EXPERIMENTAL INVITRO TREATMENT OF COCCIDIAL ORGANISMS USING ETHANOLIC EXTRACT OF RIPE PAWPAW SEED (*CARICA PAPAYA*) ON BROILER CHICKENS

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ABSTRACT

Coccidiosis is one of the most expensive and common diseases affecting poultry production in the tropics. Therefore, the need to create an effective organic measure to prevent the great loss is paramount. The organic anticoccidial effect was carried out using ripe pawpaw seed extract. Ripe pawpaw seeds were harvested, washed, air-dried and grinded. 100g of the powder was macerated in 1.5litres of ethanol and stirred at 3hours interval daily for 72hours and then filtered using whatman paper No 3. The filtrate was concentrated by evaporating the solvent at 75^0c using a rotatory eviporator (Buchi R-200) to obtain the extract. The extract was dissolved with Dimethyl sulphoxide (DMSO) and prepared at different concentration of 20, 30, 40, and 50mg/ml. The diluted extract was transferred into petric-dishes and placed equal amount of oocysts (2ml) was also added then, incubated at 28 – 30^0c for 24 – 30hours. The sporozoites were counted using molasses counting chamber. The number of non-viable and viable sporozoites were estimated by counting the number of sporozoites in a total of 100 oocysts. The results obtained showed 52.9%, 50.7%, 33.3% and 24.6% of recovered sporozoites for 20, 30, 40 and 50mg/ml concentration respectively. The result showed that the highest sporozoites inhibition was observed at concentration 50mg/ml at the rate of 24.6%. Therefore, it has shown that ripe pawpaw seed ethanolic extract can serve as an alternative to synthetic anticoccidial drugs and encouraged for use as it is cheaper and easily obtained.

Keywords*: pawpaw seed extract, sporozoites, coccidial eggs, anticoccidial effect and inhibition.*

INTRODUCTION

Broiler chicken (*Gallus gallus domesticus*) is a gallinaceous domesticated fowl, bred and raised specifically for meat production (Krutchen, 2012). However, poultry production is often confronted by avian coccidiosis, flu, and other infectious diseases as reported by Quiroz-Castañeda & Dantán-González (2015). Coccidiosis is one of the most expensive and common disease of poultry production systems, in spite of advances in chemotherapy, management, nutrition and genetics (McDougald, 2003). Avian coccidiosis is characterized as an infectious protozoan disease caused by gut parasites of the genus *Eimeria* (Coccidia subclass) (Gilbert *et al*., 2011). Various anticoccidial feed additives; most especially ionophorous antibiotics have been developed and used (Chapman *et al.*, 2005). The daily use and misuse of these drugs led to development of coccidial parasite drug resistant strains, which is detrimental to consumer health because of the presence of anticoccidial drug residues in poultry products (Danaher *et al*., 2008). Medicinal plants are considered as alternative new tools for controlling coccidiosis. For instance, the incorporation of dried leaf of creat or green chiretta (*Andrographis paniculata*) at 10, 20, 30 and 40% in feed proved to be efficient on mortality reduction (Sujikara, 2000). The anthelmintic effect of pawpaw (*Carica papaya*) led to complete mortality of *Ichthyophthirius multifilis* with in vitro treatment of *C. papaya* extract in fish (Ekanem *et al*., 2004). Okeniyi *et al*. (2007) reported the effective treatment of dried *C. papaya* seed extract against human intestinal parasites and without significant side effect. Despite the commendation of the above natural products, several challenges in the anticoccidial use of natural products such as anticoccidial efficacy, identification of active compounds, mechanism, safety, and costeffectiveness of plant extracts and compounds need to be overcome prior to further applications. The aim of this study is to test the anticoccidial effect of ripe pawpaw (*Carica papaya*) seed ethanolic extract on broiler chickens in the treatment of coccidial organisms.

MATERIALS AND METHODS

Experimental 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 21^0C and 36.5^0C (Usman, 2011).

Source of Experimental Materials

50gm of faecal samples each were collected from selected 10 different farms in Minna, Niger state, into a sterilized plastic containers and labelled properly. Fresh ripe (pawpaw) *Carica papaya* seeds were obtained from the fruit section of Kure-ultra modern market Minna Niger State.

Preparation of Raw Materials

Fresh ripe pawpaw (*Carica papaya*) seeds collected were washed thoroughly and air dried. 500g of dried ripe pawpaw (*Carica papaya*) seeds was grinded into fine powder and stored in a polythene bag until ready for use.

Preparation of Ripe Pawpaw Seed 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 4^0C until ready for use, as demonstrated by Heelan and Ingersoll (2002).

Isolation of Eimeria Oocyst

The oocysts were isolated from an infected faecal samples from ten (10) different farms within Minna metropolis. 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 musline cloth and the filtrate collected. The filtrate was added with saturated Sodium Chloride (NaCl) to bring about the 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).

Identification of Coccidial Eggs

Identification of eggs was performed by microscopic features of the oocyst morphology (shape, size, and colour of the oocysts) present in the fecal samples. The result of the aforementioned identification characteristics used were compared with the identification key by Long and Reid (1982).

Anticoccidia Effect of Extract

The extract was prepared at different concentrations of 20, 30, 40 and 50mg/ml and dissolved with Dimethyl sulphoxide (Dms). The diluted extract was transferred into petric-dishes and equal amount of oocysts (2ml) were placed in each. Then incubated at 28 -30^0c 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 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*., 2017)

RESULT AND DISCUSSION

Table 1: Result showing anticoccidial effect of ripe pawpaw seed extract on coccidial eggs

Extract Concentration (mg/ml)	Number of Oocyst Administered	%Nonviable Sporozoite	%Viable Sporozoites	% Sporozoite
Control	2ml	30.00	70.00	57.1
20mg/ml	2ml	32.00	68.00	52.9

30mg/ml	2ml	33.00	67.00	50.7
40mg/ml	2ml	40.00	60.00	33.3
50mg/ml	2ml	43.00	57.00	24.6

From the result obtained, it shows that, the sporozoites recovery rates varies at different concentrations of ripe pawpaw seed extract. Highest sporozoites recovery rate was seen in control (57.1%), 20mg/ml (52.9%) and 30mg/ml (50.7%) respectively, However, the lowest recovery rate was observed at 50mg/ml with the sporozoites recovery rate of 24.6%, which is in agreement with the work of Udo and Abba (2018) who reported the in-vitro anticoccidial efficacy *of Allium sativum* and *Carica papaya* extract at a concentration of 2.5g, 5.0g and 10g/L of distilled water when 4800 unsporulated but viable oocyst were inoculated at room temperature in the laboratory. Highest efficacy at 48hrs with the highest number of unsporulated oocysts seen at concentration 10g at 68% yield for aqueous *Carica papaya* and at 65% for powder *Carica papaya*. The ability of the extract to inhibit sporozoites growth and propagation is due to the ability of the extract concentration to lyse the cyst thereby forcing the oocyst to empty it cytoplasmic material. This work is also in agreement with the findings of Yamssi *et al*. (2017) who reported a similar trend when methanolic extract of *Psidium guajava* (guava) were applied on oocyst at 2.5mg/ml, 5mg/ml, 10mg/ml, 20mg/ml and 30mg/ ml. The highest efficacy was 88.67 ± 2.52% at the concentration of 30 mg/ml against *E. intestinalis*. The anticoccidial effect of the extract on the sporozoites can also be attributed to its ability to prevent the enzymatic process leading from oocylation to sporulation.

CONCLUSION AND RECOMMENDATION

This study was able to ascertain the effectiveness and viability of ripe pawpaw seed ethanolic extract in the treatment of coccidial organisms. 50mg/ml concentration gave the best parasite inhibition rate at 24.6%. It is recommended that ethanolic extract of ripe pawpaw seed at 50mg/ml be used in the sporulation inhibition of coccidial organisms.

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