INVITRO ANTICOCCIDIAL EFFECT OF ETHANOLIC EXTRACT OF NEEM LEAVES (*AZADIRACHTA INDICA*) ON COCCIDIA OOCYSTS

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ABSTRACT
Coccidiosis over the years has been one of the most important poultry parasitic disease which has led to huge economic losses as such the need to mitigate this menace becomes inevitable. Neem plant extract was used in this study. Neem plant leaves was harvested and dried at 50°c in a hot air oven and grinded. 100g of powder was macerated in 1.5litres of ethanol and stirred daily at 3hours interval for 72hours, and then filtered using Whatman Paper No 3. The filtrate was concentrated by evaporating the solvent at 75°C using a rotatory evaporator (Buchi R-200) to obtain the extract. The extract was prepared at different concentrations of 20, 30, 40 and 50mg/ml and dissolved with Dimethyl sulphoxide (Dmso). The diluted extract was transferred into petric-dishes and placed equal amount of oocyst (100oocyst) was also added then incubated at 28 -30°c for 24 – 48hours. The sporozoites were counted using malasses counting chamber. The number of viable sporozoites and non-viable sporozoites was estimated by counting the number of sporozoites in a total of 100 oocysts. The result showed parasites recovery rate of 63.0, 18.2, 27.6, 52.9 and 55.1 % for control, 50, 40, 30 and 20mg/ml respectively. Similarly, the result showed parasites inhibition rate of 27.0, 45.0, 42.0, 32.0 and 31.0% for control, 50, 40, 30 and 20mg/ml respectively. The findings indicate 50mg/ml concentration to have given the best parasites inhibition effect. It is established in this study that Neem plant extract can serve as an alternative to synthetic anticoccidia drugs and hence its use is encouraged as it is cheaper and readily available in the study area.

Keywords: *Neem leaves, ethanolic extract, coccidia, sporozoites and inhibition.*

INTRODUCTION
Poultry coccidiosis is considered one of the most economically important parasitic diseases worldwide that causes high mortality and morbidity rates in addition to poor food conversion and reduced weight gain in the poultry industry in spite of advances in chemotherapy, management, nutrition and genetics (McDougald, 2003). The use of several drugs, alone or in combination, has proven to be an effective alternative in the struggle against avian coccidiosis (Quiroz-Casta˜neda and Dant´an-Gonz´alez, 2015). These include costs of prophylaxis, medications, losses of productivity due to mortality, morbidity and lowered feed conversion (Chapman, 2009). The disease is clinically characterized by bloody diarrhoea, poor feed conversion ratio, low growth rate or poor weight gain. This has also been considered a contributory factor in the pathogenesis of other diseases (Bachaya *et al.*, 2012).

The public demands for residue-free meat has encouraged development of alternative control strategies (Chapman *et al.*, 2010). Phytochemicals come from various types of botanical elements have been explored as sustainable alternatives for controlling coccidiosis and seen to be quite efficacious (Allen *et al.*, 1997). One of the medicinal plants used in the treatment of Animals is *Azadirachta indica* (neem plant) that is found to be anti-helmentic in both human and animals (Schmutterer, 1990) as well as antimicrobial (Akilandeswari *et al.*, 2003). Therefore, the aim is to assess the effect of neem leave ethanolic extract in the treatment of coccidial organisms

MATERIALS AND METHODS
Study Site
This study was conducted in Animal Production Laboratory of the School of Agriculture and Agricultural Technology, Federal University of Technology, Gidan Kwano, Minna, Niger State, Nigeria. Minna lies within latitude $9^{0}30^{1}$, North and longitude $6^{0}33^{1}$, East. The annual rainfall ranges between 110mm -1600mm and a mean temperature of 21°C and 36.5°C (Usman, 2011).

Source of Experimental Materials
For ease of getting infective materials (oocysts), faecal samples were collected and screened from some Farms in Minna metropolis, into a sterilized plastic containers and labelled properly. Similarly, Fresh neem leaves were obtained from Minna metropolis, Niger State.

Preparation of Raw Materials
Fresh neem leaves collected were thoroughly washed and air dried. 500g of dried neem leaves were grinded into fine powder and stored in different polythene bags until ready for use.

Preparation of Extracts
100 g of stored powder was macerated in 1.5litres of ethanol and stirred daily at 3hours interval for 72hours, and then filtered using Whatman Paper N 3. The filtrate was concentrated by evaporating the solvent at 75°C using a rotatory evaporator (Buchi R-200) to obtain the extract which was subsequently kept in a refrigerator at 4°C until ready for use (Heelan and Ingersoll, 2002).

Isolation of Eimeria Oocyst
The oocysts were isolated from infected faecal samples. 10g of the faecal sample was dissolved in 20mls of 2.5 % potassium permanganate and mixed homogenously and allowed to stay for 30mins. The mixture was filtered using muslin cloth and the filtrate collected. The filtrate was added with saturated Sodium Chloride (NaCl) to allow for precipitation of the oocysts. The mixture was centrifuged at 350rpm for 15mins. The suspended oocysts were collected using cannula. The oocysts were suspended in the HBSS buffer until ready for use (Heelan and Ingersoll, 2002).

Sporozoites count determination
The extract was prepared at different concentrations of 20, 30, 40 and 50mg/ml and dissolved with Dimethyl sulphoxide (Dmso). The diluted extract was transferred into petric-dishes and placed equal amount of oocyst (2ml) was also added. Then incubated at 28 -30°c for 24 – 48 hours. The sporozoites was counted using malasses counting chamber. The number of viable sporozoites and non-viable sporozoites was estimated by counting the number of sporozoites in a total of 100 oocysts. The sporozoites percentage was calculated as follows:

$$\text{Percenctage (\%)Sporozoites} = \frac{\text{\% Viable Sporozoite} - \text{\%Non-Viable Sporozoite}}{\text{\%Viable Sporozoite}} \text{ X 100 (Yamssi } et\ al.\text{, 2017)}$$

RESULTS AND DISCUSSION

The result shows the parasite inhibition trends at different concentration levels as control, 50, 40, 30, and 20mg/ml respectively. Whereas, percentage viable parasites obtained for control, 50, 40, 30 and 20mg/ml were 63.0%, 18.2%, 27.6%, 52.9% and 55.1% respectively. The result of this study shows that ethanolic extract of neem leave has anticoccidial effect irrespective of the concentration added as it has shown to have the ability to kill or inhibit growth and development of sporozoite. However, the best parasites inhibition effect was obtained at 50mg/ml. This in agreement with the work of (Abdullahi *et al.*, 2006) who reported the anticoccidial efficacy of aqueous neem leaf extract in comparison to amprolium. Aqueous neem extract (800mg/kg) compared to amprolium (10mg/litre) effectively treated coccidia organisms by 100% survival rates for infected chickens. The ability of the extract to inhibit or kill the sporozoites could be attributed to its ability to inactivate the enzyme responsible for the sporolation process in helminthes (Molan *et al.*, 2003). The effectiveness of the extract can also be attributed to its ability to penetrate the cell wall of the oocyst and damaging the cytoplasm as evident by the abnormal appearance of sporozoites of the oocyst exposed to the extract in this work (Yamssi *et al.*, 2017).

Table: Anticoccidia effect of ethanolic extract of neem leave on coccidia oocysts

Extract Concentration (mg/ml)	Number of Oocyst Administered	%Nonviable Sporozoite	% Recovery rate
Control	2ml	27.00	63.0
50	2ml	45.00	18.2
40	2ml	42.00	27.6
30	2ml	32.00	52.9
20	2ml	31.00	55.1

CONCLUSION AND RECOMMENDATION

This study was able to establish the viability and the effectiveness of Ethanolic extract of neem leaves in the treatment of coccidia parasites which is an economic important disease that affect poultry farming and livestock in general with a view to providing suitable alternative to synthetic anticoccidial drugs that are expensive and could have residual effect on consumers. The best parasites inhibition effect was obtained at 50mg/ml concentration level. Therefore, it is recommended that ethanolic extract of neem leave at 50mg/ml be used in invitro treatment of coccidia organisms.

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