mixture was sterilized by diluting it with antibiotic medium (PBS with 200 IU/mL penicillin, 2 mg/mL, streptomycin, 0.05 mg/mL, gentamycin and

1,000 IU/mL nystatin at pH 7.0 - 7.4) to make 20% w/v suspension. Thereafter, the sterilized suspension was spinned in a chilled (4 - $10^0$C) centrifuge at 1,000 – 1,500 revolutions per minute (rpm) for 10 minutes to sediment tissue debris, bacteria and fungi. The supernatant was removed aseptically and incubated at room temperature for 90 minutes. To propagate the viruses, haemaglutination test was carried out and the inoculums containing 32 HA titres were inoculated in embryonated chicken eggs (ECE) through allantoic cavity as described by Senne (1998). After four days, allantoic fluids were collected and serially diluted in PBS to obtain a dilution of $10^{-6}$ of viruses which was later used to induce ND in the experimental chickens.

### *3.6.5 In vivo studies in experimental chickens*

One-day old black australorp chicks were purchased from a single farmer in the study area.Chicks were moved to experimental house at SUA where they were stabilized for 24 more months using a synthetic antibiotic (OTC-plus®), anthelmintic Kukuzole ® (mebendazole), Interchem Pharma Ltd, Moshi Tanzania and anticoccidial drug (Amprolium®, Norbrook Laboratories Ltd.). Then all chickens were vaccinated against infectious bursal disease (IBD) Lohmann Animal health GmbH & Co KG, Heinz Lohmann, Cuxhaven, Germany). After a month of brooding, the chicks were moved into rearing cages where were maintained on growers mash, given mineral supplements (Amintotal®) and *ad libitum* access to drinking water. At the age of 5 months, chickens were shifted to experimental cages, were wing-tagged and assessed for sign of diseases.

Then blood samples were taken for evaluation of HI titres for ND. Chickens which showed negative results were randomly assigned into eight groups.

### *3.6.6 Experimental animals*

Hundred and fourteen (114) one-day old black australorp chicks were purchased from a single farmer in the study area. The chicks were raised in a large brooder and maintained on maize bran-based chick mash with *ad libitum* drinking water for two weeks and then they were dewormed and vaccinated against infectious bursal disease (IBD). After a month of brooding, the chicks were moved into raring cages for four months before they were randomly assigned into different experimental groups. In the raring cages, chicks were maintained on growers mash, given mineral supplements (Amintotal®) and *ad libitum* access to drinking water. At the age of 5 months, chickens were shifted to experimental cages, were wing-tagged and randomly assigned into eight groups namely G1, G2, G3, G4, G5, G6, G7 and .G8, respectively each with 14 birds. Weekly bodyweight gains were measured and chickens were observed for any signs of diseases.

### *3.6.7 Experimental design*

Two concurrent trials were carried out in order to investigate both prophylactic and therapeutic effects of the resinous extract of *C. swynnertonii* against experimental ND infection in chickens. The eight chicken groups (G1 - G8) received different treatments as shown in Table 3. Blood samples were collected from all the chickens for serological

analysis of ND virus antibody titres before experimental infection and treatment. Chickens in G1 were neither infected with ND virus nor treated with the extract and served as negative control, whereas those in G2 were infected but were not treated serving as positive control group. Distilled water as a placebo was administered orally to chickens in the two control groups for 7 days.

Chickens in G3 to G5 were used in the prophylactic trial and received oral doses of 250, 500 and 1,000 mg resin/kg bodyweight, respectively for seven consecutive days before they were inoculated with 0.1 mL of viral suspension (containing $5.12 \times 10^2$ ND viruses per mL). Following appearance of ND clinical signs, chickens were given another round of treatment with the resin as done before for another seven consecutive days.

**Table 3:** Groups and treatments allocation

| Trial | Groups | n | Treatment with resin mg/kg bodyweight |
|---|---|---|---|
| Control | G1* | 12 | 0 |
| | G2† | 12 | 0 |
| Prophylactic | G3 | 15 | 250 |
| (treatment before infection) | G4 | 15 | 500 |
| | G5 | 15 | 1,000 |
| Therapeutic | G6 | 15 | 250 |
| (treatment after infection) | G7 | 15 | 500 |
| | G8 | 15 | 1,000 |

*Negative control - not infected; †positive control - infected

The therapeutic trial involved chickens in G6 to G8; these birds were first inoculated with 0.1 mL of the ND viral suspension and left for clinical sign of ND to appear.

Following appearance of the clinical signs, chickens in G6 to G8 were orally dosed with 250, 500 and 1,000 mg resin/kg bodyweight, respectively for seven consecutive days.

### *3.6.8 Clinical observations*

All chickens were weighed weekly and monitored daily for appearance and disappearance of clinical symptoms of ND. Clinical signs were assessed using a body condition score scale of 4 points in which 1 indicated normal healthy chickens and 4 indicated severely affected chickens (Table 4).

**Table 4:** Clinical signs score scale.

| Score | Clinical signs |
|---|---|
| 1 – Normal | No sign (healthy chickens) |
| 2 – Mild | Depression, anorexia(reduced feed intake) |
| 3 – Moderate | Depression, anorexia, greenish diarrhoea |
| 4 – Severe | All signs mention above together with anorexia, greenish diarrhoea, difficult breathing (dyspnoea), raised body temperature (> $42^0$C) and loss of bodyweight emaciation and/or death |

Dead chickens were recorded and taken for post mortem examination. Tissue sections of muscles, liver, kidney and intestines were taken from dead chickens and preserved for histopathology and microbial culture to find out whether there were any concurrent infections. The preserved tissues were processed for histopathological examination as described by Drury and Wallington (1976).

## 3.7. Investigating the effect of *C. swynnertonii* on coccidial parasite

### *3.7.1 In vivo studies in experimental chickens*

Twelve week-old local chicks were purchased from local poultry keepers in Mororgoro Municipal. Chicks were moved to experimental house at SUA where they were stabilized for 18 more weeks using a synthetic antibiotic (OTC-plus®), anthelmintic Kukuzole ® (mebendazole), Interchem Pharma Ltd, Moshi Tanzania and vaccinated against Newcastle (Lasota ® vaccine) and infectious bursal disease (IBD) Lohmann Animal health GmbH & Co KG, Heinz Lohmann, Cuxhaven, Germany). All chickens were maintained on grower's mash with *ad libitum* drinking water. At the age of 16 weeks, all chickens were assessed for any signs of diseases and faecal droppings were taken for examination of *Eimeria* spp. oocysts and other worm eggs. Chickens that showed negative results were randomly assigned into five groups according to the protocol explained in section 3.6.2 below.

### *3.7.2 Experimental animals*

About 120 chickens (approx. 12 weeks-old) were purchased from local poultry keepers in Morogoro town and moved to experimental house where they were maintained on grower's mash with *ad libitum* drinking water. All chickens were dewormed and vaccinated against Newcastle and infectious bursal disease (IBD) and left to acclimatize for four weeks prior to the experiment. At the age of 16 weeks, all chickens were

assessed for any signs of diseases and faecal droppings were taken for examination of *Eimeria* spp. oocysts and other worm eggs.

### *3.7.3 Isolation and identification of Eimeria spp. oocysts*

*Eimeria* spp. oocysts were collected through tissue scrapings from small intestines of naturally infected chickens and concentrated using floatation method (MAFF, 1986). The collected oocysts were kept in 2.5% (w/v) potassium dichromate at $27^0$C for seven days to sporulate. Following sporulation, the oocysts were re-suspended in saturated sodium chloride using floatation method. The sporulated oocysts were identified to species level under light microscope (X 40) and counted using Mc Master counting chamber. The obtained oocysts were diluted in distilled water to make a suspension of $1.5 \times 10^4$ oocysts per mL; this was kept at room temperature until used.

Oocysts dose per bird was calculated as described by (Conway *et al.*, 1993) as follows:

$$= \frac{\text{Number of oocysts to be inoculated per group of birds}}{\text{Number of oocysts per milliliter in the culture}} \quad \textbf{(1)}$$

$$= \left[\frac{\text{15,000 Oocysts to be inoculated X 100 chickens}}{\text{20,000 Oocysts in culture}}\right]$$

= 75 mL per 100 chickens

= 0.75 mL (approx. to 1mL) per chicken.

Therefore each bird was administered with 1mL contained $1.5 \times 10^4$ oocysts.

### *3.7.4 Grouping and treatments allocation*

At 18 weeks of age, the birds were weighed, wing tagged and randomly assigned into five equal experimental groups (n = 16 birds). All chickens were kept in individual cages for easy handling and sample collection. Chickens in G1 were not infected with coccidian oocysts and therefore served as negative control. All chickens in G2, G3, G4 and G5 were infected through oral administration of coccidian oocyst suspension at a dosed rate of 1.5 x $10^4$ *Eimeria* spp. oocysts per bird. This infection dose rate was calculated as described by Conway *et al. (*1993) to give a moderate coccidial infection in growing chickens. Starting from day 3 post-infection (p.i), chickens in different groups were treated for 7 consecutive days as follows: G1 and G2 (positive control) received 5 mL of normal saline as placebo, G3 and G4 were given the extract at 400 and 800 mg/kg body weight whereas G5 received anticoccidial drug (Amprolium®, Norbrook Laboratories Ltd.) as recommended by manufacturer.

**Table 5:** Groups and treatments allocation

| **Trial** | **Groups** | **n** | **Treatment with resin mg/kg bodyweight** |
|---|---|---|---|
| Control | G1* | 16 | 0 |
| | G2† | 16 | 0 |
| Resin groups | G3 | 16 | 400 |
| | G4 | 16 | 800 |
| Amprolium group | G5 | 16 | 10 mg/mL |

*Negative control - not infected; †positive control - infected

### *3.7.5 Observations and measurements*

**(i). Clinical signs and body weight**

All chickens were monitored daily for appearance and disappearance of clinical symptoms of coccidiosis. Clinical signs were assessed using a body condition score scale of 4 points in which 1 indicated normal healthy chickens and 4 indicated severely affected chickens (Table 6). Bodyweights were measured weekly throughout the experimental period using a balance.

**Table 6:** Clinical signs score with their interpretations.

| Score | Clinical signs |
|---|---|
| 1-Normal | No sign (healthy chickens) |
| 2 – Mild | Depression and ruffled feathers |
| 3 – Moderate | Depression and ruffled feathers, anorexia(reduced feed intake) |
| 4 – Severe | All signs mention above together with emaciation and brownish/ bloody diarrhea |

**(ii) Faecal oocysts count**

Faecal oocyst counts were evaluated using modified McMaster Technique (MAFF, 1986). Briefly, fresh droppings were collected from each cage early morning and processed for oocyst counts. Percentage reduction in oocysts per gram (OPG) faeces was calculated using the formula given below:

$$\% \text{ Faecal oocysts reduction} = \frac{\text{OPG before treatment} - \text{OPG after treatment} \times 100}{\text{OPG before treatment}} \ldots \textbf{(2)}$$

Deaths were recorded and all dead chickens were subjected to post mortem examination whereby livers, kidneys and intestines were preserved for histopathological examination.

## 3.8 Investigating the effect of *C. swynnertonii* on physiological parameters

### *3.8.1 Experimental animals*

Healthy adult chickens (Black Australorp) of 8 months old were purchased from a local farmer and used in this experiment. Once in the experimental house, all chickens were dewormed and vaccinated against Newcastle (ND) and Infectious Bursal disease (IBD); they were also assessed for signs of other diseases. All chickens were caged in pairs, maintained on layers mash, mineral supplements and drinking water *ad libitum*. The chickens were left for three weeks to acclimatize with experimental environment. Following acclimatization, they were weighed, wing tagged and randomly assigned into five experimental groups of 12 chickens each.

### *3.8.2 Experimental design*

Treatments allocations of chickens into the 5 groups were allocated as shown in Table 7.

**Table 7:** Grouping and treatments allocation

| Group (n =12) | Treatment given for 14 days |
|---|---|
| G1 | Negative control (not given resin extract) |
| G2 | 250 mg/kg |
| G3 | 500 mg/kg |
| G4 | 750 mg/kg |
| G5 | 1000 mg/kg |

Groups 2 - 5 chickens were given different doses of aqueous resin extract orally for 14 consecutive days. Group 1 remained as negative control i.e. chickens received normal saline only. Immediate and extended signs of toxicity and changes in body weight were observed. Blood samples were collected for evaluation of haematological and biochemical parameters.

### *3.8.3 Collection of blood for haematological and biochemical parameters*

Blood samples (approx. 3 mL) were collected from wing veins using syringe with 23G needle at regular interval from day 0, 3, 7, 14 21 and 28. About 1 mL blood was then transferred into blood sample bottles containing EDTA for haematological parameters analysis, while the remaining 2 mL were immediately centrifuged at 3000 rpm for 10 minutes to obtain fresh plasma. The plasma was used for analysis of biochemical parameters using spectrophotometer (Cole Patner 1100 rs). All sampling was carried out

in the morning between 7 a.m and 9 a.m in order to reduce on diurnal variability which might affect the parameters.

### *3.8.4 Determination of haematological parameters*

Haematological parameters assessed included packed cell volume (PCV), haemoglobin level (Hb), total red blood cell count (RBCs), white blood cell count (WBCs) and differential WBCs (lymphocytes, monocytes, heterophils and eosinophils).

**(i) Packed cell volume (PCV)**

Packed cell volume (PCV) was determined using duplicate capillary tubes. The tubes filled with blood at three quarter level and then seales at one end by the wax. The sealed capillary tubes were then centrifuged at 12,000 rpm in a microhaematocrit centrifuge (Hawksley MHC) and read using the Hawksley haematocrit reader in percentage.

**(ii) Total RBC count**

In determining RBC count, enumeration of erythrocytes was carried out by diluting blood 1:200 in a red blood cell pipette with Natt & Herrick solution and counting the number of red blood cells (RBC) using an improved Neubauer haematocytometer (Fudge, 2000). The method developed by Natt & Herrick allows for direct counting of both erythrocytes and leukocytes in avian species. Using a standard red blood cell

diluting pipette, whole anticoagulated blood was diluted with the Natt & Herrick's solution at the rate of 1:200. Dilution was made with standard pipettes and micro capillaries. The diluted blood was allowed to mix for one minute before discharging into the hemacytometer counting chamber. After charging the hemacytometer, the contents were allowed to settle for approximately 3 minutes. Then the total counting was done under the microscope at 40 0 objective.

**(iii) Total and differential WBC count**

Thin blood smears were stained with Giemsa and examined microscopically under oil immersion (Magnifications of x 100) and a minimum of 50 fields were examined for cellular characterization. Leucocytes were counted according to the estimated cell count protocol as described by Fudge, (2000). For each blood smear a minimum of 200 leukocytes were counted for determination of differential leucocytes values. To reduce variations in the differential leucocytes counts, duplicate counts were done by one person. Mean haematological values and standard errors of the mean (S.E.M) were calculated.

**(iii) Haemoglobin, mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH)**

Haemoglobin (Hb) was determined by spectrophotometer at 540 nm using Drabkin’s solution as previously described by Fudge, (2000). Blood was diluted in a solution

containing potassium cyanide potassium ferri-cyanide (Drabkin's solution), Hb is oxidized to methaemoglobin by potassium ferri-cyanide, methaemoglobin in turn combines with potassium cyanide to form cyanmethaemoglobin. The absorbance of the solution was measured in a spectrophotometer at wave length of 540 nm against Drabkin's solution as a blank. The results are expressed in g/L or g%.

This facilitated the calculation of erythrocyte indices, mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) using standard formulae given below:

$$MCH = \frac{Hb\ (g/dL)}{RBCs\ (g/dL)} \quad \textbf{(3)}$$

MCH is given in pictogram per cell (pg/cell)

$$MCHC = \frac{Hb\ (g/dL)}{Haematocrit\ (\%)}$$

MCHC is given in percentage (%)

### *3.8.5 Determination of biochemical parameters*

**(i) Determination of plasma glucose level:**

Determination of the blood glucose levels was done by the glucose-oxidase principle as explained by Trinders (1969).

**(ii) Determination of plasma total cholesterol levels:**

Determination of the plasma total cholesterol levels based in the formation of cholesterol and fatty acids principle as explained by Trinders (1969).

**(iii) Determination of plasma total protein:**

Total protein was determined by Biuretic method using Erba ® test kit as described by WHO, 2006 and International Federation of Clinical Chemistry (IFCC). Principle - Cupric ions (Cu2+) in the biuret reagent complex with the groups involved in the peptide bond. In the presence of alkaline media i.e. 3% NaOH and at least two peptide bonds a violet colored chelate is formed. Biuret also contains sodium potassium tartrate which assists in Cu2+ complex formation and further prevents their precipitation in an alkaline solution. The absorbance of the coloured chelate was measured at 546 nm. The colour intensified from pink to reddish violet due to the complexity of the peptide bond in the protein.

**(iv) Determination of plasma albumin and globulin levels:**

Albumin was measured by bromocresol green based on principles described by Peters *et al.* (1982). Principle - Bromocresol green (BCG) binds quantitatively with albumin at a slightly acid pH forming an intense blue-green complex with an absorbance maximum at 628-630 nm. The intensity of the color produced is directly proportional to the albumin

concentration in the sample. Globulin levels were determined by subtractions of albumin values from the total protein values as described by Bush (1991).

**(v) Determination of liver transaminase enzymes (ALT and AST):**

Liver enzyme (ALT and AST) were determined by the kinetic methods described by Federation of Clinical chemistry (IFCC) without Pyridoxal Phosphate using Erba ® kit. Principle - The test principle of determine the plasma transaminases is based on the oxidation of NADH to NAD+ in the conversion of alanine to pyruvate the resulting decrease in absorbance at 340-370 nm was proportional to the activity of the enzymes in the sample.

**(vi) Determination of plasma creatinine:**

Creatinine was derermined using the colorimetric Jaffe diagnostic kits (Jaffe Elitech ® Creatinine) Test Kits. Principle - Creatinine reacts with picric acid in alkaline medium forming a yellow orange color complex which was measured at 500 nm

**(vii) Determination of plasma uric acid:**

This was measured using commercial kit supplied by Biosystems® (Spain) based on Uric POD. Uric Acid is converted by uricase into allantoin and hydrogen peroxide. The hydrogen peroxide oxidizes the reaction product of 4- aminoantipyrine (4-AAP) with 3, 5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) in presence of a be peroxidase to

form a red colored dye complex. Absorbance of the colored solution was directly proportional to the uric acid concentration, and measured at 505 nm.

### 3.9 Post mortem examination

Post mortem procedures of the experimental chickens were done according to the guideline by Herenda *et al* (2000). Histopathological section slides were prepared by technique descibed by Slaoui and Fiette, (2011). Tissues were fixed with neutral formalin 10%, embedded in paraffin, and then manually were sectioned with a microtome to obtain 4-5 μm-thick paraffin sections. Dewaxed sections were then stained with Hematoxylin and Eosin (H&E) and left to dry for about 30 minutes. The histopathologial slides were examined under the microscope for different histopathological lesions.

### 3.10 Phytocemical screening of rootbark and resin extracts

Chemical tests were carried out on the aqueous and ethanolic extracts and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), and modified by Edeoga *et al* (2005).

#### *3.10.1 Test for tannins*

About 0.5 g of the dried powdered samples were boiled in 20 ml of water in test tubes and then filtered. A few drops of 0.1% ferric chloride were added to the filtrate and

observed for colour change. A brownish green or a blue-black colouration was taken as the evidence for the presence of tannins.

### *3.10.2 Test for phlobatannins*

A portion of aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid (HCl), and then allowed to cool. Deposition of red precipitate was taken as evidence for the presence of phlobatannins.

### *3.10.3 Test for terpenoids (Salkowski test)*

Five milliliters of aqueous extracts of the study plants was mixed in 2 ml of chloroform. 3 ml of concentrated sulphuric acid ($H_2SO_4$) was then carefully added to form a layer. Formation of a reddish brown colouration at the interface was an indication for presence of terpenoids.

### *3.10.4 Test for flavonoids*

Five milliliters (ml) of dilute ammonia solution was added to a portion of the aqueous extract of each study plant, this was followed by addition of concentrated sulphuric acid ($H_2SO_4$). Formation of a yellow coloration, which disappears on standing, indicated the presence of flavonoids.

### *3.10.5 Test for cardiac glycosides (Keller-Killani test)*

Five milliliters of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was followed with 1 ml of concentrated sulphuric acid ($H_2SO_4$). Formation of a brown ring at the interface indicates the presence of cardiac glycosides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the layer.

### *3.10.6 Test for steroids*

Two milliliters of acetic anhydride was added to 0.5 g ethanolic extract of each sample followed with 2 ml concentrated sulphuric acid ($H_2SO_4$). A colour change from violet to blue or green was taken as an indication for the presence of steroids.

### *3.10.7 Test for saponins*

About 2 g of the powdered / liquid sample was boiled in 20 ml of distilled water in a water bath, allowed to cool and then filtered. A 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously to form a stable persistent froth. The frothing was mixed with 3 drops of olive oil, shaken vigorously, and then observed. Formation of emulsion was taken as the evidence for the presence of saponins.

### *3.10.8 Test for anthraquinones*

Powdered plant material was boiled with 10% HCl for a few minutes, filtered and allowed to cool. Then equal volumes of chloroform were added to filtrates, followed by addition of 10% ammonia solution. Formation of rose-pink colour in the aqueous layer was taken as the evidence for the presence of anthraquinones.

## 3.11 Data analysis

Several analytical packages were used in this study. For ethnobotanical survey results were analyzed using SPSS version 16 statistical package (2011). Frequencies were tabulated and cross tabulation was used to make comparisons.

*In vitro* antibacterial, antifungal and cytotoxicity tests were analysed using MS - Excel and Kalide Graph Synergy Statistical package. Mean mortality of brine shrimp against the logarithms of concentrations was plotted using the Kaleida Graph Synergy Statistical package, which also gives regression equations. The regression equations were used to calculate $LC_{16}$, $LC_{50}$ and $LC_{84}$ values as well as confidence intervals at 95%. Extracts giving $LC_{50}$ values greater than 20µg/ mL were considered non-toxic. The results for antimicrobial (antibacterial, antifungal antiviral assays) were analyzed using MS - Excel statistical package (2007). Means and standard deviation were determined. ANOVA and Student t-test were used to compare mean values among different experimental groups. Tests were conducted at 95% confidence interval and significance level of 5%

considered significant for all experiments The HA titre values were square root transformed to normalize the data before subjecting it into regression analysis. Comparisons of means between the treated and untreated groups were done using Student's t – test.

# CHAPTER FOUR

## 4. RESULTS

### Summary

This chapter outlines the results obtained in this study. Initially, a questionnaire survey was conducted to gather information on practices and knowledge on ethno-botanical uses of *C. swynnertonii* in the study area. Results of the ethno botanical survey done to describe where *Commiphora*, the number of respondents reported to have known the plant regardless of age, gender, education and tribe Then extracts from different morphological parts of the study plant were tested against selected bacteria and fungi *in vitro* using agar well diffusion assay. Resin and root bark extracts showed significant activities ($P < 0.001$) against *S. pyogenes*, *E. coli* and *B. subtilis* compared to other extracts. The fungi, *C. albicans* and *A. niger*, were moderately inhibited. Antiviral activity of the resin and root bark extract was tested *in ovo* using embryonated chicken eggs inoculated with Newcastle disease virus (NDV). Both extracts significantly ($P < 0.001$) and effectively reduced virus titres. An animal trial was carried out using the resin and chickens experimentally infected with NDV. Results revealed significant reduction ($P < 0.05$) in clinical signs and mortality rates following administration of the resin before and after the infection. Prophylactic administration of the extract was found to be more effective than the therapeutic approach. HI titres decreased significantly ($P < 0.001$) in resin and root bark treated groups and in all chickens treated with resin irrespective of dose given and on whether the extract was administered before or after

infection suggesting that the plant materials were capable of destroying the NDV before stimulating the developing chick's immunity. Another animal trial investigated the effect of the resin against experimental coccidiosis in chickens. Results showed that oral administration of the resin significantly ($P < 0.001$) reduced mortality rate. Safety margin of the resin was also investigated by determining its effects on selected physiological and biochemical parameters in chickens. The results revealed a good margin of safety provided that the dosage ranges between 200 to 800 mg resin/kg body weights. A phytochemical study was also carried to determine major bioactive compounds in the resin and root bark extracts. The findings demonstrated the presence of several bioactive compounds, ranging from tannins to saponins. The presence of these bioactive compounds was well correlated with different biological activities observed in the various experiments.

## 4.1 Ethnobotanical survey on traditional uses of *C. swynnertonii* in Simanjiro District

### *4.1.1 Demographic information*

Out of the 106 respondents, 59% were males and 41% females; with the following age categories in years: 15-31 were 40 respondents (37%), 31-46 were 41 respondents (40%) and 47-62 were 25 respondents (24%). Tribe wise most of these respondents were Maasai (78%) followed by the Chagga (16%) and other tribes (6%). Evaluation of education level revealed that 51% did not attend any formal class, 35% attended primary education, 13% attended ordinary secondary education and 1% attended advanced secondary education. None of the participant attended the tertiary education level.

### *4.1.2 Peoples' knowledge about the plant*

Results indicate that *C. swynnertonii* is a well-known plant; it is locally known by Maasai name, "*Oltemwai*". All respondents had knowledge on the description of the plant and were able to differentiate *C. swynnertonii* from other *Commiphora* species. Thirty two percent of respondents got the knowledge from grand parents, 29% from parents, and 28% from fellow villagers while a few of them (11%) got it from herbalists and traditional healers.

### *4.1.3 Uses of C. swynnertonii*

This study revealed that *C. swynnertonii* is generally used for various purposes but 94% of the respondents mentioned the medicinal use followed by 4% who used the plant for fencing of their livestock enclosures (bomas) and fuel wood (2%).

As a medicinal plant for humans, *C. swynnertonii* was claimed to be used as (24%) disinfectant for wounds and abscesses, (24%) pulmonary diseases such as coughing, pneumonia and pulmonary tuberculosis, (18%) antifungal and other diseases as shown in Table 8. Resin, which is an exudative material from stem bark, was mentioned as the most used part of the plant; other parts such as roots and leaves were rarely used. It was also revealed that the plant parts were used alone and not mixed with anything and usually taken with hot tea, soup and /or milk.

**Table 8:** Uses of *C. swynnertonii* for treatment of human diseases in the study area

| **Disease treated** | **Plant part used** | **Application** | **Dosage** |
|---|---|---|---|
| Wound and abscesses, cellulitis (24%) | Resin, stem bark | Topical | Daily till healing |
| Fungal infections (ring worm and athletic foot) (18%) | Resin | Topical | Daily till clearance of signs |
| Pulmonary diseases and TB (24%) | Resin | With hot milk | One tea spoon till clearance of sign |
| Purgative (8%) | Resin | Orally with hot water, tea, soup | One tea spoon once |
| Arthritis / back pain (10%) | Root bark | Orally with hot, soup, tea | One tea spoon for 10 - 14 days |
| Peptic ulcers (16%) | Resin | Orally with hot milk | One tea spoon for 10 - 14 days |

In animals, *C. swynnertonii* was reported by 57% of respondents to be used as insect repellent. Other uses included; treatment of diarrhoea and as a purgative in animals suffering from anaplasmosis (8%), anthemintic agent (14%) and also as a fodder (22%) for sheep and goats. Similarly, 93% of all respondents mentioned resin as the popular plant part used followed by leaves (7%) whereas stem and roots were rarely used. Resin was claimed to be used as repellent/ anti-ectoparasitic agents against fleas (53%), mites (26%) and ticks (20%) in cattle (7%), goat and sheep (41%), chickens (18%) and dogs (7%) respectively. The topical application was the most frequently used route of administration (88%) whereas oral was the least (12%). No signs of adverse effects were mentioned in both human and animals.

A small number of respondents (27%) indicated that they use trees to supplement their monthly income by selling resin which ranged between 1,000 and 7,500 Tanzanian shillings per liter depending on the market area. All respondents reported *C. swynnertonii* to be abundantly available in other areas of Manyara Region. Agronomically, 83% reported easy and successful propagation of plant using stem cuttings.

Other medicinal plants reported to be available in Simanjiro District included *Terminalia brownie* (Orbukoi); *Aloe secundiflora* (Osukuroi)*; Commiphora africana*; (Osilalei), *Synadenium grantii* (Orkorbobi) and many others.

## 4.2 Effect of *C. swynnertonii* on brine shrimps and selected bacteria and fungi in vitro

The brine shrimp toxicity assay results are shown in Table 9. Leaf extract had the highest $LC_{50}$ value (greater than 20 µg/ml) whereas root bark showed the lowest value followed by stem bark and resin extract. Resin has shown to have moderate toxicity on brine shrimp larvae.

**Table 9:** Cytotoxicity of extracts from *C. swynnertonii* using brine shrimp lethality test

| **Plant part** | **$LC_{50}$ (µg/ml)** | **Confidence interval (CI)*$^{a-b}$** |
|---|---|---|
| Root bark extract (RB) | 3.5 | 3.4 – 3.6 |
| Stem bark extract (SB) | 13.0 | 9.4 -17.9 |
| Resin extract (RE) | 15.0 | 10.4 – 23.9 |
| Leave extract (LE) | 96.0 | 62.7 – 146.9 |

* $LC_{50}$ is defined as the concentration which resulted in a 50% mortality of brine shrimp; n =10

*** $^{a-b}$ -** Lower limit confidence - upper limit confidence interval.

The effects of various crude extracts from *C. swynnertonii* on growth of various microbes were measured by using the agar well diffusion assay, as shown in Tables 9, 10 and 11. Assessment of antimicrobial activity was based on measurement of the diameter (mm) of inhibition zones formed around the wells. The inhibition zone diameter measurements were interpreted as described by Ayo *et al.*, 2007, whereby 6 mm = no inhibition, 7 - 10 mm = weak activity, 11 - 14 mm = moderate activity and > 15 mm = strong activity. A dose-response relationship was clearly evident (combined $R^2$

= 99.92; $p < 0.001$) in all extracts and microbial species tested. Generally, gram-positive bacteria showed significantly higher ($p < 0.01$) growth inhibition zones than their gram-negative counterparts although a gram-negative bacterium, *E. coli* had the highest inhibition zone of all organisms tested (Table 10).

**Table 10:** Growth inhibition zones of various extracts from *C. swynnertonii* on growth of gram-negative bacteria (means ± SD, n = 4) in a dose dependent manner.

| **Bacteria** | **Conc. (mg/mL)** | **Inhibition zones (mm) ± SE** | | | | |
|---|---|---|---|---|---|---|
| | | **Control** | **Re** | **LE** | **Sb** | **Rb** |
| | 0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| *E. coli* | 50 | 38.0 ± 0.0 | 15.0 ± 2.0 | 11.5 ± 3.0 | 13.0 ± 2.0 | 14.0 ± 2.0 |
| | 100 | 38.0 ± 0.0 | 18.8 ± 1.8 | 13.2 ± 1.1 | 14.4 ± 1.7 | 17.6 ± 1.7 |
| | 150 | 38.0 ± 0.0 | 21.6 ± 2.9 | 13.6 ± 0.9 | 15.2 ± 1.1 | 18.0 ± 0.0 |
| | 200 | 38.0 ± 0.0 | 25.2 ± 1.8 | 14.4 ± 0.9 | 16.0 ± 0.0 | 18.4 ± 0.9 |
| | 500 | 38.0 ± 0.0 | 26.8 ± 1.1 | 15.2 ± 1.1 | 17.2 ± 1.1 | 19.6 ± 0.9 |
| *P. aeruginosa* | 0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| | 50 | 22.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.00 ± 0.0 | 6.0 ± 0.0 |
| | 100 | 22.0 ± 5.4 | 8.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| | 150 | 27.0 ± 1.0 | 9.5 ± 1.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| | 200 | 29.0 ± 0.6 | 10.0 ± 2.3 | 8.5 ± 1.0 | 9.0 ± 1.2 | 12.5 ± 1.0 |
| | 500 | 27.5 ± 1.0 | 11.5 ± 1.9 | 8.5 ± 1.0 | 11.5 ± 1.0 | 14.0 ± 0.0 |
| *S. typhimurium* | 0 | 13.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| | 50 | 13.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| | 100 | 13.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| | 150 | 13.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| | 200 | 13.0 ± 0.0 | 9.0 ± 1.15 | 6.0 ± 0.0 | 6.0 ± 0.0 | 9.5 ± 1.0 |
| | 500 | 13.0 ± 0.0 | 12.0 ± 2.3 | 6.0 ± 0.0 | 6.0 ± 0.0 | 12.5 ± 1.0 |

*Whereby Re - resin extract; Rb - root bark extract; Le - leaf extract and Sb - Stem bark extract.

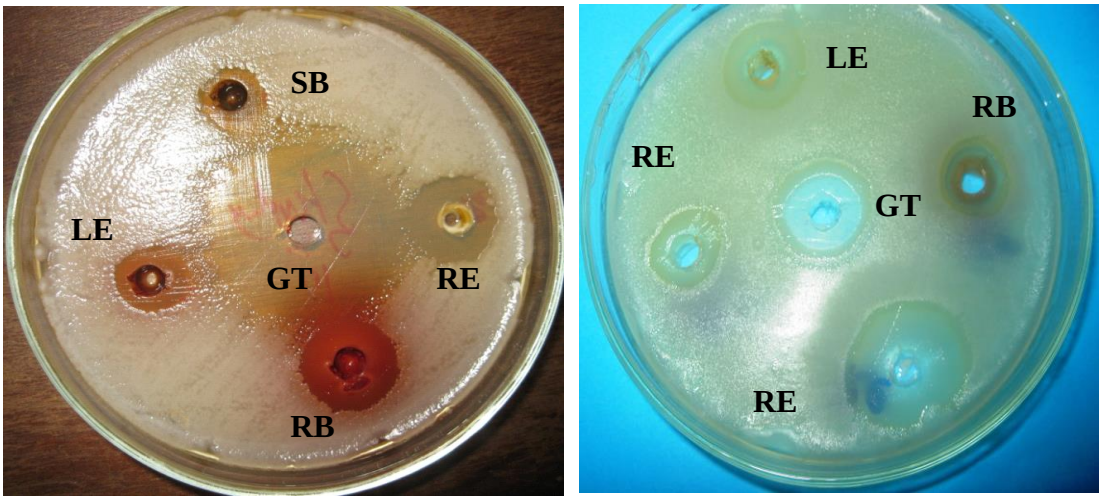


**Plate 2:** Growth inhibition zones of different ethanolic crude extracts for *S. aureus* and *E. coli.*

*Whereby LE- leaf extract; RB – Root bark; LE – Leaf extract; RE – Resin extract and GT – Gentamycin.

Resin and root bark extracts showed strong growth inhibition activity ($p < 0.001$) against *S. pyogenes, E. coli* and *B. subtilis* with inhibition zones of 23.3 ± 3.9, 21.5 ± 4.8 and 15.7 ± 3.0 mm respectively compared to the other two extracts (Table 10). *P. aeruginosa* and *S. typhimurium* were only slightly inhibited by all tested extract at the maximum concentration of 0.50 mg/mL. The fungi, *C. albicans* and *A. niger*, were moderately inhibited (Table 11).

**Table 11:** Growth inhibition zones of various extracts from *C. swynnertonii* on growth of gram-positive bacteria (means ± SD, n = 4) in a dose dependent manner.

| Bacteria | Conc. mg/mL | Inhibition zones (mm) ± SE | | | | |
|---|---|---|---|---|---|---|
| | | Control | Re | LE | Sb | Rb |
| *B. subtilis* | 0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| | 50 | 26.0 ± 0.0 | 12.0 ± 1.6 | 9.0 ± 1.2 | 10.0 ± 1.6 | 12.0 ± 0.0 |
| | 100 | 26.0 ± 0.0 | 14.0 ± 0.0 | 11.5 ± 1.0 | 13.5 ± 1.0 | 13.0 ± 1.2 |
| | 150 | 26.0 ± 0.0 | 14.5 ± 1.0 | 14.0 ± 1.6 | 13.5 ± 1.0 | 16.0 ± 0.0 |
| | 200 | 26.0 ± 0.0 | 17.0 ± 1.2 | 14.0 ± 1.6 | 16.0 ± 0.0 | 18.0 ± 2.3 |
| | 500 | 26.0 ± 0.0 | 21.0 ± 1.2 | 16.0 ± 0.0 | 17 .5 ± 1.0 | 19.0 ± 1.2 |
| *S. aureus* | 0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| | 50 | 26.0 ± 0.0 | 8.0 ± 0.0 | 6.0 ± 0.0 | 7.0 ± 1.0 | 12.0 ± 0.0 |
| | 100 | 26.0 ± 0.0 | 9.3 ± 1.2 | 7.3 ± 1.2 | 9.3 ± 1.2 | 14.0 ± 0.0 |
| | 150 | 26.0 ± 0.0 | 11.3 ± 1.2 | 8.7 ± 1.2 | 10.0 ± 0.0 | 16.0 ± 0.0 |
| | 200 | 26.0 ± 0.0 | 13.3 ± 1.2 | 10.0 ± 0.0 | 12.6 ± 1.2 | 18.0 ± 0.0 |
| | 500 | 26.0 ± 0.0 | 15.3 ± 1.2 | 12.0 ± 0.0 | 14.0 ± 0.0 | 21.3 ± 1.2 |
| *S. pyogens* | 0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| | 50 | 38.0 ± 00 | 18.0 ± 1.6 | 15.5 ± 1.9 | 16.5 ± 1.9 | 16.5 ± 3.0 |
| | 100 | 38.0 ± 00 | 21.5 ± 1.9 | 15.5± 5.7 | 14.5 ± 3.8 | 14.0 ± 2.3 |
| | 150 | 38.0 ± 00 | 23.0 ± 3.0 | 15.5 ± 2.8 | 16.0 ± 1.0 | 15.5 ± 1.0 |
| | 200 | 38.0± 00 | 13.5 ± 1.2 | 13.0 ± 3.7 | 16.0 ± 2.0 | 13.0 ± 2.0 |
| | 500 | 38.0 ± 00 | 18.0 ± 0.0 | 7.0 ± 2.0 | 12.5 ± 3.8 | 12.0 ± 1.6 |

*Whereby Re - resin extract; Rb - root bark extract; Le - leaf extract and Sb - stem bark extract

Leaf extract gave the lowest inhibition activity to all tested microbes compared to the remaining extracts (ranking: resin > root bark > stem bark > leaf). For the two tested fungi, resin and root bark extracts showed moderate activity against *C. albicans*.

**Table 12:** Growth inhibition zones of various extracts from *C. swynnertonii* on growth of selected fungi (means ± SD, n = 4) in a dose dependent manner.

| Fungi | Conc. (mg/mL) | Inhibition zones (mm) ± SE | | | | |
|---|---|---|---|---|---|---|
| | | Control | Re | LE | Sb | Rb |
| *C .albicans* | 0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| | 50 | 12.7 ± 0.7 | 9.0 ± 1.2 | 6.0 ± 0.0 | 8.0 ± 0.0 | 14.0 ± 0.0 |
| | 100 | 12.7 ± 0.7 | 12.0 ± 0.0 | 9.0 ± 1.2 | 11.0 ± 0.2 | 16.0 ± 0.0 |
| | 150 | 12.7 ± 0.7 | 13.5 ± 1.0 | 11.0 ± 1.2 | 12.0 ± 1.6 | 17.5 ± 1.0 |
| | 200 | 12.7 ± 0.7 | 16.0 ± 1.6 | 14.0 ± 0.0 | 15.0 ± 1.2 | 19.5 ± 1.9 |
| | 500 | 12.7 ± 0.7 | 21.5 ± 1.9 | 7.0 ± 2.0 | 16.5 ± 1.0 | 25.0 ± 1.2 |
| *A. niger* | 0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 | 6.0 ± 0.0 |
| | 50 | 12.7 ± 0.7 | 11.0 ± 0.6 | 10.0 ± 1.4 | 11.5 ± 0.5 | 11.0 ± 0.6 |
| | 100 | 12.7 ± 0.7 | 11.5 ± 0.5 | 11.0 ± 0.6 | 11.0 ± 1.3 | 11.5 ± 0.5 |
| | 150 | 12.7 ± 0.7 | 12.0 ± 1.0 | 13.0 ± 2.7 | 11.0 ± 0.6 | 17.5 ± 0.5 |
| | 200 | 12.7 ± 0.7 | 13.5 ± 0.5 | 13.0 ± 0.6 | 16.0 ± 1.8 | 13.0 ± 1.0 |
| | 500 | 12.7 ± 0.7 | 13.5 ± 0.5 | 10.5 ± 1.3 | 13.0 ± 1.3 | 20 .0 ± 1.6 |

*Whereby Re - resin extract; Rb - root bark extract; Le - leaf extract and Sb - stem bark extract

All plant crude extracts with MIC values < 0.0315 mg/ml are considered to possess at least some degree of inhibitory effect, and any concentration exceeding this should not be considered effective.

**Table 13** Minimum inhibitory concentrations of various plant extracts against selected microbes**.**

| | MIC in mg/mL | | | |
|---|---|---|---|---|
| **Microbes** | **LE** | **SB** | **Rb** | **Re** |
| *E. coli* | 0.250 | 0.250 | 0.0625 | 0.0019 |
| *S. pyogens* | 0.125 | 0.0315 | 0.0078 | 0.0039 |
| *B. subtilis* | 0.125 | 0.0625 | 0.0315 | 0.0078 |
| *C. albicans* | > 0.500 | 0.0625 | 0.0039 | 0.0315 |
| *S. aureus* | 0.250 | 0.125 | 0.0315 | 0.125 |
| *S. typhimurium* | > 0.500 | > 0.500 | 0.250 | 0.250 |

*Whereby Re - resin extract; Rb - root bark extract; Le - leaf extract and Sb - stem bark extract

## 4.3 Effect of resinous extract from *C. swynnertonii* against Newcastle infection *in ovo*

### *4.3.1 Time for embryo death*

Table 8 demonstrates the various times of embryo deaths observed in the study. The first embryo death was recorded in the positive control group (NDV alone) 24 hours post-inoculation (PI); by 48 hours the mortality rate in this group was 100%. This was followed by the group, which was treated with NDV virus and a diluent, DMSO whereby 100% death was attained at 48 hours posi inoculation (PI). Throughout the 4 days of observation, no embryo death was recorded from the following groups: negative control (untreated egg), bark and resin at 250 and 500 µg/mL. The first embryo death was recorded in the positive control group (NDV alone) 24 hours post-inoculation; by 48 h the mortality rate in this group was 100%. This was followed by the group, which was treated with NDV virus and a diluent, DMSO. At the lowest extract concentrations of

50µg/mL, embryo deaths were recorded at 48, 60 and 72 hours for resin and leaf, stem bark and root bark, respectively.

**Table 14:** Embryo deaths following inoculation of ECE (n=10) with NDV and incubation at different concentrations of crude extracts from *C. swynnertonii*

| Treatment | Conc. (µg/mL) | Time of embryo death (hours) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **0** | **24** | **48** | **60** | **72** | **96** |
| Untreated egg | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NDV alone | 0 | 0 | 9 | 1 | | | |
| DMSO + NDV | 0 | 0 | 0 | 10 | | | |
| Leaf extract + DMSO + NDV (LE) | 50 | 0 | 0 | 2 | 8 | | |
| | 250 | 0 | 0 | 0 | 10 | | |
| | 500 | 0 | 0 | 0 | 0 | 0 | 0 |
| Stem-bark + DMSO + NDV (SB) | 50 | 0 | 0 | 5 | 5 | | |
| | 250 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 500 | 0 | 0 | 0 | 0 | 10 | |
| Root-bark + DMSO + NDV (RB) | 50 | 0 | 0 | 3 | 3 | 4 | |
| | 250 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 500 | 0 | 0 | 0 | 0 | 0 | 0 |
| Resin + DMSO + NDV (RE) | 50 | 0 | 0 | 2 | 2 | 6 | |
| | 250 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 500 | 0 | 0 | 0 | 0 | 0 | 0 |

### *4.3.2 Mean embryo weights*

Embryo weights for ECE were taken day four post inoculation. Data on the effect of different extracts on the weights of NDV-infected embryos is presented in Fig. 2. The mean embryo weight in the positive control was significantly lower ($P < 0.01$) than that of the negative control. The embryo weights were observed to increase significantly ($P < 0.001$) with increased concentrations of almost all extracts from different parts of *C. swynnertonii*. At the concentration of 250 µg/mL of all four extracts, the mean embryo

weights were almost similar to that of negative control group ranging between 5.1 to 5.3 g; this range was not significantly different (P > 0.05) from that of the negative control which was 5.8 g. A slight decrease in the embryo weights was observed when extract concentration was raised to 500 µg/mL, particularly with the root bark, stem bark and resin ethanolic extracts. But for leaf extract the weight was increasing significantly with increased concentrations to 500 µg/mL (Fig. 2).

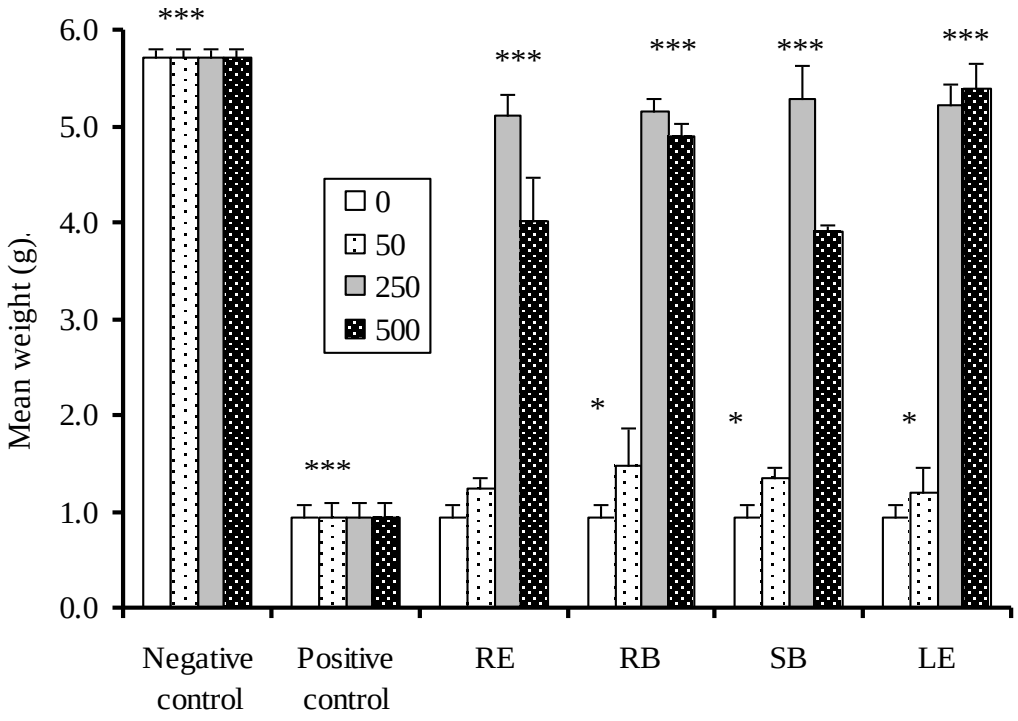


**Figure 2:** Mean embryo weights following inoculation of ECE with representative concentrations of crude extracts of *C. swynnertonii*. [Negative control – without NDV or extract; Positive control-with NDV alone; RE - resin extract; RB - root bark extract; SB - stem bark extract; LE - leaf extract. * P > 0.05; **P < 0.01; ***P < 0.001]

### *4.3.3 NDV haemaglutination (HA) titres in embryos treated with different concentrations of crude extracts.*

HA titres of NDV-infected chicken embryos following treatment with different concentrations of *C. swynnertonii* extracts are shown in Table 15. The results indicate that all tested extracts were capable of significantly reducing the HA titers in a dose dependent manner ($R^2$ = 0.63; p = 0.2). No NDV was detected in the negative control and resin extracts at 500 µg/mL concentrations. The highest HA titres of 2048 and 1020 were detected in ECE inoculated with NDV alone and NDV in DMSO, respectively.

**Table 15:** Mean HA titres in embryonated chicken eggs following inoculation with NDV and incubation in different concentrations of crude extracts from *C. swynnertonii*

| Treatment | Conc. (µg/mL) | HA titre |
|---|---|---|
| Negative control* | 0 | 0 |
| Positive control** | 0 | 2048 |
| NDV + DMSO | 0 | 1020 |
| LE + DMSO + NDV | 50 | 512 |
| | 250 | 64 |
| | 500 | 8 |
| SB + DMSO + NDV | 50 | 256 |
| | 250 | 8 |
| | 500 | 4 |
| RB + DMSO + NDV | 50 | 256 |
| | 250 | 8 |
| | 500 | 4 |
| RE + DMSO + NDV | 50 | 128 |
| | 250 | 4 |
| | 500 | 0 |

*Without NDV or extract; ** with NDV alone.

### *4.3.4 Antibodies titres against NDV in hatched chicks*

There were low mean HI titres in chicks hatched from eggs inoculated with NDV and incubated with root bark, stem bark and resin extracts (Table 16). Twelve out of fifteen chicks that were hatched had very good body condition; they had a score of 1 in the body condition score scale of 2 points. The remaining 2 chicks had lower than average body of littermates (i.e., they had a score of 3).

**Table 16:** Mean HI titres of hatched chicks treated with crude extracts of *C. swynnertonii*.

| **Treatment** | **Chick No.** | **Mean HI titres in hatched chicks** | | | |
|---|---|---|---|---|---|
| | | **Day 0** | **Day 14** | **Day 28** | **Day 32** |
| Neg. control | 15 | 0 | 0 | 0 | 0 |
| RB | 15 | 2 | 0 | 0 | 0 |
| RE | 15 | 0 | 0 | 0 | 0 |
| SB | 15 | 2 | 0 | 0 | 0 |

***RE** - resin extract; **RB** - root bark extract; **SB** - stem bark extract; **LE** - leaf extract

## 4.4 Effect of resin extract from *C. swynnertonii* against Newcastle infection in chickens

### *4.4.1 Clinical signs*

Five days following inoculation of chickens with ND virus suspension, 50% (57/114) of all infected chickens showed most of the clinical symptoms typical of ND which were demonstrated as anorexia, greenish diarrhoea, difficult breathing (dyspnoea), raised body temperature (> $42^0$C) and loss of bodyweight emaciation and/or death. This was a clear indication that the ND virus strain used was virulent. By day 10 post-infection (p.i.), most of the chickens in G2 had severe clinical signs of the disease as shown in Table 17. These clinical signs were less obvious in chickens in prophylactic trial (G3, G4 and G5). No clinical signs of ND were observed in the negative control group (G1).

**Table 17:** Body condition scores and mortality rates of chickens with experimental ND infection after 14 days post treatment with resinous extract from *C. swynnertonii*

| Trial | Group | Dose (resin mg/kg) | Body condition score | Chickens in score scale | Mortality rate (%) |
|---|---|---|---|---|---|
| Control | G1 | 0 | 1 | 12 | 0.0 |
| | | | 2 | 0 | |
| | | | 3 | 0 | |
| | | | 4 | 0 | |
| | G2 | 0 | 1 | 0 | 100 |
| | | | 2 | 3 | |
| | | | 3 | 4 | |
| | | | 4 | 8 | |
| | G3 | 250 | 1 | 4 | 40.0 |
| | | | 2 | 3 | |
| | | | 3 | 1 | |
| | | | 4 | 0 | |
| | G4 | 500 | 1 | 12 | 33.3 |
| | | | 2 | 3 | |
| | | | 3 | 1 | |
| | | | 4 | 1 | |
| Prophylactic (treatment before infection) | G5 | 1,000 | 1 | 13 | 33.3 |
| | | | 2 | 2 | |
| | | | 3 | 1 | |
| | | | 4 | 0 | |
| | G6 | 250 | 1 | 1 | 73.3 |
| | | | 2 | 3 | |
| | | | 3 | 3 | |
| | | | 4 | 8 | |
| | G7 | 500 | 1 | 1 | 66.7 |
| | | | 2 | 3 | |
| | | | 3 | 7 | |
| | | | 4 | 5 | |
| Therapeutic (treatment after infection) | G8 | 1,000 | 1 | 2 | 40.0 |
| | | | 2 | 4 | |
| | | | 3 | 7 | |
| | | | 4 | 3 | |

From the results, there was significant difference ($p<0.05$) in mortality rates among the positive control, prophylactic and therapeutic trials. Administration of the extract before inoculation of chickens with NDV significantly ($P < 0.001$) reduced mortality rates to

40% (G3) and 33.3 % (G4 and G5) on day 7 post treatment. Mortality rates of chickens in therapeutic groups were reduced ($P < 0.05$) to 73.3% (G6), 66.7% (G7) and 40 % (G8).

### *4.4.2 Post mortem and histopathological lesions*

Post-mortem examination of dead chickens from the positive control group (G2) revealed lesions typical of ND, namely, emaciation, facial and peri orbital oedema, conjunctivitis and whitish mucoid creamy caseous material in trachea. The proventriculi were severely swollen with button ring-like reddish lesions.

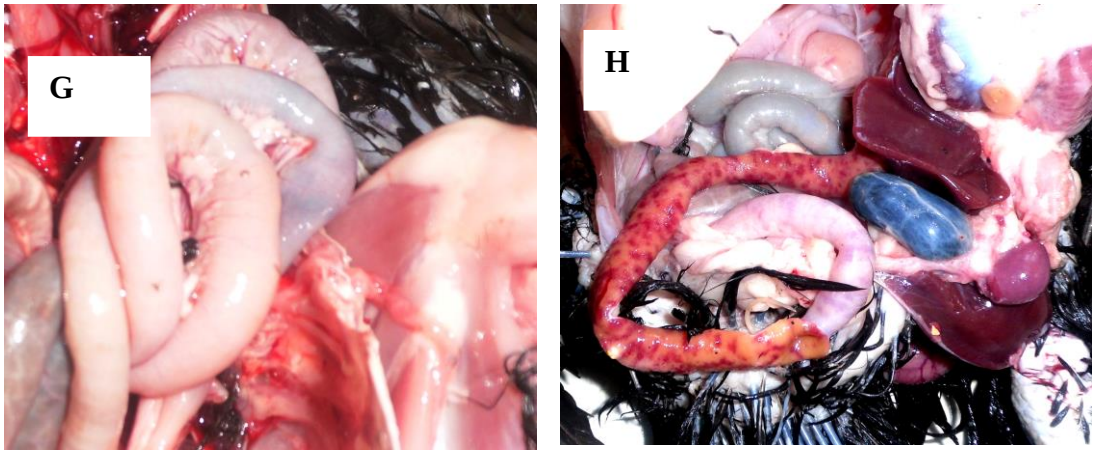


**Plate 3:** Normal intestines with no lesions from negative control group, G1 [G] and severe enteric hemorrhages in untreated NDV infected chickens, G2 [H].

Histopathological findings included congested livers, lungs and intestines; perivascular cuffing with mononuclear cellular infiltrations were seen in tissues of all chickens

infected with NDV. The lesions became less severe in all chickens, which received the resin extract treatment regardless of the dose given.

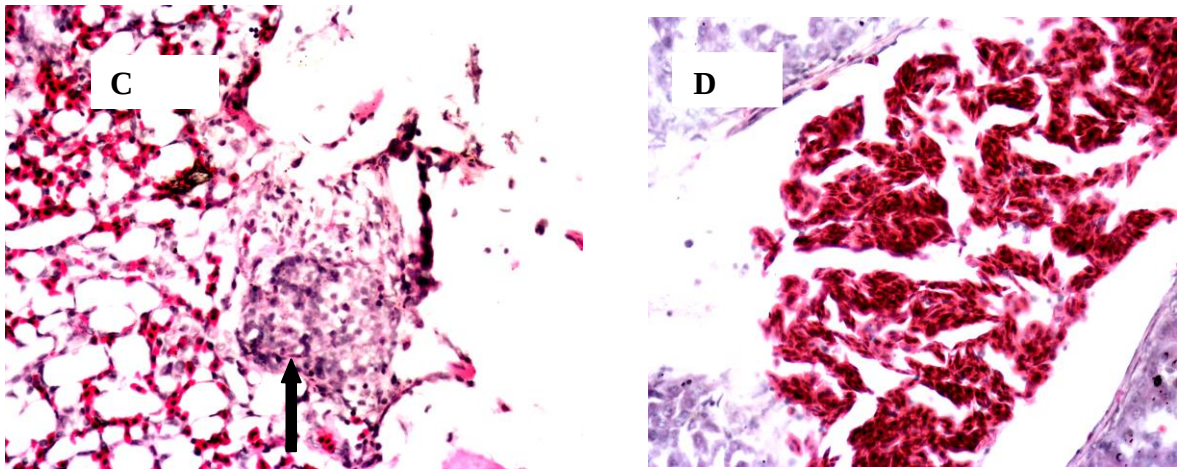


**Plate 4:** A section of lung tissue: an arrow showing a granular material [C], congestion in the interstitial tissues [D] and mononuclear cell infiltration (x 40 magnifications).

### *4.4.3 Body weight changes*

Mean body weights of chickens in the prophylactic trial (G3, G4 and G5) decreased gradually with time until seven days after treatment before there was an increase towards 14 days when treatment was terminated. In the therapeutic trial (G6, G7 and G8), the decrease in body weight with time was significant ($P < 0.01$). Furthermore, comparison of bodyweights on day 7 post treatment showed a decrease in bodyweight in a dose dependent manner ($R^2 = 0.98$; $P = 0.07$). The body weight of chickens in negative control group (G1) increased significantly ($P < 0.001$) to the end of experiment (Table 18).

**Table 18:** Body weight changes

| | | | Days on treatment | | | | |
|---|---|---|---|---|---|---|---|
| **Trial** | **Group** | **Dose** | **Day -7** | **Day 0** | **Day 7** | **Day 14** | **Day 21** |
| | G1 | 0 | 998 | 987.7 | 1,288.2 | 1,431.4 | 1,734.0 |
| Control | G2 | 0 | 1,014 | 1,013.5 | 918.8 | 858.3 | 612.0 |
| | G3 | 250 | 1,301 | 1,202.3 | 1,151.5 | 1,098.0 | 1,132.0 |
| | G4 | 500 | 1,308 | 1,073.1 | 973.0 | 890.4 | 976.0 |
| Prophylactic | G5 | 1,000 | 1,260 | 1,243.3 | 1,062.5 | 991.6 | 1,030.0 |
| | G6 | 250 | 1,243 | 1,030.1 | 1,000.3 | 916.3 | 900.0 |
| | G7 | 500 | 1,073 | 1,081.4 | 920.3 | 857.1 | 798.0 |
| Therapeutic | G8 | 1,000 | 1,273 | 1,184.0 | 965.4 | 783.7 | 745.0 |

### *4.4.4 Antibody titre changes*

Antibody titres were detected starting from day 5 post infection in all infected groups. There was highly significant ($P < 0.001$) difference in levels of antibodies titres between positive control (G2) and the treated group irrespective of the dose of the extract given. The HI titres of chickens in G2 rose to a maximum of 2,048 by day 14 pi. However, in the prophylactic trial, results showed that administration of the extract reduced antibody titres in groups G3, G4 and G5 to $2^3$ which is similar to a value of 8 (Fig. 3). For the therapeutic trial (G6, G7 and G8), the antibody titres decreased in a dose dependant manner ($R^2 = 0.79$; $P = 0.08$) from day 7 post-treatment (Fig. 4). Negative control groups (G1) remained ND virus antibody negative throughout the entire period of the experiment.

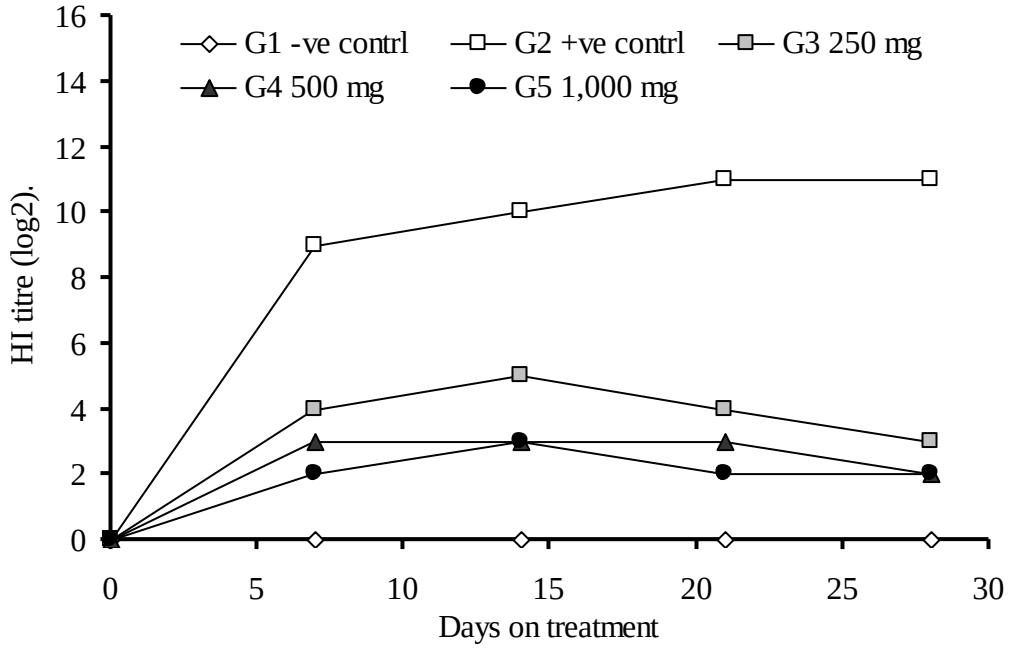


**Figure 3:** HI titre profiles of chickens infected with ND virus and treated with different levels of *C. swynnertonii* resinous extract before the infection (prophylactic trial).

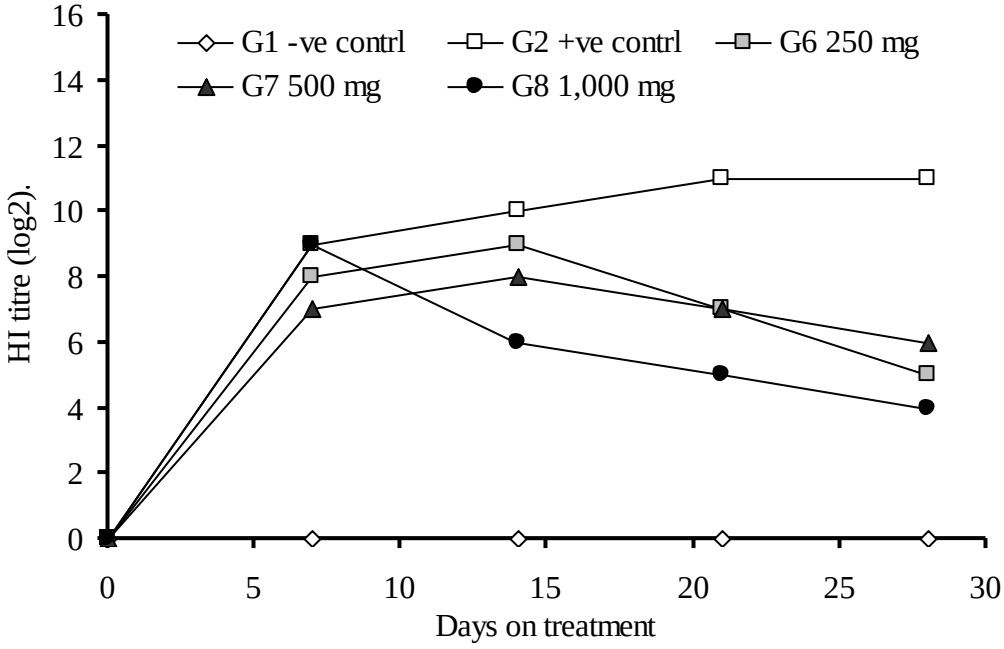


**Figure 4:** HI titre profiles of chickens infected with ND virus and treated with different levels of *C. swynnertonii* resinous extract after the infection (therapeutic trial).

## 4.5 Effect of resin extract from *C. swynnertonii* on chickens experimentally infected with mixed coccidia

### *4.5.1 Clinical signs*

Following sporulation, examination of the oocysts revealed that the natural infection was composed of three *Eimeria* species identified as *E. tenella*, *E. necatrix* and *E. mitis*. Upon oral administration of infective oocysts 62% (40/64) of all infected chickens showed clinical signs typical of coccidiosis from day 5 post infection. These signs ranged from depression, ruffled feathers, and loss of appetite to dark brownish diarrhea.

Post-mortem examination of dead chickens from G2 revealed typical coccidiosis lesions. Clinical signs became less obvious following treatment with the resin extract and the anticoccidial drug as shown in Table 19. Administration of the extract significantly ($p < 0.001$) reduced mortality rates from 93.8% (G2) to 25.0% (in both G3 and G4).

**Table 19:** Body condition scores and mortality rates of chickens with experimental coccidiosis after 7 days of treatment with resinous extract from *C. swynnertonii*

| Group | Body condition score | No. of chickens in score scale | No. of dead chickens in group | Mortality rate (%) |
|---|---|---|---|---|
| G1- Negative control | 1 | 16 | 0 | 0.0 |
| | 2 | 0 | | |
| | 3 | 0 | | |
| | 4 | 0 | | |
| G2- Positive control | 1 | 0 | 15 | 93.8 |
| | 2 | 0 | | |
| | 3 | 5 | | |
| | 4 | 11 | | |
| G3- 400 mg/kg | 1 | 0 | 4 | 25.0 |
| | 2 | 8 | | |
| | 3 | 5 | | |
| | 4 | 3 | | |
| G4- 800 mg/kg | 1 | 0 | 4 | 25.0 |
| | 2 | 9 | | |
| | 3 | 7 | | |
| | 4 | 0 | | |
| G5- Anticoccidial drug | 1 | 12 | 1 | 6.3 |
| | 2 | 3 | | |
| | 3 | 1 | | |
| | 4 | 0 | | |

### *4.5.2 Body weight*

Mean body weight of chickens in the negative control group (G1) and those treated with anticoccidial drug (G5) did not differ significantly ($p > 0.05$). Body weight of chickens in groups treated with the extract (G3 and G4) decreased significantly ($p < 0.01$) during treatment (day 7) compared to chickens in G5, in which the body weight increased gradually (Fig. 5).

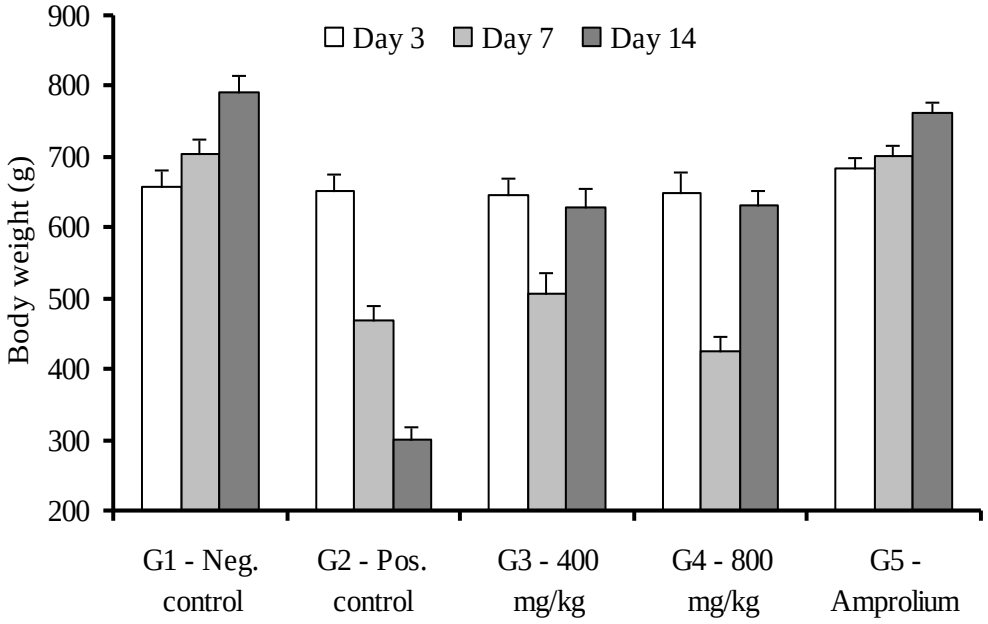


**Figure 5:** Body weight changes of chickens with experimental coccidiosis on 7 before (day 3), during (day 7) and after (day 14) treatment with two levels of resinous extract from *C. swynnertonii*.

### *4.5.3 Faecal oocysts count (FOC)*

Administration of the resinous extract to chickens with coccidiosis significantly reduced ($P < 0.001$) faecal oocyst counts (FOC) in dose dependent manner (correlation coefficient = 0.88). After 4 days of treatments, FOC was reduced from $1.03 \times 10^5$ (G2) to $6.55 \times 10^3$ (G4) oocysts per gram faeces and by day 11 after treatment the oocysts count was less than $2.80 \times 10^3$ (Fig. 6).

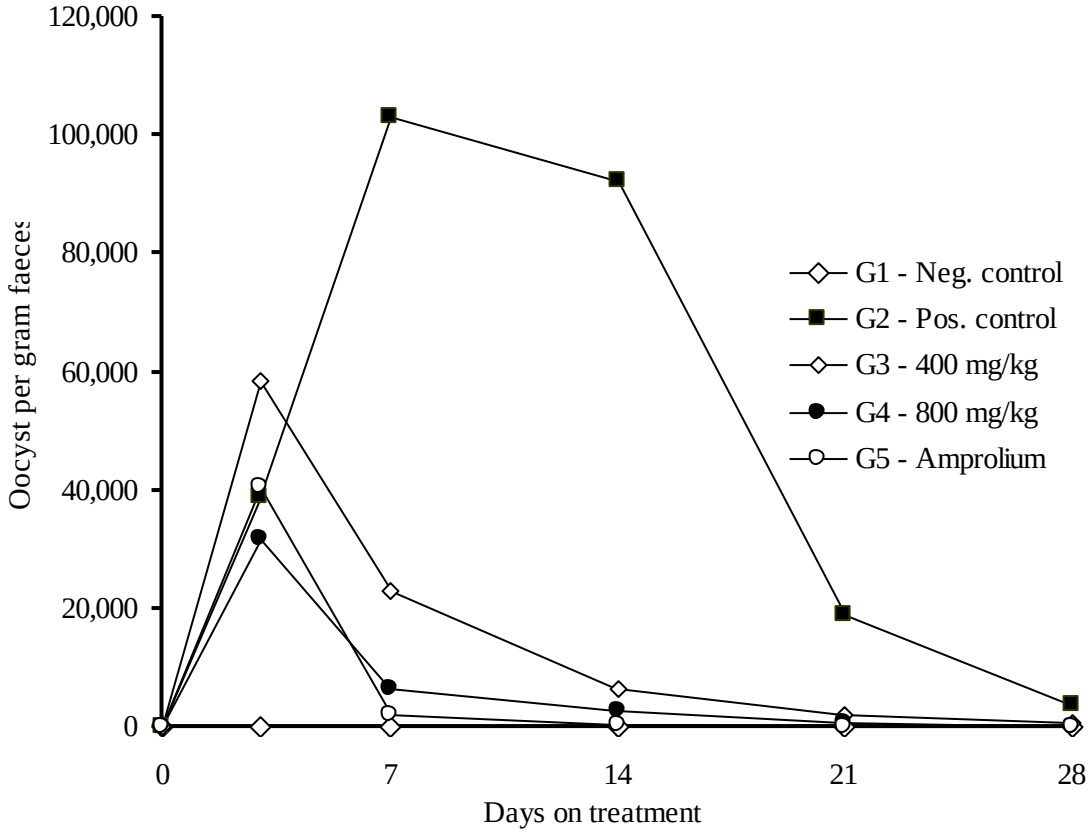


**Figure 6:** Faecal oocyst counts in chickens experimentally infected with coccidian oocysts and treated with different levels of resinous extracts from *C. swynnertonii*.

### *4.5.4 Pathological and histopathological results*

Dead chickens from each group were taken for post mortem examination. Different tissues such as the livers, kidneys and intestines were taken for histopathology and microbial culture to see whether there was any opportunistic bacteria involvement. Grossly, the intestines had mild petechiation and mucus mixed with intestinal contents particularly in the resin treated chickens group.

The livers were slightly enlarged with pale yellow appearance, seen both on the capsule and cut surface. This uniform change was consistent with fatty metamorphosis (fatty changes). Wide hepatic infarcts enlarged and congested spleen and kidneys, petechiation of visceral organs and intestines were also seen. Histopathological changes in untreated control chickens observed in the intestine were haemorrhagic enteritis, epithelial necrosis leading to loss of glandular villi with sub mucosal mononuclear cells infiltration in the intestines. All these changes were not seen in the chickens treated with resinous extract and the anticoccidial drug (Plate 5).

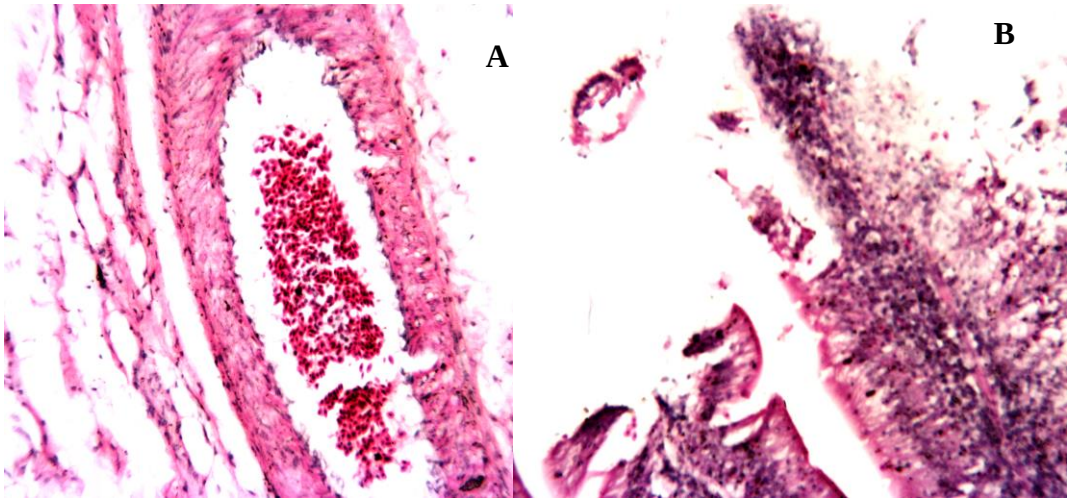


**Plate 5:** Intestinal section (x 40 magnifications H&E stain) showing [A] congestion and hemorrhagic enteritis, sloughing off of glandular villous and mononuclear cell infiltration [B].

## 4.6 Effect of *C. swynnertonii* resin on various physiological parameters in chickens

### *4.6.1 Clinical signs*

No signs of sickness or death were observed in the G1, G2 and G3 group throughout the experimental period of 28 days. Twenty one percent (5/24) of chickens from G4 and G5 showed signs of dullness and voided loose whitish faeces. These signs were evidently seen from day 7 of treatment.

### *4.6.2 Mean body weights*

Mean body weights of chickens in G1 increased gradually ($P < 0.001$) throughout the experimental period compared to resin treated groups. From day 3 of treatment, the body weights of chickens in G2, G3, G4 and G5 decreased significantly in a dose dependent manner ($R^2 = 0.85$; $P = 0.02$). From day 14 post treatment the mean body weights were almost constantly decreased till the end of experimental period (Fig.7).

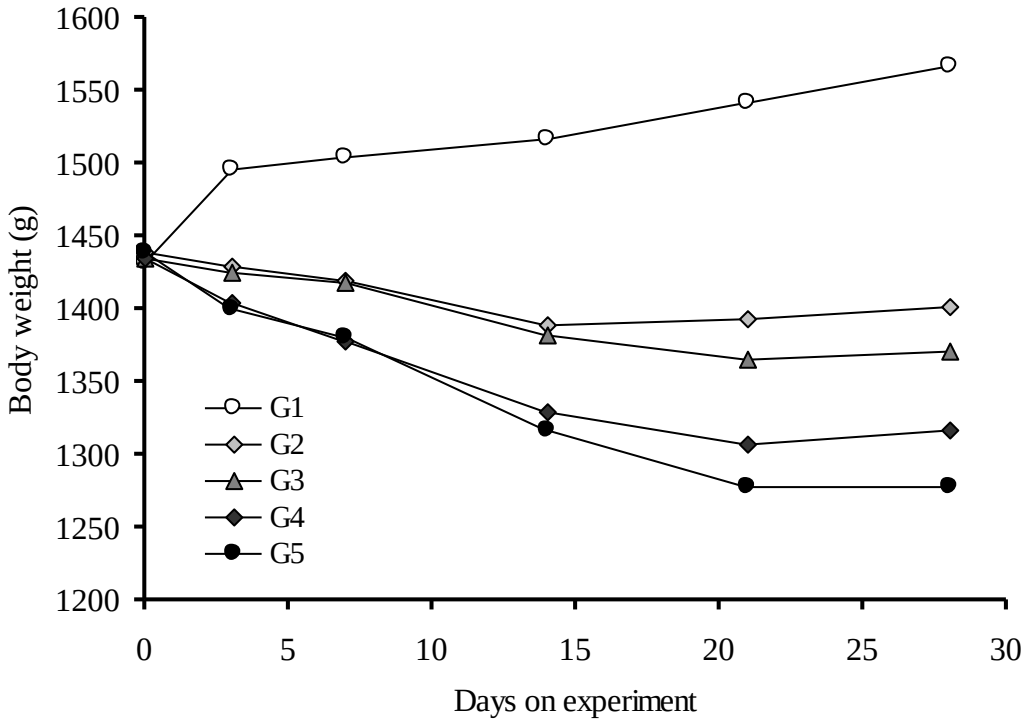


**Figure 7:** Changes in body weight following resin administration for 14 days with different levels of *C. swynnertonii* resinous extract.

### *4.6.3 Haematological parameters*

Haematological parameters are presented in Table 20. PCV values of chickens in the negative control group (G1) significantly ($P < 0.05$) higher compared to those of resin treated groups (G2, G3, and G5). There was no significant difference in the levels of PCV among the resin treated groups.

Hb levels for all treated groups were significantly ($P < 0.001$) lower than to that of the negative control group (G1). Similar trend was observed with total RBC count whereby the levels for all groups treated with aqueous resin extract were lower than that of G1.

The values for the calculated MCH were observed to be significantly higher in $G_2$ and G5 as compared other treated groups. While the MCHC was observed to decrease in all groups but more the decrease was highly noted in G2 and G5.

Total WBC count of chickens in G1 and G2 was significantly ($P < 0.001$) lower as compared to G3, G4 and G5. This significant difference was attributed to increased levels of monocytes and lymphocytes compared to other cells such as heterophils, eosinophils. The levels of heterophils and eosinophils were similar in all groups regardless of the treatment given.

**Table 20:** Mean haematological parameters of chickens following oral administration of *C. swynnertonii* resinous extract.

| | | Group | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Day** | **G1** | **G2** | **G3** | **G4** | **G5** |
| PCV (%) | 0 | 23.0 ± 1.1 | 24.2 ± 0.7 | 24.9 ± 0.8 | 24.2 ± 0.8 | 26.2 ± 1.0 |
| | 14 | 26.8 ± 1.5* | 24.8 ± 0.7 | 23.9 ± 1.4 | 21.9 ± 1.4 | 24.2 ± 1.4 |
| Hb (g/dL) | 0 | 8.1 ± 0.4 | 7.9 ± 0.8 | 8.2 ± 0.4 | 7.9 ± 0.4 | 9.1 ± 0.4 |
| | 14 | 8.5 ± 0.3 | 6.3 ± 0.5* | 5.6 ± 0.5** | 5.5 ± 0.5** | 5.4 ± 0.5*** |
| RBC count ($x10^6$ µL) | 0 | 3.9 ± 0.4 | 3.7 ± 0.2 | 3.8 ± 0.2 | 3.6 ± 0.2 | 3.9 ± 3.0 |
| | 14 | 3.9 ± 0.1 | 2.8 ± 0.3* | 2.8 ± 0.1** | 2.3 ± 0.2** | 1.9 ± 0.3*** |
| MCH (pg/cell) | 0 | 21.9 ± 1.7 | 21.4 ± 1.8 | 21.4 ± 1.6 | 22.2 ± 1.8 | 25.3 ± 2.5 |
| | 14 | 21.7 ± 1.2 | 26.4 ± 4.2* | 22.7 ± 1.7 | 24.7 ± 2.4 | 37.4 ± 6.9* |
| MCHC (%) | 0 | 36.8 ± 3.4 | 33.1 ± 3.1 | 33.0 ± 2.7 | 32.7 ± 1.4 | 35.4 ± 2.4 |
| | 14 | 32.7 ± 2.3 | 23.5 ± 2.1* | 28.9 ± 4.3 | 28.7 ± 2.8 | 23.0 ± 2.2** |
| Total WBC ($x10^3$ µL) | 0 | 1.8 ± 0.2 | 1.5 ± 0.2 | 1.1 ± 0.2 | 1.7 ± 0.1 | 2.1 ± 0.3 |
| | 14 | 1.7 ± 0.2 | 1.5 ± 0.1 | 1.7 ± 0.1 | 2.0 ± 0.1 | 2.2 ± 0.3 |
| Lymphocytes (%) | 0 | 57.2 ± 1.9 | 57.5 ± 1.7 | 57.5 ± 1.7 | 54.9 ± 2.1 | 56.6 ± 2.*1* |
| | 14 | 57.1 ± 1.9 | 61.3 ± 2.4* | 60.5 ± 2.7* | 63.0 ± 2.1** | 62.5 ± 1.8** |
| Heterophils (%) | 0 | 32.1 ± 1.7 | 29.6 ± 1.4 | 30.5 ± 2.1 | 29.6 ± 1.4 | 29.6 ± 1.4 |
| | 14 | 26.8 ± 1.6 | 30.2 ± 1.9 | 30.3 ± 1.6 | 31.2 ± 1.8 | 32.8 ± 1.6 |
| Monocytes (%) | 0 | 7.0 ± 0.5 | 6.0 ± 0.4 | 5.9 ± 0.7 | 6.0 ± 0.5 | 5.3 ± 0.4 |
| | 14 | 6.8 ± 0.4 | 7.6 ± 0.4* | 8.3 ± 0.3*** | 7.8 ± 0.3** | 7.5 ± 0.3*** |
| Eosinophils (%) | 0 | 3.7 ± 0.5 | 4.3 ± 0.5 | 3.3 ± 0.4 | 4.3 ± 0.5 | 4.3 ± 0.5 |
| | 14 | 3.8 ± 0.6 | 4.4 ± 0.4 | 3.7 ± 0.4 | 4.4 ± 0.4 | 4.4 ± 0.4 |

Tabulated values are the mean ± standards error of the mean for 12 determinations; *P< 0.05; ** P<0.01; ***P<0.001

### *4.6.4 Biochemical parameters*

Plasma glucose levels of chickens treated with aqueous resin extract are shown in Fig. 8. Daily treatment with resin extract at dosages of 250, 500, 750 and 1000 mg/kg produced significant ($P < 0.01$) decrease in the plasma glucose levels. This decrease in plasma glucose level was dose and time dependent ($R^2 = 0.83$; $P = 0.01$). For instance, the glucose levels on day 14 after the last resin dose was 228.3 to 131.8 mg/dL and 222.4 to 143.3 mg/dL for G4 and G5 respectively.

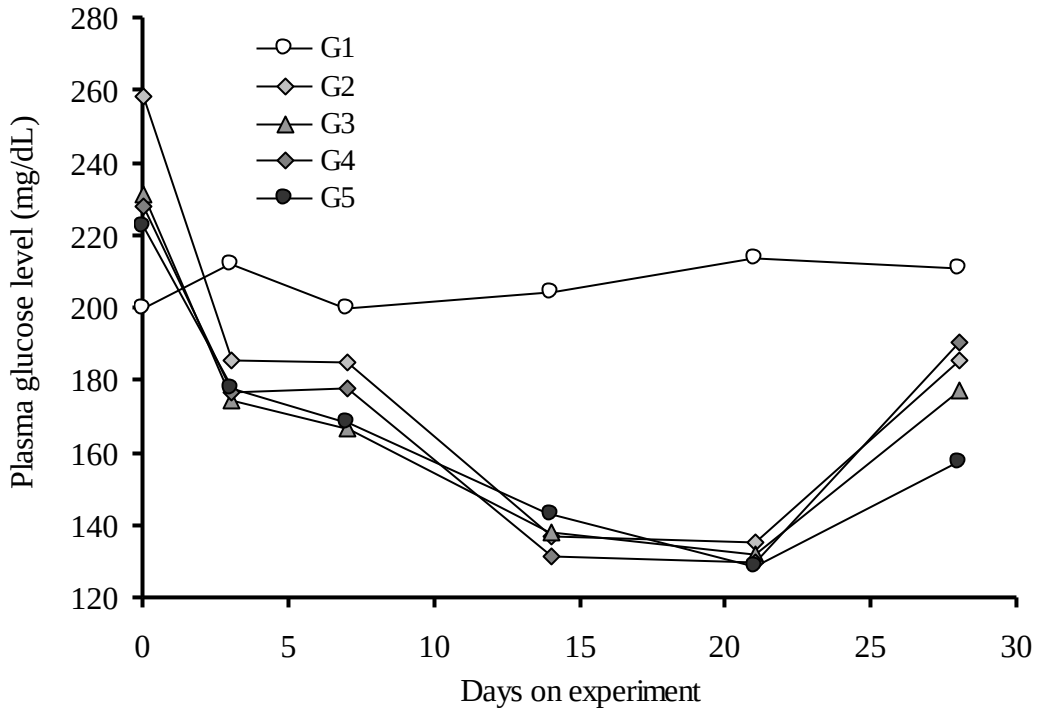


**Figure 8:** Total plasma glucose profiles of chickens following administration of *C. swynnertonii* resinous extract.

The total plasma cholesterol decreased in a dose dependent manner as shown in Fig. 9. By day 3 of treatment, levels in the treated groups were significantly ($P < 0.01$) lower than that of the negative control group (G1). There was negative correlation between the increases doses of resin with that of plasma total cholesterol level (correlation coefficient = - 0.84).

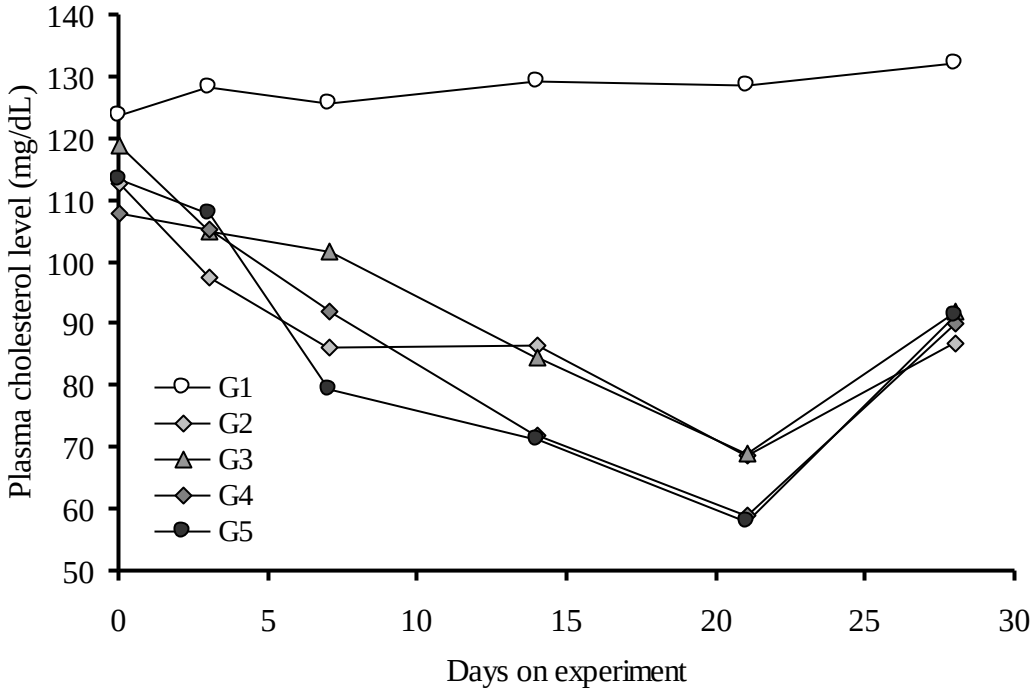


**Figure 9:** Total plasma cholesterol profiles of chickens following administration of *C. swynnertonii* resinous extract.

The resin extract did not induce any significant variations in the levels of total plasma protein including those of albumin and globulin (Table 21). That is, similar patterns were observed across the groups regardless of resin treatment. For instance, levels of all the three parameters in all groups were the lowest around day 15 of treatment and increased steadily towards the of observation period.

The effect of the extract on liver function markers (ALT and AST) was not significant although between day 14 and 21 levels in G3, G4 and G5 were higher ($P = 0.08$) than in the control group (Table 22).

**Table 21:** Mean biochemical parameters following oral resin extract administration.

| | | Days on experiment | | | | | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **Group** | **0** | **3** | **7** | **14** | **21** | **28** |
| Total protein (g/dL) | G1 | 4.9 ± 0.1 | 5.9 ± 0.3 | 5.0 ± 0.2 | 4.4 ± 0.3 | 4.7 ± 0.3 | 5.0 ± 0.2 |
| | G2 | 5.1 ± 0.1 | 4.9 ± 0.2 | 5.1 ± 0.1 | 4.5 ± 0.2 | 4.6 ± 0.2 | 5.2 ± 0.1 |
| | G3 | 5.2 ± 0.2 | 5.8 ± 0.4 | 4.8 ± 0.3 | 4.5 ± 0.2 | 4.7 ± 0.3 | 5.6 ± 0.2 |
| | G4 | 5.3 ± 0.4 | 5.3 ± 0.5 | 4.9 ± 0.2 | 4.5 ± 0.2 | 5.1 ± 0.3 | 5.9 ± 0.5 |
| | G5 | 5.2 ± 0.3 | 5.5 ± 0.2 | 4.9 ± 0.3 | 4.8 ± 0.3 | 4.9 ± 0.3 | 5.8 ± 0.3 |
| Globulin (g/dL) | G1 | 3.4 ± 0.2 | 3.9 ± 0.2 | 3.0 ± 0.3 | 3.1 ± 0.3 | 3.1 ± 0.3 | 3.3 ± 0.3 |
| | G2 | 3.5 ± 0.1 | 3.2 ± 0.2 | 3.4 ± 0.1 | 2.8 ± 0.2 | 2.5 ± 0.2 | 3.8 ± 0.1 |
| | G3 | 3.6 ± 0.2 | 4.0 ± 0.4 | 3.1 ± 0.2 | 2.7 ± 0.2 | 2.7 ± 0.3 | 3.9 ± 0.2 |
| | G4 | 3.7 ± 0.4 | 3.8 ± 0.5 | 3.1 ± 0.2 | 2.8 ± 0.2 | 3.0 ± 0.3 | 4.1 ± 0.5 |
| | G5 | 3.6 ± 0.2 | 3.8 ±0.2 | 3.1 ± 0.3 | 2.9 ± 0.3 | 2.8 ± 0.3 | 4.0 ± 0.3 |
| Albumin (g/dL) | G1 | 1.5 ± 0.1 | 2.0 ± 0.1 | 2.0 ± 0.1 | 1.7 ± 0.1 | 2.1 ± 0.1 | 1.7 ± 0.1 |
| | G2 | 1.6 ± 0.1 | 1.7 ± 0.1 | 1.8 ± 0.0 | 1.7 ± 0.1 | 2.1 ± 0.1 | 1.6 ± 0.1 |
| | G3 | 1.6 ± 0.1 | 1.8 ± 0.1 | 1.7 ± 0.1 | 1.8 ± 0.1 | 1.9 ± 0.1 | 1.7 ± 0.1 |
| | G4 | 1.6 ± 0.1 | 1.6 ± 0.1 | 1.8 ± 0.1 | 1.8 ± 0.1 | 2.2 ± 0.1 | 1.9 ± 0.1 |
| | G5 | 1.6 ± 0.1 | 1.7 ± 0.1 | 1.9 ± 0.1 | 1.9 ± 0.1 | 2.1 ± 0.1 | 1.8 ± 0.1 |

Tabulated values are the mean ± standards error of the mean for 12 determinations; *P< 0.05; ** P<0.01; ***P<0.001

**Table 22:** Markers of liver enzymes profiles of chickens following administration of *C. swynnertonii* resinous extract

| Parameters | Group | Treatment Days | | | | | |
|---|---|---|---|---|---|---|---|
| | | Before | During | | | After | |
| | | 0 | 3 | 7 | 14 | 21 | 28 |
| ALT (IU/L) | G1 | 39.3 ± 5.2 | 40.9 ± 3.7 | 34.6 ± 3.9 | 40.4 ± 2.8 | 41.0 ± 2.6 | 39.9 ± 1.7 |
| | G2 | 36.8 ± 4.8 | 36.2 ± 3.5 | 36.8 ± 3.5 | 35.6 ± 2.5 | 48.6 ± 7.0 | 35.7 ± 2.4 |
| | G3 | 30.4 ± 3.0 | 37.3 ± 4.2 | 45.2 ± 4.3 | 48.6 ± 4.6 | 50.3 ± 6.5 | 35.9 ± 1.9 |
| | G4 | 37.6 ± 3.8 | 38.1 ± 4.4 | 39.9 ± 3.9 | 46.6 ± 4.0 | 47.2 ± 5.1 | 34.3 ± 2.0 |
| | G5 | 33.5 ± 3.1 | 34.3 ± 3.1 | 43.8 ± 4.4 | 44.8 ± 4.7 | 50.6 ± 6.6 | 34.0 ± 1.4 |
| AST (IU/L) | G1 | 122.1 ± 12.2 | 123.5 ± 12.1 | 128.0 ± 11.0 | 125.0 ± 9.7 | 121.5 ± 10.0 | 122.5± 10.3 |
| | G2 | 100.1 ± 13.5 | 115.4 ± 14.9 | 116.3 ± 14.0 | 116.6 ± 10.5 | 134.3 ± 12.5 | 109.5 ± 9.9 |
| | G3 | 113.5 ± 13.2 | 111.7 ± 12.8 | 122.9 ±4 9.8 | 128.2 ± 11.0 | 141.6 ± 12.3 | 115.0 ± 7.8 |
| | G4 | 117.1 ± 11.4 | 118.3 ± 9.4 | 124.3 ± 11.4 | 132.4 ± 10.9* | 135.3 ± 11.1 | 116.9 ± 7.4 |
| | G5 | 114.3± 12.4 | 116.8 ± 12.9 | 118.6 ± 9.0 | 137.4 ± 12.6* | 137.6 ± 11.4 | 114.8 ± 7.8 |

Tabulated values are the mean ± standards error of the mean for 12 determinations; *P< 0.05

Plasma creatinine levels increased in a dose dependent manner for all treated groups as compared to the negative control groups. The levels were significantly ($P < 0.01$) increased were more obvious observed from day 7 of treatment. Uric acid levels did not differ significantly ($P > 0.05$) among treated groups though the increased level was seen from last day of treatment with resin extract. For plasma creatinine and uric acid, the levels resumed immediately to the normal state following cessation of drug administration (Fig. 10).

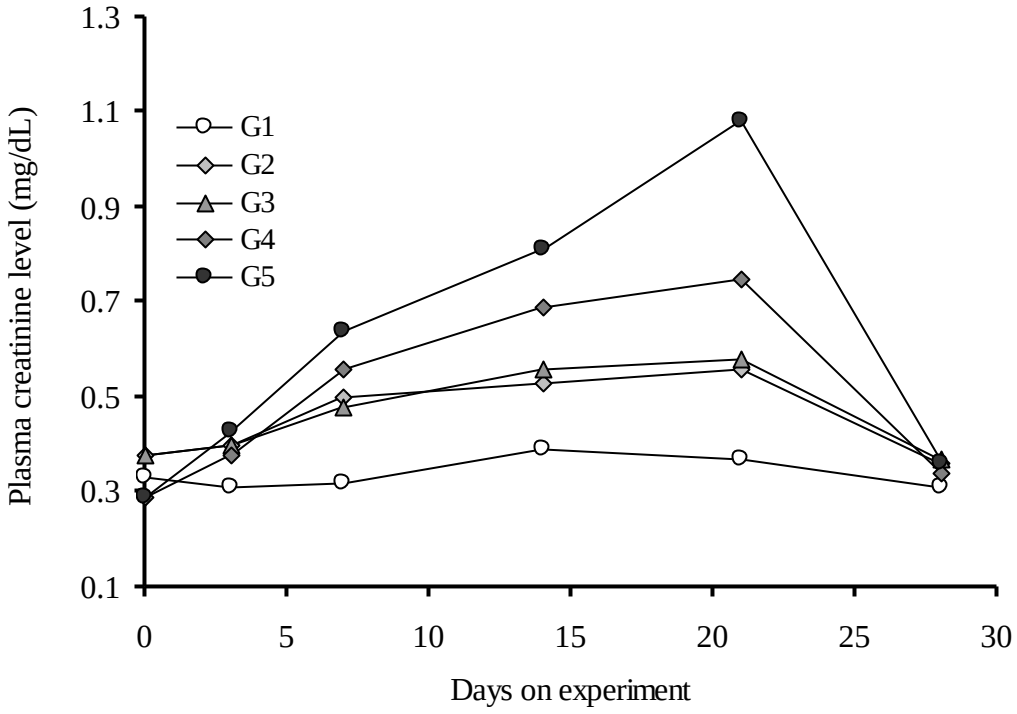


**Figure 10:** Plasma creatinine profiles of chickens following administration of *C. swynnertonii* resinous extract.

### *4.6.5 Pathological and histopathological findings*

Two chickens from each group were sacrificed and examined for macroscopic and histopathological lesions. Gross examination of liver showed marked enlargement in the G4 with numerous yellowish patches of different sizes (Plate 6).

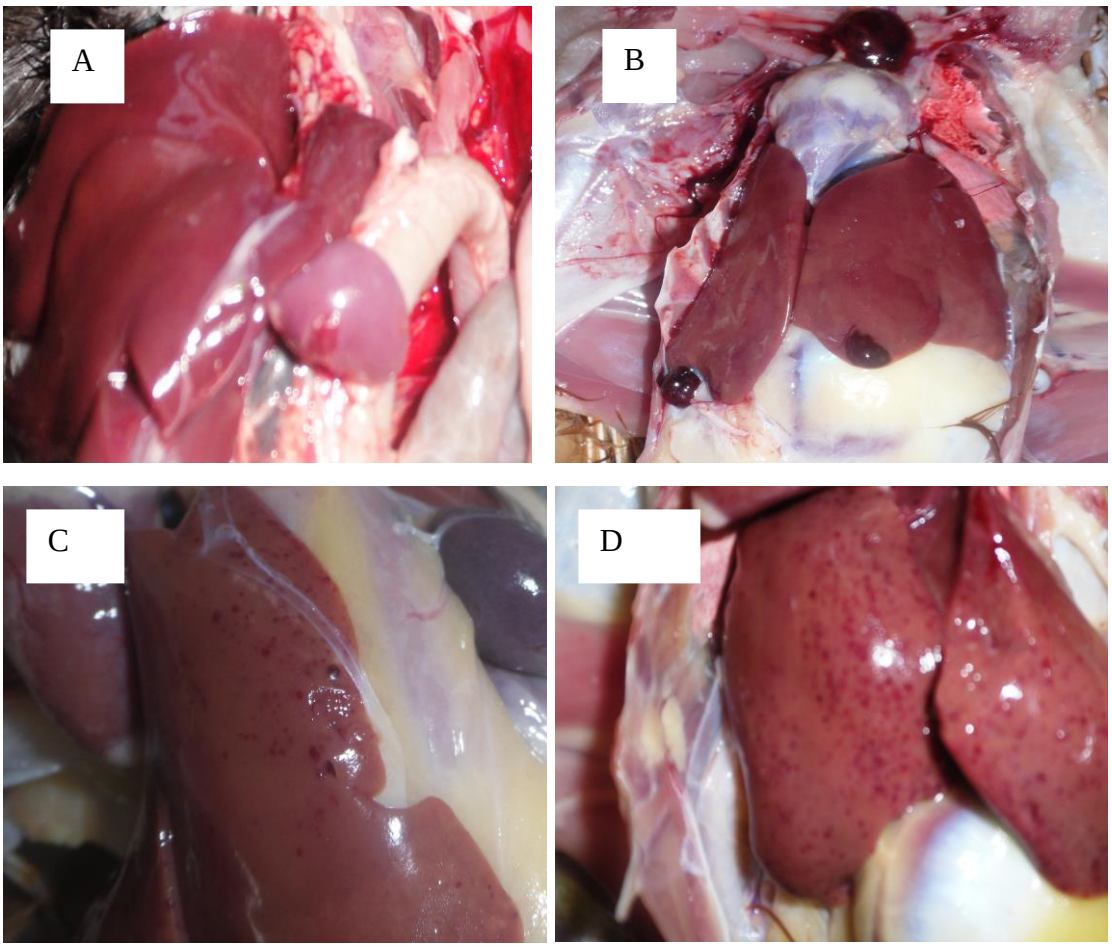


**Plate 6:** Liver section showing [A] normal liver from the untreated groups. Liver of the chicken treated with resin at 500 mg/kg bodyweight [B] and mottled appearances of the liver treated with resin at concentration of 750 mg/kg [C]: and [D] 1000 mg/kg**.**

No macroscopic changes were observed in the intestines, kidneys, lungs, spleen, muscles or other vital tissues/organs in normal and chickens treated with resin extract at doses lower than 500 mg/kg. Histopathological results showed that there was no noticeable structural damage to the liver and kidney tissues of chickens administered extract at lower doses such as 250 and 500 mg/kg. However, at higher doses of 750 and 1000 mg/kg bodyweight, mild congestion, fatty degeneration, as well as, infiltration of mononuclear inflammatory cells around blood vessels (perivascular cuffing) was observed at subcapsular and around portal triad of the liver as shown in (Plate 7) below.

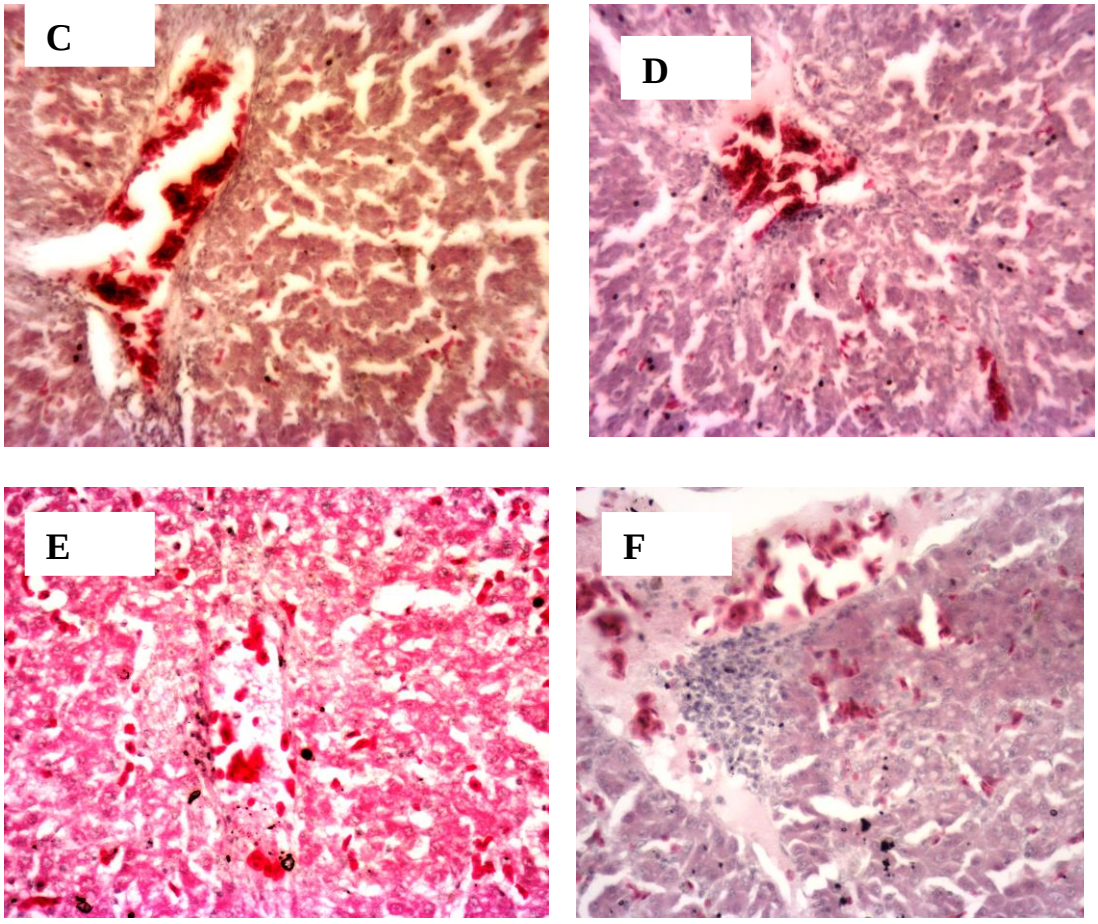


**Plate 7:** Liver tissue (X 40 magnifications) showing congestion [C and D], mild fatty changes [E] and mononuclear cell infiltration shown in [F].

In addition, cortical haemorrhages, medullary congestion, hydropic degeneration of the cortical - tubular epithelium and glomerulus were seen in the kidney (Plate 8 and 9), in the group administered 750 mg/kg bodyweight (G4). The group treated with 1000 mg/kg bodyweight (G5) also showed acute glomerulonephritis. Lungs showed passive pulmonary congestion with mild atelectasis (collapse of alveolar tissues). Hyperplasia of lymphocytic white pulps was identified in the spleen.

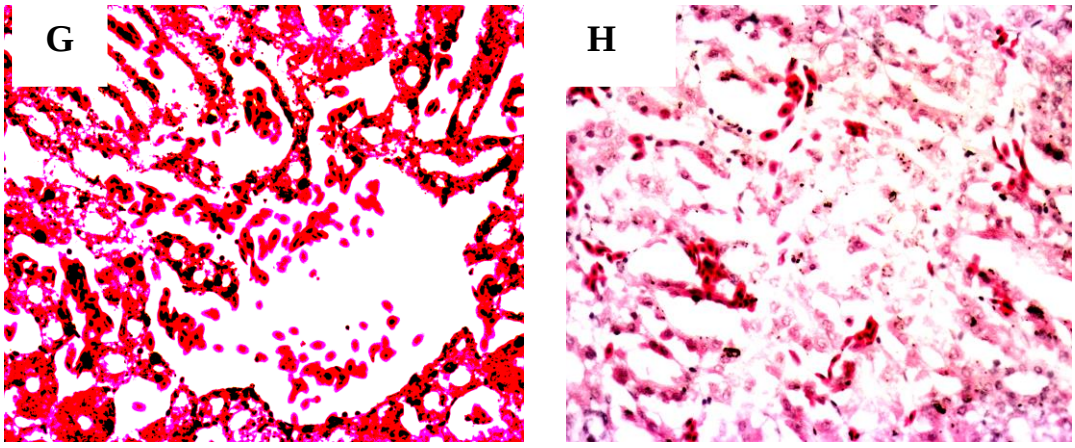


**Plate 8:** A section of chicken's kidney [20 x magnifications, H&E stain] following infection with NDV and then treated 1000 mg/kg per day *C. swynnertonii* resin extract 14 days post-treatment showing severe congestion [G] and normal kidney [H].

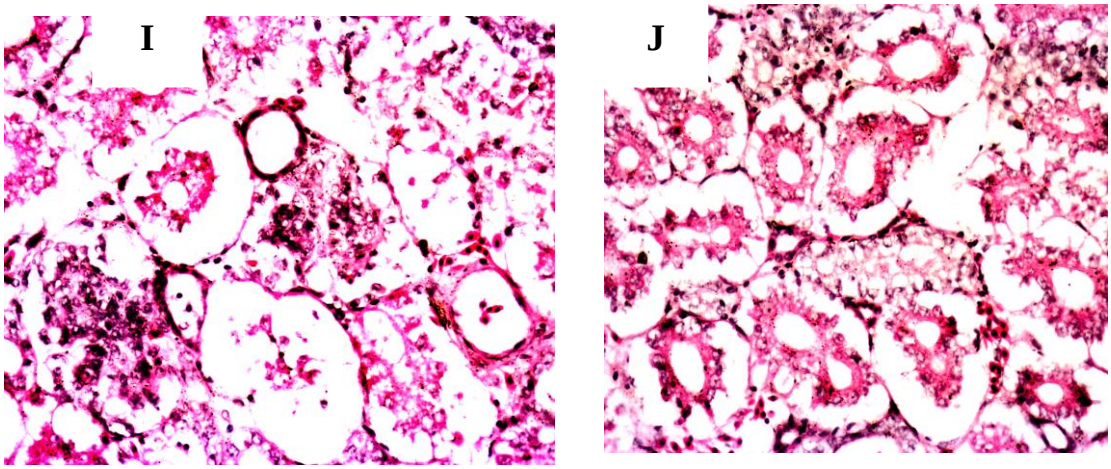


**Plate 9:** A section of chicken's kidney [40 x magnification, H&E stain] showing glomerular and hydropic tubular degeneration (I) with cystic luminal dilatation (J) and others at the cortex.

### 4.7 Bioactive constituents of the rootbark and resin extracts

Following extraction with ethanol, yields of different extracts were obtained as shown in Table 23. Leaves (81.1%) have shown to give more extract yield as compared to other four morphological parts (resin, stem bark and root bark). Root bark (23.9%) gave the least amount of extract among the four.

**Table 23:** Extraction yield from different morphological parts of *C. swynnertonii*

| **Plant part** | **Amount soaked (g)** | **Yield (g)** | **Percentage yield (%)** |
|---|---|---|---|
| Root bark (RB) | 519.38 | 124.5 | 23.9 |
| Stem bark (SB) | 694.92 | 302.0 | 43.5 |
| Leaf extract (LE) | 114.04 | 92.46 | 81.1 |
| Resin extract (RE) | 434.82 | 211.3 | 48.6 |

The results of the phytochemical analysis (Table 23) revealed the presence of tannins, saponins, flavonoids, glycosides, steroids and terpenoids. The phytochemical screening of the *C. swynnertonii* root bark extract showed the presence of saponins, flavonoids cardiac glycosides and terpenoids while it was negative for tannins, flavonoids, terpenoids steroid, anthraquinones and phlobatannins. Resin extract was positive for terpenoids, saponins and cardiac glycosides and negative for the rest. *C. swynnertonii* leaves were positive for tannins, flavonoids, terpenoids, cardiac glycosides, steroids and saponins and negative for the phlobatannins and antraquinones for resin exract.

**Table 24:** Phytochemicals in different morphological parts of *C. swynnertonii*

| **Phytochemical** | **Root bark** | **Resin** |
|---|---|---|
| Tannins | ++ | + |
| Phlobatannins | - | - |
| Flavonoids | +++ | +++ |
| Terpenoids | ++ | +++ |
| Anthraquinones | +++ | - |
| Steroids | +++ | ++ |
| Cardiac glycosides | +++ | +++ |
| Saponins | + | +++ |

Key: - = absence of a compound; + = Low amount; ++ = Moderate; +++ = Abundant

# CHAPTER FIVE

## 5. DISCUSSION

### Summary

The use of medicinal plants in treatment of diseases has generated renewed interest in recent years; herbal preparations are increasingly being used in both human and animal healthcare systems**.** Use of the crude extracts from *Commiphora* plants for treatment of various diseases has been reported worldwide. However, there is little scientific evidence validating the use of *C. swynnertonii* despite its extensive use particularly within pastoral communities in Tanzania. The main objective of this study was to establish ethno-botanical information and investigate biological activities of crude extracts from various morphological parts of *C. swynnertonii* against selected microbes (protozoan, bacteria, fungi and viruses) of veterinary importance in chickens. Safety margin of the most potent extract was investigated *in vitro* and *in vivo* using brine lethality test and haematological and physiological parameters respectively. These objectives were achieved through a series of in *vitro* and *in vivo* experiments. The current study has clearly demonstrated that resin from *C. swynnertonii* has strong antibacterial, anticoccidial, antifungal and antiviral activities. These findings are expected to be a base for further research that will validate the use of *C. swynnertonii* in managing important chicken diseases such as Newcastle, coccidiosis and bacterial infections. This chapter discusses major finding from the current work and their practical

implications. The prospects of using *C. swynnertonii* in ethno-veterinary practices in controlling several chicken diseases in local chicken keepers are also considered.

**5.1 Ethnobotanical survey**

Findings from this survey revealed that most of the respondents in Simanjiro were Maasai pastoralists who are also engaged in agricultural activities. Low participation of women in this study was due to the Maasai custom, which bars women from being interviewed in absence of their spouses.

The test plant, *C swynnertonii*, is widely distributed across a wide geographical area with varying climatological conditions. The plant is abundantly available in Simanjiro, Manyara, Dodoma, Kilimanjaro, Mwanza, Tanga and other parts of Tanzania. The reliable availability of the plant in the study area explains the fact that all respondents were aware of the plant and its medicinal properties. The information regarding the uses of *C. swynnertonii* in humans was similar to that reported in other studies (Hanus *et al.*, 2005; Paraskeva *et al.*, 2008). Treatment of wounds, abscesses, painful swellings, pulmonary and fungal infections were among the frequently mentioned medicinal uses. Also in all these studies, *Commiphora* spp were reported to be used as anti microbial and anti-parasitic agents. Addition of resin and/or root bark in tea, soup or milk for oral administration were the main preparations used in human beings .It is speculated that

this route of administration was a way of neutralizing some toxic compounds and reduce microbes that could be present in the plant material.

Other reported uses of *C. swynnertonii* in animals include; insect repellency particularly against ticks, lice, mites and fleas (Minja, 1989; Sambuta and Masola, 2008) and as anthelmintic agent (Massoud *et al.*, 2001, Haridy *et al*., 2003). These effects were also reported by more than half of the respondents in the current study. The more frequent use of resin and root bark by respondents compared to the other plant parts is attributed to high concentrations of active compounds present in those morphological fractions. Furthermore, findings in the current study revealed that *C. swynnertonii* was used as a source of income through selling of stem products mainly the resin. Elsewhere, trading on *Commiphora* spp products has been reported (Hanus *et al.*, 2005). In Somalia, resin (myrrh) from *C. molmol* is used as the source of income by the rural people whereby a kilo sells between USD 3.50 (cleaned type) and USD 3.00 (natural myrrh).

Although *C. swynnertonii* is drought resistant and well adapted to its environment, the current findings indicate that its exploitation will increase significantly in the future. Apart from its medicinal uses, *C swynnertonii* is used as a building material (poles) and for fencing of animal enclosures. In Simanjiro District, there is over – exploitation of the plant habitats through mining, overgrazing, urbanization and other agriculture activities. All these factors together with lack of awareness on which plant part to be used contribute to disappearance of several medicinal plant species including *C. swynnertonii*.

Findings from the current ethnobotanical survey and experiments revealed potential of using resin as medicinal plants because of its potency, low cytotoxicity, reliable availability and the fact that its harvesting does not damage the plant and the environment compared to other parts such as roots and cuttings of the stem barks. Also it was observed that propagation of plant using stem cuttings is simple and successful therefore conservation programs must be carried out to conserve the biodiversity for the sustainable availability of resin extract.

In conclusion, *C. swynnertonii* is well known in the study area and has a wide range of uses including medicinal, fodder tree, fencing as a well as well as source of income through selling of resin. The literature supports some of the claimed uses of the plant as they are used in traditional medicine. However, more studies to validate the claimed uses of this plant are required. Also the advice on the importance of protecting the study plant for sustainable use should be given a community concerned.

### 5.2 Effect of diferrent *C. swynnertonii* extracts against bacteria and fungi

Results of the current study have clearly shown that crude extracts from different morphological parts have varying antimicrobial activity *in-vitro* in a dose dependent manner. Our findings are in agreement with previous studies done by El-Ashry *et al*., (2003) and Abdallah *et al*., (2009) who found that several *Commiphora* species had considerable antimicrobial activity against some gram positive and gram negative bacteria. Furthermore, *in-vitro* studies by Paraskeva *et al*. (2008) using selected South

African *Commiphora* species, showed more activity against gram positive bacteria than gram negative. Similarly, in the current study, gram positive bacteria showed significantly higher ($P < 0.01$) growth inhibition zones than their gram negative counterparts although a gram negative bacterium, *E. coli* was most sensitive to the four extracts compared to all organisms tested. The difference in susceptibility between gram positive and gram negative has been associated with their cell wall structure (Parekh and Chanda, 2007). In general, the Gram-negative bacteria displayed the least sensitivity towards the extracts, and all of the plant extracts exhibited poor and unvaried activity against *Pseudomonas aeruginosa* and *Salmonella typhimurium*, indicating the resistance of this bacterium to the plant extracts. This could be due to their outer membrane / cell wall acting as a barrier to many environmental substances, including antibiotics. Some studies reported that the specialized cell wall structure and especially the presence of the outer envelope resulted in the impermeability of these micro-organisms to biocides and antibiotics, and at times, resulting in regulation and prevention of their passage to the target region (Paraskeva *et al.,* 2008). Resistance to the plant extracts is, thus, exhibited to a far greater extent by the Gram-negative bacteria than by Gram-positive bacteria (Lin *et al.,* 1999).

Resin extract ranked the highest in inhibiting the growth of the tested microbes; with largest inhibition zones against *S. pyogenes*, *E. coli* and *B. subtilis* in that order. Similar studies using *C. quadricincta* (Salamah and Zaid, 1999) also showed higher activity of resin against the three bacteria in comparison to other extract tested. Akor and Anjorin,

(2009) reported highest activity of *Commiphora africana* against *E. coli*, *S. aureus* and *C. albicans*. Also Musa, (2008) demonstrated a good activity of *Commiphora kerstingii* against *S. aureus*. Furthermore, Akor and Anjorin (2009) reported that *E. coli* and *B. subtilis* were the most susceptible among microorganism treated with crude ethanolic root extract from *C. africana. S. typhimurium* and *P. aeruginosa* were the least affected by the crude extracts. Resistance of these two bacteria to crude plant extracts and even commonly used antibiotics has been documented in other studies. Parekh and Chanda, (2007) tested twelve species of Indian medicinal plants and found that *S. typhimurium* and *P. aeruginosa* were resistant to all tested plants. Also *P. aeruginosa* was shown to be resistant to root extract of *C. africana* (Musa, 2008; Akor and Anjorin, 2009).

Resistance of the two bacteria to various antibiotics has been reported by Brisabois *et al*. (1997); Wang *et al*. (2006). This resistance was associated with presence of resistant genes, PSE and CARB-type, in both the bacteria and animals. These genes are located on an integron, a new family of genetic components into which many resistance agents can fit (Brisabois *et al*., 1997). The antibacterial activity of various *Commiphora* spp. has been attributed to presence of different active constituents. The commonly reported active constituents include phenolic compounds, alkaloids, saponins, tannins, flavonoids, anthraquinones and cardiac glycosides, terpenes, sesquiterpenes, esters cumunic aldehyde, eugenol, steroids, resin acids and proteins (Hanus *et al.,* 2005, Aliyu *et al*., 2007; Musa, 2008 and Abdallah *et al*., 2009). The antibacterial activity of *C. molmol* was attributed to presence of terpenes in its oleo-resin (Rahman *et al*., 2008).

The brine shrimp lethality assay was carried out to assess toxicity of extracts from different morphological parts of *C. swynnertonii*. Results from this study indicated that all tested extracts (with exception of leaf extract) had $LC_{50}$ values below 20 µg/mL suggesting that exposure to high concentrations can be acutely toxic to biological systems. Brine shrimp $LC_{50}$ values of medicinal plants have been used to predict anti-carcinogenic activity when values are less than 20 µg/mL (Meyer *et al*., 1982; Moshi *et al.*, 2006). Studies done by (Moshi *et al.*, 2004, 2006) on different plants extracts on brine shrimp results provide a circumstantial evidence that plant extracts with $LC_{50}$ values below 20 µg/ml have a likelihood of yielding anticancer compounds. The resin extract, apart from being the most potent, seems to be more appropriate because it's harvesting causes minimal damage to the plant and also showed less cytotoxic effect than the root and stem barks.

The current study has clearly demonstrated that crude extracts, especially resin, from *C. swynnertonii* have strong antimicrobial activity. These findings support the traditional use of the resin in treatment of various infectious diseases. Further *in vivo* investigations using the resin are recommended to validate the use of *C. swynnertonii* as an antimicrobial agent against infectious diseases caused by the tested pathogens.

### 5.3 Effect of different *C. swynnertonii* extracts against ND virus *in ovo*

The present study has demonstrated, for the first time, antiviral activity of crude extracts from *C. swynnertonii* against Newcastle disease virus using an *in ovo* technique. In this study, death of all chicken embryos within 2 days (48 hours) PI with the NDV was a clear indication that the virus strain was highly virulent. Similar findings were also reported by Sulaiman *et al.* (2011) that all eggs inoculated with the virus control and treated with 5 and 2 mg/ml extracts from Baobab tree (*Adansonia digitata* Lin) died between 48 and 72 h. However, addition of any of the four tested *C. swynnertonii* crude extracts significantly prolonged the survival time of the embryos in a dose-dependent manner. From the embryo weight data, it was further observed that the highest dose of crude extract inclusion (>500 µg/mL) was associated with a reduction in embryo weights. This suggested that high concentrations of the extracts could be lethal to the embryo as well and therefore, the concentration of 200 to 250 µg/mL should be the optimum level of the extract inclusion. The antiviral activity of different *C. swynnertonii* extracts was further demonstrated in the current study by data obtained from the haemaglutination (HA) test, which quantified the amount of viruses in the allantoic fluid of live chicken embryos. The highly significant reductions in virus populations in extract-treated embryos, also in a dose dependent manner, suggested a strong viricidal effect. All the four types of extracts were most toxic to the NDV at the concentration of 500 µg/mL. The antiviral activity of the crude extracts was finally confirmed by the haemaglutination inhibition (HI) data whereby very low levels of antibodies against NDV were detected in chicks hatched from resin, stem bark and root bark extract treated

eggs. The low or absence of antibodies in chicks was an indication that viruses were neutralized or destroyed before stimulating the immune system. Similar observations were also recorded by Waihenya *et al*. (2002) who found that the crude *Aloe* spp. extract inhibited NDV multiplication in embryonated chick eggs. Also, Wafaa *et al.* (2007) showed that neem tree (*Azadirachta indica*) extract at concentrations ranging from 3 to 4 µg/mL had a significant inhibitory action against both NDV and infectious bursal disease virus (IBDV) *in ovo*.

The mechanisms through which the crude extracts from *C. swynnertonii* inhibit NDV multiplication *in ovo* are not yet known. This indicates inhibitory rather than virucidal effect of the extract on the ND virus at these doses (*in ovo*). However, many traditional medicinal plants used to treat viral diseases have been shown to contain high levels of compounds such as coumarins, flavonoids, alkaloids, terpenes, naphthoquinones and anthraquinones (Hanus *et al*., 2005; Sulaiman *et al.,* 2011). Same classes of compounds have been found in some *Commiphora* spp. (Hanus *et al*., 2005). These compounds exert their effect by killing the virus and/or interfering with viral multiplication (Jassim and Naji, 2003). Specifically, some of these compounds exhibit protease inhibition, hence interferes with cleavage of haemaglutinin neuramidase and fusion protein, which are important glycoproteins for NDV attachment and multiplication Zhirnov *et al*. (1985).

This study demonstrated the antiviral activity of various extracts of *C. swynnertonii* against ND virus *in ovo*. Although all of the four tested extracts had similar efficacy, the

use of resin was recommended because its harvesting is less aggressive and does not interfere much with normal growth of the tree.

### 5.4 Effect of resin in chickens experimentally infected with NDV

The current findings have demonstrated significant antiviral activity of crude resinous extracts of *C. swynnertonii* against experimental Newcastle infection in local chickens. The typical clinical signs, which were observed following infection, were a clear indication that the ND virus strain used was virulent.

Significant reduction in all clinical parameters, including mortality rates and pathological lesions of Newcastle infection suggested that crude resinous extract from *C. swynnertonii* had significant antiviral effect. Other *Commiphora* spp have been implicated in reducing severity of various viral infections in humans (*http://www.dickcontino.com/myrrhinfections.htm*) although the exact mode of action was not explained. Further indications of antiviral activity of the extract came from measurements of antibody titres against the ND virus during the chicken trials. Comparison of antibody titres between the two trials showed that the levels of titres were significantly lower in the prophylactic than in the therapeutic trial. This observation suggests that administration of the resin extract before the infection helped to reduce/interfere with virus multiplication with consequent reduced immunological response against the virus. Similar finding were observed when embryonated chicken

egg were infected with ND virus and then treated with different extracts from *C. swynnertonii* (Bakari *et al.,* 2012a). Also, the reduction of antibody titres by the extract to the ratio of 1:8, which is regarded as protective antibody titre (Awan *et al.,* 1994; Numan *et al.,* 2005; Musa *et al.,* 2009, Wambura, 2011; IOE, 2012), is a further indication that the *C. swynnertonii* extract could be modulating the immune system of the chickens by promoting the production of infection-fighting white blood cells, as well as having a direct antimicrobial effect of its own. Abdalah *et al.* (2009) reported increased white blood cell counts following administration of aqueous suspensions of the resins of *Commiphora molmol* Engl. Ex Tschirc in Winster albino rats. Other studies (Mtambo *et al*., 1999; Waihenya *et al.,* 2002) in which medicinal plants were tested against ND virus in chickens revealed that there were no significant differences in the levels of antibody titres between treated and untreated groups.

In the current study, it was also observed that an increase in the dose of the extract had a negative dose-dependent effect on bodyweight gain. This could be associated with the extract because some *Commiphora* spp have been used as anti-obesity remedy to reduce body weights in humans (Ojha *et al.,* 2008, Rayalam *et al.,* 2009). The weight reduction effect has been associated with reduction in plasma cholesterol and glucose levels (Scott, 2005) through stimulation of thyroid hormone function thus interfering with basal metabolic rate leading to loss of body weight (Scott, 2005). Administration of *C. swynnertonii* resinous extract in healthy chickens significantly reduced concentrations of glucose and cholesterol in plasma (unpublished data).

The mechanisms through which the crude extracts from *C. swynnertonii* inhibit NDV multiplication in chicken's body are not yet known. However, many traditional medicinal plants used to treat viral diseases have been shown to contain high levels of compounds such as coumarins, flavonoids, alkaloids, terpenes, naphthoquinones and anthraquinones. Same classes of compounds have been found in *C. swynnertonii* and other *Commiphora* spp. (Hanus *et al*., 2005). These compounds exert their effect by killing the virus and/or interfering with viral multiplication (Jassim and Naji, 2003). Specifically, some of these compounds are speculated to exhibit protease inhibition, hence interferes with cleavage of haemaglutinin neuramidase and fusion protein, which are important glycoproteins for ND virus attachment and multiplication Zhirnov *et al*. (1985). Other classes of compounds such as flavonoids from *C. africana* have been reported to act by inhibiting production of prostaglandin (signaling molecule) and phosphosdiesterases involved in cell activation Tiwari *et al.* (2011), the effect which predominantly depend upon biosynthesis of protein cytokines that mediate migration of circulating leucocytes to site of injury (Manthey *et al.*, 2001) thus promoting healing.

Findings from this study have demonstrated significant antiviral activity of resinous extract from *C. swynnertonii* against experimental ND infection in local chickens. Prophylactic administration of the extract could be a better approach in mitigating the effects of ND infection in endemic areas. Furthermore, therapeutic administration of resin extract after an outbreak could also be used to reduce disease severity and

mortalities. Field trials are recommended as a way of validating the use of *C. swynnertonii* extract against Newcastle disease in chickens.

### 5.5 Effect of resin against chickens experimentally infected with coccidia

In this study, resin was chosen because findings from our previous work (Bakari *et al.*, 2012 a, b) had shown that resin was more appropriate than other morphological fractions of the plant owing to its easy harvesting, potency to other microbes (like bacteria, fungi and Newcastle virus) and low cytotoxic effect. The current findings have demonstrated anticoccidial activity of the crude resinous extracts of *C. swynnertonii* against coccidial infection in local chickens. In this study, it was also shown that *Eimeria* spp. tested (*E. necarix, E. tenella* and *E. mitis*) were virulent and able to cause typical coccidial infection in chickens.

Significant reduction in all parameters of coccidial infection was a clear indication that crude resinous extract from *C. swynnertonii* had significant anticoccidial effect. Similar findings were reported by Baghdadi and Al-Mathal, (2010) who showed that treatment with crude oleo-gum-resin (myrrh) extract and mirazid (a commercial extract) from *Commiphora molmol* resulted in significant recovery of rabbits infected with *Eimeria stiedae* from all symptoms of infection compared to the untreated infected group. The anticoccidial activity of various *Commiphora* spp. has been attributed to presence of different bioactive constituents in the resin extract (Baghdadi and Al-Mathal, 2010). The

most commonly reported active constituents include phenolic compounds (tannins), alkaloids, saponins, anthraquinones cardiac glycosides, and terpenes (Hanus *et al.*, 2005; Baghdadi and Al-Mathal, 2010). Tannins bind proteins on mucous surfaces so that they become less permeable and thus poorly absorbed and produce a protective layer of coagulated protein, which stops diarrhea, reduces gut motility and numb nerve ending of parasites thus making elimination of parasites easier (Max *et al.*, 2009; Baghdadi Al-Mathal, 2010; Tiwari *et al*., 2011).

Studies with other *Commiphora* spp have also showed activity against other protozoa pathogens. Fathy, (2011) reported significant efficacy of *C. molmol* against *Trichomonas vaginalis* and *Cryptosporidium* spp. in human beings. Also *C. molmol* was found to reduce parasite loads in rats through a direct toxic effect on *Giardia lamblia* trophozoite and also by reversal of the observed mucosal damage associated with the infection (Fathy, 2011).

Coccidiosis is known to cause severe damage of intestines in chickens (Sousby, 1982). In this study post-mortem examination of dead chickens from extract-treated-group had milder intestinal mucosa damage compared to the untreated group (G2). Furthermore, there was thick mucus in the intestinal lumen of chickens from the extract-treated group which was not observed in the anticoccidial-treated group (G5). This observation was also reported by Olivier, (2009) who suggested that mucus formation is a protective mechanism of the some *Commiphora* species against the necrotizing effects of various

agents on the intestines and other organs such as the liver. Another study (Al-Harbi *et al.,* 1997) reported an increase in both nucleic acid production and concentration of non-protein sulfhydryl compounds in maintaining gastro duodenal integrity. Other plants species also reported to have same activities in reducing oocysts count in chickens artificially challenged with mixed *Eimeria* spp. Nwosu *et al.,* 2011 showed that *Khaya senegalensis, Anona senegalensis* or / and anticoccidial drug significantly reduced or eliminated faecal oocysts output and improved live weight of pullet chicks following oral treatment of infected with 120,000 sporulated *Eimeria* oocysts per chick.

It is concluded that the crude resinous extract from *Commiphora swynnertonii* has significant anticoccidial effect against an experimental infection in chickens. These findings indicate the potential of using the extract for treating coccidiosis in chickens. Further studies are needed to validate the veterinary use of the extract, isolate responsible active chemical component(s) and to elucidate their mechanism(s) of action.

## 5.6 Effect of resin on selected haematological and biochemical parameters in chickens

The present study has demonstrated the effect of aqueous crude resin extracts from *C. swynnertonii* on various haematological and biochemical parameters in chickens. Only chickens receiving higher resin doses (G4 and G5) showed signs of toxicity including dullness and loose faeces. This was an indication that extended administration of high doses of the *C swynnertonii* resin extract could be detrimental to the gastrointestinal tract of chickens. Similar observations have been reported in rats (Scott, 2005) and humans (Olivier, 2009) who noted increased mucus production in the intestinal tract following treatment with resin from some *Commiphora* spp. These findings suggest that the resin can stimulate production of mucus in the GIT. The negative effect of resin on body weight of chickens was clearly evident and was dose dependent. The weight reduction effect has been associated with reduction in plasma cholesterol and glucose levels through stimulation of thyroid hormone (T3 and T4) function thus interfering with basal metabolic rate leading to loss of body weight (Scott, 2005).

Thyroid hormones (T3), stimulates the production of RNA polymerase I and II and, therefore, increases the rate of protein synthesis, potentiates the effects of the β-adrenergic receptors on the metabolism of glucose. Therefore, it increases the rate of glycogen breakdown and glucose synthesis in gluconeogenesis. Also stimulates the breakdown of cholesterol and increases the number of LDL receptors, thereby increasing

the rate of lipolysis (Guyton, 2006). In the current study, the metabolite reduction caused by the resin extract could be a probable cause of decreased body weight in chickens.

Administration of the resin to chickens affected some of the hematological parameters in different ways. PCV, differential WBC count (neutrophils and eosinophils) were not affected. Hb, total RBC count, MCH and MCHC decreased with increasing concentration of the resin dosage. This significant decrease could be a result of presence of saponins in the resin. Saponins are known to cause red blood cell breakdown by dissolving their membranes hence causing haemolytic crisis (Kayser *et al.,* 2002). Similar studies on other *Commiphora* species reported no significant changes in PCV, Hb, MCH, MCHC and RBC counts in experimental animals (Bakhiet and Ibrahim, 2006; El-Naggar, 2011).

The significant increase in lymphocytes and monocytes counts indicated that *C. swynnertonii* has ability to activate the defence mechanism in chickens. This is supported by the pathological changes observed in the body where increases mononuclear cell infiltration was seen in different tissues in chickens' body.

The significant dose dependent reduction in plasma glucose and total cholesterol can be referred to as antiglycemic and hypolipidaemic effect respectively. Other studies using various *Commiphora* spp. also reported antiglycemic effect in Wistar rats (Sheela and Augusti, 1995; Helal *et al.,* 2006; Goji *et al.,* 2009). This effect has been associated with

increased glycogen intake by increasing insulin level. Helal *et al.* (2006) attributed the antiglycemic effect of *Commiphora* with a decreased production of glucose precursors in the liver, suggesting the usefulness of this therapy in treating non-insulin dependent diabetes mellitus. Another interesting finding in the current study was the reduction in total plasma cholesterol following administration of the resin. Other studies involving differential cholesterol determination revealed reduction in total cholesterol, low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein (VLDL-c) cholesterol at the same time elevating the high density lipoprotein cholesterol (HDL-c) (Wang e*t al.*, 2004; Adebayo *et al.*, 2006, Bellamkonda *et al.*, 2011). The exact mechanism through which the *Commiphora* spp. resin reduces plasma cholesterol levels is yet to be known. Some *Commiphora* spp. (e.g., *C. mukul*) contains some compounds such as guggulsterone, which act by antagonizing the effect of the nuclear farnesoid X receptor (FXR) (Tu, 2000; Thrall *et al.*, 2006). The FXR is a key transcriptional regulator for the maintenance of cholesterol and bile acid dynamics. FXR has been shown to regulate cholesterol metabolism by binding directly to the chenodeoxycholic acid (CDCA), a primary bile acid, which mediates the feedback suppression by bile acids of cholesterol 7-alpha-hydroxylase, thus limiting the enzyme in bile acid biosynthesis from cholesterol. Secondly, the FXR participates in the activation of intestinal bile acid binding protein, which is involved in the enterohepatic circulation of bile acids. Thus, according to Tu (2000), FXR constitutes a potential therapeutic target that can be modulated to enhance the removal of cholesterol from the body. Another possible mechanism is through the presence of ketosteroid, an active compound of *C. mukul* which acts by stimulating the

thyroid gland and has also found to increase the activity of catecholamine and dopamine –p- decarboxylase that are involved in lowering plasma cholesterol (Wang *et al.*, 2004).

The observed reduction in body weight of resin-treated chickens is well connected with the observed levels of plasma glucose and total cholesterol. High carbohydrate (glucose) and cholesterol intake are known to increase body fats, hence increased body weight and eventually obesity (Kaur and Kulkarni, 2000; Scott, 2005).

The insignificant variation in the levels of total protein, albumin and globulin in the resin-treated groups was an indication that resin has no modulatory effect on the immune system. Liver enzymes transaminases (AST and ALT) are often used as specific markers of active hepatic injury and represent markers of hepatocellular necrosis (Davern and Scharschmidt, 2002; Thrall *et al.*, 2006). Whereas ALT activity is primarily localised in the liver and largely specific for parenchymal diseases (Gatsing *et al.*, 2005; Thrall *et al.*, 2006), AST activity is present in a wide variety of tissues including heart, skeletal muscle, kidney, brain and the liver (Gatsing *et al*., 2005). In the current study, liver transaminases (ALT and AST) levels increased to the maximum peak by day 21-post treatment before decreasing significantly to levels similar to pretreatment values. A similar study reported no change in levels of AST and ALT activity after treating rats with *C. molmol* for 24, 48 and 72 hours. (Rao *et al.*, 2001; Aliyu *et al.*, 2007). In this study, it was observed that prolonged use of resin extract caused damage of the liver thus led to increased concentrations of liver enzymes in the blood.

The effect of the resin on kidneys was assessed through determination of plasma creatinine levels, which usually increase when there is significant renal impairment (Thrall *et al.,* 2006). Thus, the significant increase in plasma creatinine levels observed in chickens in groups G4 and G5 after 14 days of treatment concur with the noticeable damage of the kidney to renal cortices and glomeruli as seen in histopathological sections (Plate 9). Similar findings were reported by Aliyu *et al.* (2007) that prolonged use of ethanolic leaf extracts in rats caused noticeable damage to the cortex and glomerulus. The significant increase in creatinine at high doses may possibly be due to some regenerative mechanisms by the kidney in response to the effect of resin extract. It is known that for any markers of kidney function (creatinine or uric acid) to significantly appear in blood, about 75% of the nephrons must have been damaged (Boyd, 1983; Thrall *et al*., 2006), suggesting that administration of high doses of *C. swynnertonii* resin could be detrimental to kidneys.

Some secondary plant metabolites such as coumarin, flavonoid, terpenoid, arginine and glutamic acids have been shown to confer antiglycaemic and cholesterol lowering effects in various experimental animal models (Akah and Okafor, 1992; Marles and Farnsworth, 1995). The significant antiglycaemic and anticholesterol observed in the current study can therefore be explained by the fact that *Commiphora swynnertonii* contain remarkable amounts of terpenoids and flavanoids. Terpernoids and flavonoids appear to be involved in the stimulation of the ß-cells and the subsequent secretion of preformed insulin (Goji *et al.,* 2009; Bellamkonda *et al.,* 2011).

In conclusion, this study has demonstrated that chickens can tolerate oral administration of *Commiphora swynnertonii* resin at doses less than 750 mg/kg bodyweight whereby haematological parameters tested and liver functions were not significantly affected. Administration of higher doses had negative effects on liver, kidney and lung functions, which included acute glomerulonephritis and pulmonary congestion respectively. The observed antiglycemic, anticholesteremic and body weight lowering effect were interesting findings, which can be used as a template for further research in humans. Further studies on the resin extract are needed to isolate the bioactive component(s), elucidate its exact mechanism(s) of action and validate its uses in the chickens and other animal species.

### 5.7 Bioactive constituents in rootbark and resin extracts from *C. swynnertonii*

It has been established that, plants produce some secondary metabolites, which are responsible for biological activities in man and animals (Nwanjo, 2005). Some of these chemical compounds have been reported to have inhibitory effects on some bacteria, fungi, protozoa, helminths and viruses (Cowan, 1999). The antibacterial activity of various *Commiphora* spp. has been attributed to presence of different active constituents. The commonly reported active constituents include phenolic compounds, alkaloids, saponins, tannins, flavonoids, anthraquinones and cardiac glycosides, terpenes, sesquiterpenes, esters cumunic aldehyde, eugenol, steroids, resin acids and proteins (Hanus *et al.*, 2005; Aliyu *et al*., 2007; Rahman *et al*., 2008; Musa, 2008: Abdallah *et*

*al*., 2009). In *C. swynnertonii*, same compounds were also observed to be present in the root bark and resin. Antibacterial activity of these could probably be due to their ability to complex with extracellular and soluble proteins, complex with bacterial cell walls and disruption of microbial membranes (Cowan, 1999).

For antiviral activity, many traditional medicinal plants used to treat viral diseases have been shown to contain high levels of compounds such as coumarins, flavonoids, alkaloids, terpenes, naphthoquinones and anthraquinones. Same classes of compounds have been found in some *Commiphora* spp. (Hanus *et al*., 2005) including *C. swynnertonii*. These compounds were also reported by Cowan, 1999 to be active against Human Immunodeficiency Virus (HIV) by inhibiting HIV reverse transcriptase enzymes (Cowan, 1999). Others exert their effect by killing the virus and/or interfering with viral multiplication (Jassim & Naji, 2003). Specifically, some of these compounds exhibit protease inhibition, hence interferes with cleavage of haemaglutinin neuramidase and fusion protein, which are important glycoproteins for NDV attachment and multiplication (Zhirnov *et al*., 1985; Cowan, 1999). However, the same compounds particularly flavonoids were also reported to plays some roles as antioxidative and anti-inflammatory effects. These compounds act as free radical scavengers thus providing protection against myocardial necrosis, inhibition of platelet aggregation, as well as increased fibrinolysis. This is helpful in preventing or slowing the progress of various oxidative stress-related diseases such as cancer, coronary heart disease and even altitude sickness (Paraskeva, 2008). Saponins are glycoside components often referred to as

"natural detergent" because of their foamy nature (Edeoga *et al.*, 2005) and are reported to posses anticarcinogenic properties, immune modulation activities and regulation of cell proliferation as well as health benefits such as cholesterol lowering activity (Edeoga *et al.*, 2005). The study of resin extract from *C. swynnertonii* on biochemical parameters demonstrated the cholesterol and glucose lowering effects. Furthermore, resin from *C. swynnertonii* has been shown to have saponins as one of its bioactive compounds. This explains why the resin showed activities against the selected microbes and protozoa.

It is concluded that root bark and resin extracts contained several bioactive compounds, which correlated with different biological activities observed in the various experiments. Therefore, further investigations on the isolation and identification of most active compounds of this plant, which may lead to chemical entities, are suggested.

# CHAPTER SIX

## 6. CONCLUSIONS AND RECOMMENDATIONS

### 6.1 Conclusions

The findings obtained from different studies undertaken in this thesis have clearly demonstrated significant biological activity against the tested organisms; namely bacteria, fungi, ND virus and protozoa (coccidian spp.). This measured biological activity information concurs with the gathered ethno-botanical information (section 4.1), which supports the use of *C. swynnertonii* in treating various disease conditions in humans and animals.

Studies were carried out to determine the most potent morphological part of the plant against the claimed uses. Among the tested morphological fractions of *C. swynnertonii,* resin was chosen because of its potency to all tested microbes, low cytotoxicity to biological systems, reliable availability and the fact that it's harvesting does not damage the plant and the environment.

All of the tested extracts showed significant activity against most of the tested microbes as reported in section 4.2. The fact that resin was very potent against the tested opportunistic microbes (*E. coli, S. aureus, S. pyogenes, B. subtilis* and *C. albicans*) is a good indication that *C. swynnertonii* can be used to suppress infections which normally

accompany immunosuppressive conditions. This could also be used against opportunistic infections in HIV/AIDS patients.

Following promising results of the *in ovo* experiment, *in vivo* trials were carried out to investigate the prophylactic and therapeutic effects of *C. swynnertonii* resin on artificial ND infection in chickens (section 4.3). These experiments have clearly demonstrated that the resin from *C. swynnertonii* has a strong antiviral activity which managed to protect ND virus-infected- embryos to successful hatching. This was an indication that the crude extract could be inhibiting multiplication of or neutralizing the virus. Firther more findings from the *in vivo* trials (section 4.4) revealed important information as far as timing of resin administration is concerned. Prophylactic administration proved to be a more appropriate approach because it led to less severe clinical parameter, lower HI titres and mortality rates. This reduced severity seems to be as a result of the *C. swynnertonii* resin against opportunistic microbes, which usually exacerbates viral infections. It is also possible that the prophylactic administration helped to boost the chickens' immune system; this is supported by findings in section 4.6 which showed significant increase in total and WBC cell counts, particularly lymphocytes and monocytes.

The current study evaluated the long and short term effects of resin extract on selected physiological and biochemical parameters. The results indicated that in chickens, the resin has a good margin of safety if it is used at a dose lower than 800 mg/kg body

weight. Higher doses than the later were found to cause damage of the kidney and other vital organs therefore the plant should be used with caution not to exceed the recommended doses when used in chickens. Also in the current study, administration of resin from *C swynnertonii* significantly lowered blood cholesterol and glucose. This finding could be of significant in managing conditions such as diabetes mellitus, coronary heart and atherosclerosis.

It is concluded that all these measured biological activities observed in the various experimentscorrelated with different bioactive constituents present in the tested morphological fractions of *C. swynnertonii.*

## 6.2 Recommendations

Although plants from *Commiphora* spp have been known and used since 2000 years B.C., only few species have been scientifically verified. In Tanzania, *Commiphora* plants are known to be widely distributed but there is scanty scientific information concerning their use. Screening to assess their medicinal potential in controlling other poultry and animal diseases should continue. This could be possible through collaborative research among ethno-botanists, pharmaceutical industry, natural chemist, biomedical and those with ethno medical and/or ethno veterinary knowledge. The current study has clearly demonstrated that crude extracts, especially resin, from *C. swynnertonii* have varying degrees of antimicrobial activity (antibacterial, antifungal, anticoccidial and antiviral activities). Based on the findings from the current studies, the following are recommended:

- Further on-station and field studies are needed and should focus on other microbes of medical and veterinary importance such as HIV/ AIDS (targeting opportunistic microbes), colibacilli, mycoplasma, infectious bursal disease (IBD), fungi and ectoparasites.
- Also research should be geared to isolate active chemical component(s) and elucidate their exact mechanism(s) of action, safety margin and efficacy.
- Formulation of products from *Commiphora swynnertonii* such as medicines, cosmetics and food supplements (nutriceuticals) will require collaboration between researchers and the pharmaceutical industry.

- Conservation of *C. swynnertonii* biodiversity should be an important aspect to ensure sustainable availability of the plant. Therefore, agronomical studies should be carried out on propagation and on whether it can be introduced in other geographical areas.

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## APPENDIX 1

**Appendix 1:** Structured questionnaire guarded for villagers, traditional healers and key person in villages on Simanjiro District, Manyara RegionAppendix 1: Biological activity of extracts from *Commiphora swynnertonii* against microbes of veterinary importance in chickens

i. Questionnaire no…….
ii. Study site……
iii. Date of interview……
iv. Name of interviewee……..
v. Language used to interview……

**Section 1: Demographic data**

| No | Question | Coding category |
|---|---|---|
| 1. | How old are you? | |
| 2. | What is the highest level of education you have attended? | 1= none<br>2= primary(std 1-7)<br>3= secondary (std 9-12)<br>4= tertiary(std 12+) |
| 3. | What is your religion? | 1= Christian<br>2= Muslim<br>3= traditional<br>4= others specify |
| 4. | What is your current occupation? | 1= housewife<br>2= farmer<br>3= business<br>4= traditional healer<br>5= others specify |
| 5. | What is your tribe? | 1= Maasai<br>2= Meru<br>3= Chaga<br>4= Mbulu<br>5= Others specify |

**Section 2: Information about *Commiphora Swynnertonii***

| No | Question | Coding category |
|---|---|---|
| 6. | Have you ever heard about *C. swynnertonii?(oltemwai)* | 1= yes<br>2= no |
| 7. | Where did you get the information about *C. swynnertonii?* | 1= traditional healer<br>2= fellow villagers<br>3= relatives<br>4= friends<br>5= parents<br>6= nobody<br>7= others specify |
| 8. | Do you know the use of *Commiphora swynnertonii* in human beings? (can circle more than one response) | |
| 9. | How is it used and part involved | |
| 10. | Do you know the use of *Commiphora swynnertonii* in livestock? (can circle more than one response) | |
| 11. | How is it used and part involved | |
| 12 | Is the plant (*Commiphora swynnertonii*) available here? | 1= yes<br>2= no<br>3= don't know |
| 13. | Where else can the plant be found? (can list more than one response) | |
| 14. | Which part is mostly used ?(circle more than one response) | 1= leaves<br>2= stem<br>3= roots<br>4= resin<br>5= all parts<br>6= none |
| 15. | In which state is it used? | 1= fresh<br>2= dried<br>3= mixed with other plants |
| 16. | In which preparation form is it used? | 1= powder<br>2= juice<br>3= paste<br>4= other specify |
| 17. | How is it used in human beings? | |
| 18. | How is it used in livestocks? | |
| 19. | Is there any undesirable effect when using *Commiphora swynnertonii* as a medicinal plant? (If no/ don't know skip Q18) | 1= yes<br>2= no<br>3= don't know |

| | | |
|---|---|---|
| 20. | Which are those undesirable effects in livestock/human beings? | |
| 21. | Do you sell the plant as medicine? | 1= yes<br>2= no (If no skip Q15) |
| 22. | If yes, how much? In what amount? | |
| 23. | Are there any other medicinal plants in this area? | 1= yes<br>2= no (If yes go to Q24) |
| 24. | Mention the plants | |

**“Thank you for your cooperation”**