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ANTICOCCIDIAL EFFEICACY OF *AZADIRACHTA INDICA* AND *LIPPIA ADOENSIS* METHANOLIC LEAF EXTRACTS IN EXPERIMENTALLY INFECTED BROILER CHICKENS

ADDIS ABABA UNIVERSITY

COLLEGE OF VETRINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF MICROBIOLOGY, PARASITOLOGY AND POULTRY HEALTH

MASTER OF SCIENCE IN VETERINARY PARASITOLOGY

MSc THESIS

BY

EJARA ENSA

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Anticoccidial Efficacy of *Azadirachta Indica* and *Lippia Adoensis* Methanolic Leaf Extracts in Experimentally Infected Broiler Chickens

A thesis submitted to College of Veterinary Medicine and Agriculture, Addis Ababa University, in partial fulfillment of the requirements for the degree master of Veterinary Science in Parasitology

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APPROVAL

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As members of the examining board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by: Ejara Ensa Tola entitled "**Anticoccidial effeicacy of *azadirachta indica* and *lippia adoensis* methanolic leaf extracts in experimentally infected broiler chickens**" and recommend that it be accepted as fulfilling the thesis requirement for the degree of masters of Veterinary science in parasitology.

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Internal examiner

Final approval and acceptance of the contingent up on the submission of its final copy to the ADPG I hereby certify that I have read the revised version of this thesis prepared under my direction and recommended that its accepted as the fulfilling the thesis/dissertation requirements.

STATEMENT OF AUTHOUR

I comfirm that the research titled "**Anticoccidial effeicacy of *azadirachta indica* and *lippia adoensis* methanolic leaf extracts in experimentally infected broiler chickens"** is entirely my own work, and I have appropriately acknowledged all sources used in this study. This research is being submitted as the part of requirements for an advanced degree of master of Veterinary science at Addis Ababa University, College of Veterinary Medicine and Agriculture and I will made available in the University's library borrowing according to library rules. I affirm that this research has not been submitted for the purpose of obtaining any academic degree, diploma or certificate from any other institution.

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Date of submission ____________________

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LIST OF ABBREVIATIONS

ACI	Anticoccidial Index
LS	Lesion Score
OPG	Oocysts shed per gram of feces
P.I	Post-infection
POAA	Percent Optimum Anticoccidial Activity
RLS	Reduction of Lesion Score
ROP	Reduction of Oocysts Production
InNT:	Infected but was not receive any treatment
UiUT:	Uninfected and untreated negative control

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ABSTRACT

Coccidiosis is a persistently prevalent parasitic disease in the poultry industry. Currently a range of anticoccidial drugs are employed to prevent and treat the disease. However, their diminishing effectiveness has raised serious concern compelling researchers to exploring for safer, plant-based alternatives. This study has evaluated anticoccidial effect of methanolic leaf extracts of *Azadirachta indica* and *Lippia adoensis* in experimentally infected broiler chickens. Toxicity assessments indicated that both plant extracts were safe for use in poultry, with no adverse effects or mortality observed during trials. A randomized controlled trial was conducted using 105 one day old cobb500 broilers chickens. The chickens were divided into seven groups (15 per pen), each group with three replicates. On the 19^{th} days of age all chickens except those in UiUi (negative control) were orally challenged with 1x 10^5 sporulated Eimeria Oocysts. Treatment commenced on 4^{th} PI. Lip300 and Lip600 were infected and treated with *L. adoensis* at 300 mg/kg and 600 mg/kg respectively, Azd300 and Azd600 were infected and treated with A. *indica* at 300 mg/kg and 600 mg/kg respectively. Koxd was infected and treated with koksidex at1g/L of water, InNT was infected untreated, while UiUT served as non-infected & non-treated. Clinical observations, mortality, oocyst shedding, weight gain, and lesion scores were recorded throughout the study period. The findings revealed that lower oocyst output, higher weight gain, fewer clinical symptoms, and better survival rates were observed in herbal treated group compared to InNT group. Moreover, *A. indica*, especially at 600mg/kg dose, exhibited the most significant improvements across all parameters. Using these parameters efficacy status of herbal extracts was also determined based on anticoccidial index, reduction of oocyst productions, reduction in lesion score and percent optimum anticoccidial activity. All indices showed that *A. indica* was effective against Eimeria infections, standing out as promising candidate for future drug development. Further study is recommended to optimize route of administration and extract concentrations.

Keyword: *Anticoccidial index, Azadirachta indica, Coccidiosis, Lippia adoensis*

1. INTRODUCTION

Poultry farming plays a crucial role in fulfilling global nutrition requirements in terms of meat and eggs (Mohsin *et al*., 2021). Poultry are the primary source of valuable animal protein around the world (Govoni *et al*., 2021). Chicken meat provides not only high-quality protein, but also essential vitamins and minerals (Yousaf, 2018). Additionally, eggs provide an excellent amino acid profile, a good source of energy, essential fatty acids and high levels of many vitamins and minerals (Bohrer, 2017). Demand for poultry meat has increased substantially over the past several decades because of agrowing population, and greater per-capita consumption (Korver, 2023). Nevertheless, the economic efficiency of poultry production is challenged with various constraints that may undermine poultry health and thus meat production, leading to economic loss. One of the most prominent challenges to the poultry industry is coccidiosis, which accounts for approximately $13 billion in global losses (Blake *et al*., 2020).

Poultry coccidiosis is an economically devastating disease because it causes high morbidity and mortality rates in both young and adult birds. The Eimeria spp. parasites invade the intestinal cells of the host at different sites damaging the intestinal walls leading to economic loss due to poor nutrient absorption, reduced weight gain, bloody diarrhea, poor feed conversion ratio, and dehydration (Pawestri *et al*., 2020. In addition to seriously harming epithelial cells, Eimeria infection significantly disrupts the gut microbial communities in the gastrointestinal tract (GIT), leading to the colonization and expansion of other pathogens. This increases the susceptibility of infected chickens to secondary infections, ultimately resulting in high mortality rates (Macdonald *et al*., 2019).

Different strategies are currently employed to control the disease, such as improving management and hygiene, using anticoccidial products, and applying various types of anticoccidial vaccines. Of these measure strategies, anticoccidial drugs (chemotherapy) are commonly used to prevent and treat coccidiosis (Soutter *et al.,* 2020). The effective use of anticoccidial drugs over the past five decades has significantly accelerated the growth of the poultry industry, improving the availability of high-quality, affordable poultry products to consumers. However, indiscriminate and long-time use of these drugs has led to the emergence of drug resistance and adverse side effects (Zaheer *et al*., 2022). Besides the resistance, there is also a problem of the potential presence of drug residues in poultry meat and other products, being sought alternative methods in many countries for safer control methods which reduce dependence on synthetic chemical products (Sundar *et al.*, 2017).

Among alternative approaches, the uses of herbal extracts are the potential candidates for control of avian coccidiosis. Herbal extracts are an alternative and safe anticoccidial medication because they do not leave tissue residue or produce drug resistance (Ghafouri *et al.*, 2023). A number of studies have explored the potential of herbs and plant extracts as alternative treatments for poultry coccidiosis. *Artemisia annua*, *Origanum vulgare* (Oregano), *Allium sativum* (Garlic), *Azadirachta indica* (Neem), *Curcuma longa* (Turmeric) and different species of *Aloe* (Muthamilselvan *et al*., 2016). Extracts of these plants' species may have natural compounds containing metabolites capable of inhibiting the life cycles of various Eimeria species (Cobaxin-Cárdenas, 2018). Some are known to have antimicrobial, antioxidant, and anti-inflammatory properties, effectively mitigating the severity of coccidiosis in poultry (Adaszyńska-Skwirzyńska and Szczerbińska, 2017).

1.1. Statement of the Problem

Despite the rapidly growing poultry industry the significant economic burdens posed by poultry coccidiosis and the growing reports of anticoccidial drug resistance, the search for alternative control strategies including herbal medications remains limited in Ethiopia. *Azadirachta indica* (Neem) and *Lippia adoensis* (commonly knowm as Koseret) are two medicinal plants that are abundantly available across Ethiopia. These plants has been long been used in traditional medicine and are recognized for their broad therapeutic properties. Azadirachta indica in particular, is considered one of the most promising herbal candidates for promoting growth and health in poultry. It is said to possess a wide range of pharmacological properties, including antifungal, anti-helminthic, anti-inflammatory, immune stimulant and antibacterial properties (Alzohairy, 2016).

Similarly, *Lippia adoensis* (syn. *Lippia* a*byssinica*), known locally as Koseret, is widely used in Ethiopian tradictional medicine and cusine. Leaf oil extracts of *Lippia adoensis* were reported to have potent antimalarial, antibacterial and antifungal properties (Pascuala *et al*., 2001; Shiferaw *et al*., 2023). However, while some preliminary investigation suggests that *Lippia adoensis* may hold promise for veterinary appilications, no published studies to date have specifically evaluated its anticoccidial efficacy in poultry, particularly with in the study region. Similarly, leaf extracts of *Azadirachta indica* are known for their antimicrobial, antimalarial and anti-inflammatory activities (Subapriya and Nagini, 2005). However, except very few studies (Islas *et al*., 2020) investigation on their anticoccidial properties has never been given adequate attention. The currents knowledge gaps highlight the need for more scientific investigations to assess the effectiveness and safety of these plant-based treatments as alternative to conventional anticoccidial drugs in Ethiopia. Therefore, this study was carried out with the following objectives:

General objective

The main objective of this study is to evaluate and compare the efficacy of extracts from two plant species (*Azadirachta indica* and *Lippia adoensis*) for the treatment of experimentally induced coccidiosis in broilers.

Specific objective

- To evaluate anticoccidial effect of *Azadirachta indica and and Lippia adoensis* extract treatment in experimentally infected broiler chickens.
- To compare the relative efficacy of extracts of the two-plant species based on Relative Oocyst Production (ROP), Percent Optimum Anticoccidial Activity (POAA), Anticoccidial Index (ACI) and Reduction of Lesion Score (RLS).

2. LITERATURE REVIEW

2.1. Causative Agents of Coccidiosis

Coccidiosis is an infectious protozoan disease, caused by Eimeria, in the intestinal epithelium of chickens with high morbidity and mortality. This disease is one of the most significant challenges facing the development of poultry farming (Mohsin *et al*., 2021; Jamil *et al*., 2022). The infection impairs intestinal epithelial cells and tissues, destabilizing the gut of chickens (Madlala *et al*., 2021). Nine Eimeria species, *E. acervulina, E. brunetti, E. maxima, E. necatrix, E. praecox, E. mitis, E. tenella, E. mivati, and E. hagani*, have been identified from chickens. *Eimeria brunette, Eimeriamaxima, Eimeria necatrix, Eimeria tenella* are the most pathogenic (Abudabos *et al*., 2017). The pathophysiology of Eimeria spp. infection varies, however *Eimeria tenella, Eimeria necatrix*, and *Eimeria brunetti* are more hazardous and cause serious disease outbreaks in poultry. *Eimeria acervulina, E. maxima*, and *E. tenella* are the most economically significant for broiler chickens (Mnisi *et al*., 2022).

Table 1: Poultry coccidiosis and its predilection site

Eimeria species	Predilection Site
E. tenella	Caecum
E. praecox	Anterior gut
E. mitis	Anterior gut
E. necatrix	Mid gut
E. maxima	Mid gut
E. acervulina	Duodenal loop
E. brunette	Lower intestine
E. mivati	Duodenal
E. hagani	Anterior gut

Source: (Quiroz-Castañeda and Dantán-González, 2015).

2.2. Epidemiology

Poultry coccidiosis is distributed worldwide, where ever poultry are kept (Biratu, 2021) in (table 2). In Ethiopia despite the immense research works done by several scholars in the area of poultry coccidiosis in different parts of the country, the disease is still continued being a major problem demanding much research and investigation (Gari *et al.*, 2008). The prevalence of poultry coccidiosis has been documented in different parts of Ethiopia in different years. Variations in prevalence may be attributed to differences in study time, breed, management systems, and the possibility of drug resistance (Negash *et al*., 2015).

The occurrence of the disease depends on both the species of Eimeria and the size of the infecting dose of oocysts. Poultry coccidia exhibit a significant capacity for reproduction within the host, resulting in a rapid increase in the parasite population within susceptible hosts and subsequently high levels of environmental contamination (Abdisa *et al*., 2019). Several environmental and management factors have been associated with the presence and severity of coccidiosis. These include bird age, flock size, geographical location, litter depth (Singh *et al*., 2021), poor sanitation conditions, and inadequate environmental management (Van-Limbergen *et al*., 2020).

Table 2: Prevalence of Eimeria in domestic chicken

Author	**Country**	**Sample size**	**Infected**
Pajic *et al*. (2023)	Serbia	100	59
Adem *et al*. (2023)	Ethiopia	450	122
Carrisosa *et al*. (2021)	United States	80	50
Flores *et al*. (2022)	South Korea	388	291
Jan *et al.* (2022)	India	780	293
Nan-Mariam *et al*. (2023)	Nigeria	204	74
Montes-Vergara *et al*. (2021)	South America	535	362
Györke *et al*. (2013)	Romania	23	21
Dinka and Tolossa (2012)	Ethiopia	300	215

2.3. Life Cycle and Transmission of Chicken Coccidiosis

Protozoan of genus Eimeria are highly host specific and site specific. Eimeria spp. Have a direct life cycle that not involves vectors or carries for their transmission (Abbas *et al.*, 2023). The infection can be potentially transmitted through several routes including direct bird to bird contact, clothing and foot wear, as well as wild birds that come in to contacts with poultry facilities, breeding equipment and non-sterilized contaminated feed bags (Serbessa *et al.*, 2023). This life cycle includes stages of asexual and sexual multiplication and consists of three developmental phases: shizogony, gametogony and sporogony (McDougald *et al.,* 2020). The life cycle of this parasite is intricate and starts when sporulated oocysts from litter are inadvertently consumed. Then its progresses to the daily excrera of millions of non sporulated oocysts from infected hens. Sporocysts enter the host organism, breaking free and infiltrating intestinal mucosal epithelial cells, where they undergo schizogony and gametogony (Blake and Tomley, 2014). Numerous oocysts are produced at the end of life cycle, which expelled with the chicken's faeces and sporulated in the surrounding environments (Nahed *et al.,* 2022).

2.4. Economic Importance of Poultry Coccidiosis

Coccidiosis is among poultry`s most important parasitic disease, causing heavy economic loses to this sector (Mesa-Pineda *et al*., 2021). Large numbers of resources are spent on treatment, prevention, prophylactic drug and vaccine can diminish the profitability of poultry farms (Blake *et al*., 2020). Poultry mortality due to disease is estimated to be between 20 and 50%, but it can be as high as 80% during epidemic seasons (Engidaw and Getachew, 2018). Among these diseases, coccidiosis is recognized as the major parasitic infection with the greatest economic impact on poultry industries worldwide (Allen and Fetter, 2002).

The disease affects chickens, with infection rates reaching 5% and 20% for clinical and sub-clinical cases, respectively. Economic losses from coccidiosis arise not only from clinical outbreaks that cause low production and high mortality rates but also from subclinical coccidiosis, which may not present visible clinical signs. Subclinical coccidiosis reduces nutrient absorption, feed conversion, performance and vulnerability of secondary disease (Teng *et al*., 2021). The disease poses substantial threat to food security, birds' welfare and global economy. Therefore, effective managing and preventing this disease is essential to ensure sustainable poultry production and to meet the growing demand for inclusion of poultry products in human diets (Adhikari *et al*., 2020).

2.5. Methods used to Control Avian Coccidiosis

2.5.1. Control of coccidiosis in chickens with anticoccidial agents

Chemotherapeutic (anticoccidial) drugs are commonly used as prophylactic and therapeutic drugs effectively in commercial farms (Noack *et al*., 2019). They are target different stages of the Eimeria life cycle, aiming to arrest the parasite at that stage and ultimately control of coccidiosis (Adams, 2021). Based on their mode of action, anticoccidial agents are classified as coccidiostats or coccidicides. Coccidiostats inhibit the development of parasites, limiting their replication and growth; however, their effects can be reversible, as removing them from the diet may allow the disease to re-emerge. In contrast, coccidicides act by destroying or imposing irreparable damage to the parasites (Nahed *et al.*, 2022). For a long time, the coccidiosis disease in chicken was successfully controlled by synthetic medications such Amprolium, Nicarbazin, Diclazuril, Clopidol, Salinomycin, and Toltrazuril. The medications specifically target the parasite's sporozoite/merozoite growth stage (Fatoba and Adeleke, 2018). However, in the current era, multiple drugs have been restricted from being used because of their public health issues or reports of resistance (Martins *et al*., 2022).

2.5.2. Control of coccidiosis in chickens by vaccination

As the challenges of drug resistance in Eimeria and drug residues in the animal-derived products intensify (Attree *et al*., 2021), anticoccidial vaccines have become essential tools in the control of coccidiosis. Vaccination is an effective and successful method against coccidiosis because Vaccines offer a high level of protection against coccidiosis and replace the populations of Eimeria, thereby reducing the issue of drug resistance in chickens (Akanbi and Taiwo, 2020). The primary goal of vaccines is to expose the birds to *Eimeria* species to elicit protective immunity against them, which is adequate to prevent future infections (Chapman and Blake, 2022). Although vaccination against several *Eimeria* spp. was very promising, but side effects such as post-vaccination mild infection and reduction in weight gain and feed conversion are discouraging this practice.

Additionally, the high cost of anticoccidial vaccines limits their widespread adoption, leading to increased interest in various nutraceutical and phytochemical remedies with anticoccidial properties (McDougald *et al*., 2020).

2.5.3. Natural alternative to controlling of coccidiosis

Residual effects and increased resistance are associated with conventional anticoccidials. Although development of vaccines against poultry coccidiosis has benefited technology and immunology, but they still need to meet farmers' expectations as effective, safe, and economical (Kim and Lillehoj, 2019). Therefore, safe and effective anticoccidial alternative drugs are needed. Natural treatments, such as prebiotics, probiotics, plant and fungal extracts, and essential oils, serve as examples of these alternative therapeutic options (Abd El-Hack *et al*., 2022). Among these natural treatments, the use of herbal medicines for controlling poultry coccidiosis has the potential to offer several benefits over chemical agents, as they are generally considered safe and have a lower risk of developing antiparasitic resistance compared to chemical agents (Cheng *et al*., 2022). Furthermore, using herbal medicines can help reduce the environmental impact of animal production by reducing chemical residues in animal products (Withers *et* al., 2015). They are also are less toxic and considered ideal feed additives in food animal production (Puvača *et al*., 2018).

Azadirachta indica is a tree commonly known as neem which fast-growing tree *belongs to the family* Meliaceae (Girish and Shankara, 2008), valued as a medicinal plant, as a source of organic pesticides, and for its timber. Neem is likely native to the Indian subcontinent and to dry areas throughout South Asia. It has been introduced to parts of Africa. Neem trees can reach 15–30 metres in height and have attractive rounded crowns and thick furrowed bark. The compound leaves have toothed leaflets and are typically evergreen but do drop during periods of extreme drought. *A. indica* is a medicinal tree rich in phytochemicals such as tannins, alkaloids, flavonoids, terpenoids, reducing sugars, glycosides, tannins, cyclic trisulphide, unsaturated sterols, and saponins (Susmitha *et al*., 2013). These active compounds in neem are responsible for its anti-coccidial properties by inhibiting the growth of Eimeria parasites in chickens (Onyiche *et al*., 2021). Neem can also serve as an immune booster and support gut health, which is crucial in preventing poultry diseases, including coccidiosis (Dkhil *et al*., 2013).

Lippia adoensis Hochst also known as *L. multiflora* is a herbaceous plant belonging to the family Verbanaceae (WCSP, 2017). Traditionally, the dried leaves of L. adoensis were used in Ethiopian society as a constituent in the preparation of spiced butter, preservation of cow butter, and food flavoring. The plant possesses white sweet-scented flowers stalked on cone-like heads in a terminal panicle nearly 120 mm long. Lippia species in Ethiopia occurs as an erect woody shrub which grows up to 1-3m tall. It is endemic medicinal plant and cultivated variety commonly found in home gardens in different regions of Ethiopia with altitudinal range of 1600-2200m. Two varieties are recognized in Ethiopia, the wild variety (var. adoensis) and the cultivated variety locally known as koseret (var. koseret sebsebe). L. adoensis var. koseret sebsebe widely grown in the central and southern highlands of Ethiopia (Godeto *et al*., 2023). The plant has been used in traditional medicine to treat several diseases. Additionally, extracts from the plant have shown potential as a fumigant against several insect pests that affect cultivated crops (Kanko *et al*., 2004).

3. MATERIALS AND METHODS

3.1. Description of Experimental Site

The study was conducted at College of Veterinary Medicine and Agriculture of the Addis Ababa University located in Bishoftu town (figure1). Bishoftu town is located at an altitude of 1850 meters above sea level in the central part of Ethiopia (ADARDO, 2007). The annual average temperatures range from 12.3°C to 27.7°C, with an overall average of 18.7°C. The highest temperatures are typically recorded in May (CSA, 2001). The room temperature in the experimental unit was however, adjusted to 25 to 33ºC to suit the needs of the chicken which changes with their age. Similarly, relative humidity was regulated at a maximum of 61.3%.

Figure 1: Geographical location of experimental site
Source: (Abunna *et al*., 2018).

3.2. Plant Collection and Identification

Fresh leaves of *Lippia adoenensis* (koseret) and Leaves of *Azadirachta indica* (neem) tree were harvested from middle-aged green trees grown in and around Bishoftu. The plants were gathered from November to December 2024/2025, during the active growing season when the leaves were green and tender. Plant identification and authentication were conducted at the National Herbarium of Addis Ababa University and deposited with voucher specimen ID: EE001 for *Lippia adoensis* and EE003 for *Azadirachta indica*. The harvested leaves were transported to the laboratory in clean plastic bags, washed under running tap water to remove dust particles and rinse with distilled water. Then the samples were placed on clean plastic plates and air-dried at room temperature until a constant weight was obtained to ensure that they were fully dried. Finally, the dried leaves were pulverized using a blender, and a 2mm mesh diameter sieve was employed to obtain fine dust. This dust was stored in an airtight container until it was used for solvent extraction.

3.3. Herbal Crude Extraction

Herbal crude extractions were conducted in the laboratory of the Department of Biomedical Science (CVMA, Addis Ababa University). Methanol extracts were prepared by soaking 200g of each powdered plant material in a conical flask to which 1 liter of 70% methanol solvent is added. The mixture was allowed to macerate for 72hr at room temperature with frequent agitation after which it was strained using a muslin cloth and filtered using a Whatman No.1 filter paper. The filtrate was collected in a flask and later concentrentrated using rotary evaporator (Rota vapor: BUCHI R-200, Labor-technik, Switzerland) at 60^{o}C under reduced pressure (700mmHg) and rotation speed of 20rpm. The extract was then dried on a petridish in an incubator at 40°C, scraped off of the petridish, weighted and kept in labeled bottles at 4^{o}C until used (Tabassam *et al*., 2008).

3.4. Herbal Product Acute Toxicity Test in Chicken

Acute toxicity test study was adapted from OECD (2022) guideline for acute oral toxicity determination of chemicals in mice. Fifteen healthy broiler chickens, aged 21 days old chickens were randomly divided in to three groups, with five chickens in each group. Prior to administration, all birds were fasted for two hours. The first groups received distilled water and served as a negative control. The second and third groups were orally administered 2000mg/kg body weight of *Azadirachta indica* and *Lippia adoensis* leaf extract respectively. Following administrations, the chicken were closely observed for first four hours for any signs of toxicity, such as behavioral changes, drooling, lethargy, or mortality. Daily monitoring was continued for three consecutive days to evaluate the safety and possible adverse effect of the plant extracts.

3.5. Isolation, Sporulation and Propagation of Eimeria Oocysts

Fresh litter samples were collected from commercial farms confirmed positive for coccidiosis in Bishoftu town. Oocysts were purified from the faeces following a modified protocol described by (Eckert *et al*., 1995). Briefly, the faecal samples were transferred to a 1 Littre beaker, two volumes of tap water were added and the material was homogenized. The homogenate was then filtered through a 250- μm-pore size sieve over double layer of gauze and the filtrate transferred into 1liter of cylinder and allowed to sediment overnight. On the next day, the supernatant was discarded and the sediment resuspended in saturated saline solution followed by centrifugation of the resuspension at 1300×g for 10 min. Suspended oocysts were collected and washed three times with tap water by centrifugation at 1300×g for 10 min.

After washing the sediment containing the oocysts were collected, 2% (w/v) $K_2Cr_2O_7$ solution was added and allowed to sporulate at room temperature on a petridish for seven days with regular stirring. The sporulated oocysts were orally inoculated to five chickens for oocyst multiplication. The chickens were monitored daily for clinical signs and for the presence of Eimeria oocysts in the feces. Once the chickens developed patent infection, litter samples were collected, processed as described before and sporulated oocysts were collected. Then, 2% $K_2Cr_2O_7$ solution was added and the materials were refrigerated at 4 ºC until further use for the actual experiment. The $K_2Cr_2O_7$ solution was removed through centrifugation followed by washing and the sporulated Eimeria oocysts were suspended in distilled water at the time of oral administration.

3.6. Study Animals and Managements

A total of 105 one-day-old Cobb 500 unsexed broiler chickens, weighing between 47 and 50.9 grams, were procured from Alema Commercial Hatchery for use in this experimental study. Before the arrival of the chicken, the poultry house was thoroughly cleaned and disinfected using hydrogen peroxide. Environmental conditions, including temperature, humidity, and lighting were managed in accordance with the Cobb 500 Management Guide (Cobb Germany, 2012) to provide optimal brooding condition. Upon arrival, all chickens were housed together and reared under uniform conditions until 18 days of age. During the experimental period, they were fed with a commercial coccidiostat free starter feed followed by grower and then finisher feed formulas according to their body weight. Clean potable water was available ad libitum throughout the experiment. The birds were maintained under strict biosecurity measure including, the use of footbath at entry points and mandatory personnel protective clothing. In line with standard poultry healthy management practices, all chicks were vaccinated against Newcastle disease according to recommended vaccination schedule.

3.7. Experimental Design, Infection and Treatment

A randomized control trial was designed involving seven groups: four treatment groups, one negative control group, and two positive control groups (one for infection only and one for treatment with a commercial drug). On day 18, the chicken were first tagged by the use of leg ring and randomly assigned to groups by a lottery method. Each group consisted of 15 birds per pen with three replicates of five chickens (Table 3). All the birds were provided with similar management conditions like floor space, temperature, ventilation and lighting. This ensured that environmental factors did not influence the outcomes. The study aimed to evaluate the treatment efficacy under controlled conditions.

Table 3: Experimental groups

Experimental groups	Treatments
Lip300**:** Infected and treated with *Lippia adoensis* (koseret) at 300mg/kg body wt.	
Lip600**:** Infected and treated with *Lippia adoensis* at 600mg/kg body wt.	
Azd300**:** Infected and treated with *Azadirachta indica* (neem) at 300mg/kg body wt.	
Azd600: Infected and treated with *Azadirachta indica* at 600mg/kg body wt.	
Koxd: Infected and then treated with Koksidex (Amprolium +Sulfaquinoxaline +vitamin K3) at 1gm/liter of water (Koxd)	
InNT: Infected but was not receive any treatment (positive control)	
UiUT**:** Uninfected and untreated negative control	

At 19 days of age all chickens except those in group UiUT control were orally challenged with 1x 10^5 sporulated mixed Eimeria oocysts diluted in 1 ml of distilled water using a syringe without a needle, following the protocol used in previous study by (Rajput *et al*., 2014). Once infected chickens started showing clinical signs and oocysts were detected in the litter samples, the treatment regimen was commenced. Herbal treatments were administered to the chicken in groups Lip300, Lip600, Azd300 and Azd600 at the dose of (300mg/kg and 600mg/kg) body weight orally with 1ml of distilled water. Group Koxd received the commercial drug Koksidex (Amprolium+Sulfaquinoxaline +vitamin K3 (powder) at1g/L of water (Medion, Bandug-Indonesia). Treatments were given for the first three consecutive days, then two days interruption followed by another 3-day treatment. Fecal examination, body weight measurement, clinical observations, mortality and postmortem lesion scoring were recorded.

3.8. Herbal Extracts Efficacy Evaluation

The efficacy of the herbal extracts in experimental coccidiosis in broiler chickens were evaluated using several parameters which includes oocysts count per gram of feces (OPG), body weight measurement, postmortem lesion scoring (LS) survival rate, anticoccidial index (ACI), reduction of oocyst productions (ROP), reduction in lesion score (RLS) and percent optimum anticoccidial activtity (POAA). Daily observations were made to monitor clinical signs and mortality following infection. Data collected from treated groups were systematically compared with those from the control groups to assess efficacy.

3.8.1. General clinical examination

During the experimental period, the birds in all groups went through daily monitoring for clinical sign, and the symptoms were recorded. Signs such as depression, weakness, bloody diarrhea, reduction in feed intake, mortality etc. were recorded.

3.8.2. Quantification of oocysts per gram of feces

Pooled fecal materials were collected on the day of initial treatment (Day 0PT), One day post treatment (Day 1PT), three day post treatment (Day 3PT), five day post treatment (Day 5PT) and eight day post treatment (Day 8PT). The oocysts counting were done through Mc Master Techniques as described (Pajić *et al*., 2018). On this matter, 3g of feces was mixed with 42ml of water and shaken vigorously to obtain a uniform mixture. Then 15 ml of this mixture is centrifuged at a speed of 2000 rpm for 10 min, and in the next step, the supernatant is discarded while saturated salt solution was added to the formed sediment to bring the volume to 15 ml. In the next step, the chambers of the McMaster slide were filled with the sample and allowed to stand undisturbed for 5 min so that the oocysts came into the same focus level. The oocysts are counted with a 10 × magnification of the compound microscope, and expressed as OPG in that sample.

3.8.3. Body weight measurement

Chickens were weighed individually to the nearest gram on day of oocysts drenching (Day 0PI) and on the day of initial treatment (Day 0PT). Additional body weight measurements was conducted one day post treatment (Day 1PT), three day post treatment (Day 3PT), five day post treatment (Day 5PT) and eight day post treatment (Day 8PT) of days to evaluate the effect of treatment on this parameter. In each group, the average weight for each pen was recorded. The values were used for evaluation of anticoccidial efficacy of the herbal extracts based on efficacy indices described previously (Lan *et al.*, 2017) as follows:

Relative rate of body weight gain (RRBWG) = $\frac{\text{\% change in BWt of treatment group}}{\text{\% change in BWt of UiUi group}}$

Growth and survival rate (GSR) = $\frac{\text{Final BWt on day (8PT)}}{\text{Initial BWt on day (0PT)}}$

3.8.4. Determination of chicken survival rate

Mortality was recorded starting from the initial day of treatments up until the end of the treatments.

Data was used to calculate chicken survival rate as follows (Ojimelukwe *et al*., 2018):

Survival rate = $\frac{\text{Number survived at day (8PT) x100}}{\text{Number survived at day (0PT)}}$

3.8.5. Lesion score

Two chickens per replicate were randomly selected and humanely euthanized by cervical dislocation on the 8th day post-infection (pi). Lesions scores (LS) was conducted by employing the grading system described by Johnson and Reid (1970). The intestines were carefully incised and examined for gross lesions (wall thickening , mucosal sloughing, haemorrhages, discolorations) and changes in the intestinal content (mucoid accumulations, bloody content, fecal consistency), According to the severity of lesions, lesions was graded from 0 to 4.

3.8.6. Extract efficacy estimation

Using the above measurments, efficacy status of the herbal products was determined based on four major indices: on anticoccidial index (ACI), reduction of oocyst productions (ROP), reduction in lesion score (RLS) and percent optimum anticoccidial activtity (POAA) as clearly described by Lan *et al.* (2017).

i. ROP = $\frac{\text{OPG of InTR x100}}{\text{OPG of InNT}}$ where InTR= infected and treated, InNT= infected nontreated

ROP <15% = the product is effective

ii. ACI = (RRBWG + SR) – (LS + ROP), where RRBWG: Rate of relative body weight gain, SR: Survival rate, LS: Lesion score,

ROP: Reduction Oocyst Production,

- ACI ≥ 160 indicates = the product is effective

iii. POAA = $\frac{\text{GSR of InTR groug-GSR of InNT x100}}{\text{GSR of NiNT – GSR in InNT}}$

Where NiNT= non-infected, non-treated, GSR: Growth and survival rate

- POAA > 50% = shows product effectiveness

iv. RLS = $\frac{\text{Mean LS of InNT- Mean LS of Tre group x100}}{\text{Mean LS of InNT}}$

Where LS: Lesion Score

- RLS ≥ 50= implies product efficacy

3.9. Ethical Review

All procedures involving animals were approved by ethical review committee of institutional animal care and use committee (IACUC) of the College of Veterinary Medicine and Agriculture. This work is part of a bigger thematic research project entitled integrated management of poultry coccidiosis which has an ethical review certificate (Ref. No.VM/ERC/03/31/16/2024) granted from the research ethics review board of the College of Veterinary Medicine and Agriculture.

3.10. Statistical Analysis

The data was entered and stored into a Microsoft Excel spreadsheet then transferred into Software R4.3.1. Evaluation of the different groups were made by using one-way analysis of variance (ANOVA) and multiple comparison was made by Tukey's test. The difference group means between treatment and control groups were considered significant at ($P < 0.05$). Comparation of herbal efficacy index like anticoccidial index, reduction of oocyst productions, reduction in lesion score and percent optimum anticoccidial activtity made by using descriptive statistics.

4. RESULTS

4.1. Herbal Product Acute Toxicity Test in Chicken

Acute toxicity test of *A. indica* and *L. adoenensis* conducted on chickens revealed that the products pose no significant health risks to the chickens. The administration of 2000mg/kg of body weight of each plant extracts did not results in any mortality or observable acute toxic effect during the three days of observation.

4.2. General Clinical Examination

The chickens were perfectly healthy on the day of infection with sporulated oocysts. Four days after challenge however, infected chicken started to show signs of clinical coccidiosis and oocysts were detected in their feces. Clinical signs such as depression, weakness, bloody diarrhea and ruffled feathers were also seen in these infected groups of chickens (figure 2). Decrease in food consumption was observed among the infected birds, prompting the initiation of treatment in all treatment groups according to the scheduled protocols, except for the negative control group (UiUT). After starting treatments, these clinical signs become decreased in treated groups and manifestations of symptoms of coccidiosis become milder as treatment progressed were as it continued in the infected untreated group (InNT). Bloody diarrhea and other signs were less severe in *A. indica* treated groups than *L. adoensis* treated groups. Overall, *A. indica* appeared more effective in alleviating the severity of coccidiosis.

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Figure 2: Clinical sign observed in infected groups of chickens

A. Bloody diarrhea B. Depression of chicken with ruffled feathers

4.3. Body Weight Measurement

Chickens in all groups were weighted to the nearest grams (g) using weighting balance. Weight measurements were taken at six different time periods: on the day of infection (0PI), on the day of first treatment (0PT), one day post treatment (1PT), three day post treatment (3PT), five day post treatment (5PT) and eight day post treatment (8PT). The mean body weight showed no differences among groups on day of infection (0PI). On 4th day post-infection, at the starts of first treatment, the mean body weight remained similar across groups. Although the negative control group tended to have higher values, the difference among the groups was not significant (P>0.05). On 5th day post-infection (1PT) significantly increased body weight score was registered for the UiUT control groups (Table 4).

On the 3rd days post treatment (3PT), the body weight gain of all treated groups showed a significant improvement ($p < 0.05$) when compared to the InNT control group. Of the birds treated with herbal extracts, group Azd600 (Treated with *A. indica* 600 mg/kg) has shown the highest body weight gain among the herbal treatment groups which was comparable to that of group Koxd (treated with koksidex). On the 5th and 8th days post-treatment, the treated groups showed a significantly higher body weight gain compared to the non-treated positive control group.

Table 4: Body weight gain in (g) of broilers chicken with experimental coccidiosis

Groups	Days					
	0PI	0PT	1PT	3PT	5PT	8PT
Lip300	370.93 ± 22.4	429.87±22.5	433±29.7^{b}	465.67±39.1^{dc}	514.67±20.6^{d}	592.83±24.3^{d}
Lip600	370.33 ± 22.6	429.86±30.4	436.27±31.5^{b}	482.25±32.8 ^{c}	544.8±19.46^{cd}	627.8±34.69^{cd}
Azd300	370.53 ±26.78	430.53±33.3	437±29.74^{b}	503±21.79^{bc}	582.7± 23.18^{bc}	644.43±17.2^{c}
Azd600	369.87 ± 25.17	430.2 ±23.56	439.3±30.37^{b}	523.57±28 ^{b}	614.1±33.5^{b}	701.6±17.3^{b}
Koxd	370.2 ±17.59	430 ± 20.1	440.87±27.7^{b}	533.73±26.3^{b}	622.5±18.5^{b}	717±16.5^{b}
InNT	370.4 ±22.36	430.33 ±19.6	428.66 ±27.58^{b}	407±20.8^{e}	419.2± 51.36^{e}	471.66±14.9 ^{e}
UiUT	370.47 ±18.38	455.06±34.98	494.73±19.3^{a}	617.6±45.56^{a}	705.7±29.2^{a}	822.67±18.8^{a}

Values are represented as mean ± SD. Mean with different superscript letter compared to infected – untreated control in the same column are significantly different ($p<0.05$). **Key:** PT: post treatment, PI: post infection.

4.4. Quantification of Oocysts per gram of Feces

The UiUT control group showed no fecal oocyst throughout the study period. Following oocyst gavage on their 19th days of age, Eimeria oocysts began to appear in the feces of infected groups on the 4th day post infection. To visualize the trend of oocyst production, oocyst count data were registered over five sampling stages: on the day of first treatment (0PT), One day post treatment (1PT), three day post treatment (3PT), five day post treatment (5PT) and eight day post treatment (8PT) as showed in (Table 5). Accordingly, oocysts count in treated groups was significantly lower than counts in the InNT group ($P<0.05$). On the other hand, 600mg of *Azadirachta indica* (Azd600) had reduced oocyst counts to the level achieved by the commercial drug Koksidex (Koxd group) 8 days post treatment. When the effect of the two herbal extracts on oocysts count are compared, at both treatment doses, *A. indica* has shown significant reduction compared to those treated with *Lippia adoensis (P<0.05)* at the end of the experiment.

Table 5: Fecal oocyst count in broilers with experimental coccidiosis

Groups	Counting days (PT)				
	0PT	1PT	3PT	5PT	8PT
Lip300	39000±300	92766.6±57.7	68233.33±513.1[a]	48266.67±58[a]	4200±100[a]
Lip600	39033.3±208.2	92533.3±57.7	66666.67±251.6[a]	47700±100[a]	3933.3±152.7[a]
Azd300	39000±300	92100 ±100	60800±854.40[b]	40700 ±173.2[b]	2300 ±100[b]
Azd600	39033.33±208.2	91266.67±152	58800±854.4[bc]	40166.6±57[bc]	2066.67±57[bc]
Koxd	39033.3±208.16	91000 ±100	57333.3±709.5[c]	39666.7±58[c]	1966.7±57.7[c]
InNT	39000 ±200	93533.3±57	298800±1907.8[e]	198300±608.3[d]	51000 ±200[d]
UiUT	0 ±0	0 ± 0	0 ± 0 [f]	0 ± 0 [e]	0 ± 0[e]

Values are represented as mean ± SD. Mean with different superscript letter compared to infected untreated control in the same column are significantly different (p<0.05). PT: post treatment. Superscripts with different letters show that values are statistically different

4.5. Intestinal Lesion Score

Gross intestinal lesions were absent in the negative control, while variable degrees of damages were observed in all oocyst-challenged groups. The severity of lesion scores was highest in the InNT control group (LS=3.75), indicating high infection rates compared to those in the treated groups. Among the herbal-treated groups, the lesion scores were better in the *A. indica* -treated groups (Azd300 and Azd600) than in the *L. adoensis* -treated groups (Figure 3). High score lesions were observed always from the cecum.

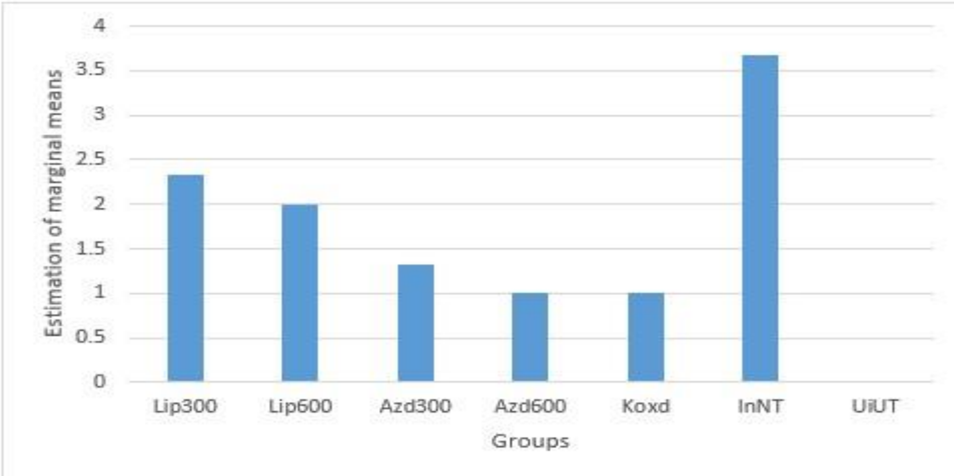

Figure 3: Lesion scores caused by Eimeria infection in different treatment groups

4.6. Survival Rate

Treatment was best (100% survival rate) in Azd600 group (Treated with *A. indica* 600 mg/kg) which was comparable to that of group Koxd (treated with koksidex), while Azd300, Lip600, and Lip300 groups were showed 93.3%, 86.7% and 80% survival rate respectively. In case of positive control (InNT) group 53.3% of survival rate was recorded.

4.7. Extract Efficacy Estimation

In this study, the anticoccidial efficacy of *Azadirachta indica* and *Lippia adeonsis* were evaluated using four indices: anticoccidial Index (ACI), Relative Oocyst Production (ROP), Relative Lesion Score (RLS), and Percent Optimum Anticoccidial Activity (POAA). Among the herbal treatment groups, Azd600 group (Treated with *A. indica* 600 mg/kg) showed highest efficacy with all indices indicating strong anticoccidial activity and an ACI of 173, which closely matching with standard drug koksidex (Koxd group) which had an ACI of 175. The 300mg/kg dose of *A. indica* in group Azd300 also demonstrated overall effective results. In contracts, *L. adoensis* at 600mg/kg in group Lip600 has shown moderate efficacy performing better in two of the four indices, while the lower dose has proven ineffective (Table 6). Overall, *A. indica* particularly at higher dose, proved to be a promising herbal alternative for coccidiosis control.

Table 6: Anticoccidal evaluation of L. *adoensis* and A. *indica* based on efficacy assessment indices

						ROP		POAA		RLS		ACI		Overall
Groups	GSR	RBWG	Survi %	RRBWG	LS	Value	Efficacy	Value	Efficacy	Value	Efficacy	Value	Efficacy	
Lip300	1.39	37.9	80	46.92	2.33	8.23	E	41.35	NE	36.5	NE	116	NE	NE
Lip600	1.46	46.5	86.7	57	2	7.71	E	51	E	45.5	NE	134	NE	ME
Azd300	1.49	49.68	93.3	61.45	1.33	4.51	E	59	E	63.7	E	149	NE	E
Azd600	1.63	63.08	100	78.1	1	4.05	E	75.1	E	72.7	E	173	E	E
Koxd	1.67	66.74	100	80.62	1	3.86	E	80.45	E	72.7	E	175	E	E
InNT	1.09	9.45	53.3	11.69	3.67	100	-	-	-	-	-	-	-	-
UiUT	1.81	80.78	100	100	0	0	-	-	-	-	-	-	-	-

GSR: Growth and survival rate, RBWG: Body weight, RRBWG: Rate of relative body weight gain, RLS: Reduction of Lesion score, ROP: Relative Oocyst Production, RBWG**:** Rate of body weight gain, SUVIR%: Survival rate, E: Effective, NE: Not effective, ME: Moderately effective.

5. DISCUSSION

The present study was conducted to investigate the efficacy of aqueous methanolic leaf extracts of *A. indica* and *l. adoensis* against experimental infection of broiler chickens with local isolates of Eimeria oocysts. Coccidiosis was well known for producing severe illness in poultry farms, by destroying intestinal epithelial cells. Following experimental infection, drenched chickens exhibited signs of weakness, reduced appetite, ruffled feathers and bloody diarrhea. These clinical signs were decreased in bird treated with herbal extracts while they continued in infected and untreated birds. This finding agrees with the reports of Biu *et al*. (2006) and Kurkure *et al.* (2006). The improvement with herbal treatment may be attributed to the presence of active metabolites or compounds that might have anticoccidial or anti-inflammatory effects.

Body weight measurement is a crucial indicator of the effectiveness of anticoccidial treatments (Lan *et al.,* 2017). The present study revealed that all treated groups had higher body weights than the infected nontreated control group suggesting that the extracts had some potency against Eimeria infection. A number of herbal products have been reported to be effective against poultry coccidiosis. Compounds derived from Artemisia, Garlic Neem and Aloe have proven to have potent anticoccidial efficacy. Such plant products may act directly on the parasite or boost the immune system of the chicks and reduce intestinal pathology (Muthamilselvan *et al.,* 2016). Treatment with *A. indica* and *L. adoensis* leaf extracts have also improved body weight gains and chicken survival rates while they reduced oocysts production and lesion scores compared to the InNT control group. This implies that the effect of these plant products is multifactorial.

In this study, when the efficacy of the products from the two plant species is compared, *A. indica* was found more effective than *L. adoensis* in improving body weight gain and survival rate while significantly reducing oocysts count and caecal lesion scores. These findings agree with the reports of Onyiche *et al* (2021). These authors have also indicated that the level of improvement was extract dose-dependent.

In agreement with the above reports, the current study has also demonstrated that both herbs exhibited a dose-dependent response, with higher dose (600 mg/kg) resulting in better outcomes compared to lower doses (300 mg/kg). However, *A. indica* even at a lower dose of 300mg /kg outperformed *L. adoensis* at 600mg/kg, indicating that *A. indica* possesses stronger anticoccidial and growth promoting properties. Although its anticoccidial effect has never been documented, Buli *et al.* (2015) and Germame and Mekuria (2021) have reported that *L. adoensis* has antimicrobial effect against various species of bacterial pathogens. The latter also revealed that the phytochemical screening has shown presence of various chemical compounds like alkaloids, flavonoids, and tannins and Thin Layer Chromatography analysis of the methanol, petroleum ether and ethyl acetate fraction of the leaves of *Lippia adoensis* var. koseret revealed bands indicating the presence of various compounds that could act as potential antimicrobial agents. Since, we have demonstrated that methanolic extract from this plant had some anticoccidial activity at the higher dose (600mg), further study is required to confirm its best performance as the dose increases beyond the current concentration.

In a similar situation, the higher performance of *A. indica* extract suggests that the plant has compounds that can limit the development of Eimeria in the intestinal tissue which could be complemented by its immune potentiating and anti-inflammatory potentials. Saleem *et al.* (2018) reported that the tree is popular for its pharmacological attributes such as microbicidal, anti-inflammatory, nematicidal, antiulcer, antioxidant, and antileishmaniasis properties. Similarly, Onyiche *et al.* (2021) ascribed the anticoccidial efficacy of *A. indica* to the presence of tannins, saponins, cardiac glycosides, alkaloids, flavonoids, and steroids in different extracts of the plant. Biu *et al.* (2006) have also documented that ethanolic neem leaf extracts showed protective effect in broilers against coccidiosis, resulting in better weight gain. The results of this study also align with Sarag *et al*. (2001), who found the highest body weight gain and best feed conversion ratio in broilers given neem leaf extracts compared to control group.

The findings in the current study are in line with reports of Tipu *et al*. (2006), Guha *et al.* (1991), Abbas *et al*. (2006), Toulah *et al.* (2010), Hady and Zaki (2012), Landi *et al*. (2011) and Oyagbemi and Adejinmi (2012) who reported leaves extract of *A. indica* induced significant reductions in oocyst counts and intestinal lesion scores. Evaluation of the overall contribution of the two plant extracts as measured by the ACI, ROP, POAA, and RLS and has clearly shown that both concentrations of *A. indica* extract have potent anticoccidial effect.

6. CONCULIONS AND RECOMMENDATIONS

The results of the present study suggest that although higher dose of *L. adoensis* extract has shown moderate efficacy as measured by percent optimum anticoccidial activity, treatment with herbal extracts of *A. indica* has shown dose-dependent alleviation of the negative impacts of the Eimeria in broiler chickens. Indices calculated from the number of oocysts per gram of feces, weight gain, gross lesion and chicken survival rate have also witnessed the potential of *A. indica* in reducing mortality, intestinal damage, and oocysts production and ameliorating the body weight gain of treated chicken which was comparable to the effect of the commercial drug, Koksidex.

Based on the above conclusion the following recommendations are forwarded:

- Different routes of administration of *A. indica* leaf extracts should be tested for easy, chipper and effective delivery of the product.
- This finding should be validated through extensive field trial in different poultry operation.
- As Ethiopia has very rich biodiversity, the search for locally produced botanical products should continue to be supported.

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8. APPENDICES

Annex 1: Post mortem examinations

GIT of normal control (left) Thickened, hemorrhagic wall (right)

Slightly thickened cecal wall, petecheal hemorrhage (left)   Thickened wall (healing) (right)

Annex 2: Photo captured during laboratory and farm works

Air-dried neem leaf (left) Air-dried koseret leaf (right)

Herbal crude extraction using rotary evaporator

Annex 3: Ethical clearance

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/02/39/16/2024

Name of Applicant: **Geremew Haile (DVM, MSc, Assistant Professor)**

Address*:* Department of Pathology and Parasitology, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project*: Investigation towards alternative anticoccidial compounds and vaccine development for integrated management of poultry coccidiosis in intensive and semi-intensive production systems*

Date of application:	**January, 2024**
Nature of the project:	**Experimental study**
Target animal species:	**chicken**
Number of animals involved:	**500**
Study area:	**CVMA-Bishoftu, Ethiopia**

Minutes No. and date of review: **VM/ERC/02/16/024, 20/02/2024**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of **Geremew Haile.**

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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Annex 4: Plagiarism report

THE EFFICACY OF AZADIRACHTA INDICA AND LIPPIA ADOENSIS AGAINST COCCIDIOSIS IN EXPERIMENTALLY INFECTED BROILER CHICKENS

ORIGINALITY REPORT

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SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Seyed Ali Ghafouri, Abolfazl Ghaniei, Soheil Sadr, Amir Ali Amiri et al. "Anticoccidial effects of tannin-based herbal formulation (Artemisia annua, Quercus infectoria, and Allium sativum) against coccidiosis in broilers", Journal of Parasitic Diseases, 2023 Publication	2%
2	www.tandfonline.com Internet Source	2%
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5	D.R. Korver. "Review: Current challenges in poultry nutrition, health, and welfare", animal, 2023 Publication	1%
6	www.thaiscience.info	