



Oocysts Output of Broilers Experimentally Infected with *Eimeria tenella* And Treated with N- Butanol Leaf Extract of *Khaya senegalensis*

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SUMMARY

A study to determine the anticoccidial efficacy of *Khaya senegalensis* in relation to oocyst shedding by broilers experimentally infected with *Eimeria tenella* was conducted. The development of drug-resistant field strains of *Eimeria* species has prompted the exploitation of alternative methods for controlling coccidiosis and there is an increasing use of medicinal plants as alternatives to orthodox medicine. Fresh leaves of *Khaya senegalensis* (KS) were collected, dried under shade and the extract prepared using the maceration method in 70% methanol. The dried crude extract was partitioned into petroleum ether, chloroform, n-butanol and aqueous portions, dried with phytochemical analysis conducted on them. One hundred and twenty birds reared under standard management practice were divided into six groups (A, B, C, D, E and F). All the groups except group F (uninfected untreated group) were infected at four weeks old with sporulated *Eimeria tenella* oocysts (1.0×10^5 sporulated oocysts / ml / bird) obtained locally from the intestinal scrapings of experimentally infected broilers. Groups A, B and C were given calculated three dose levels of 11mg/kg, 33mg/kg, 99mg/kg respectively of the prepared n-butanol methanol extract twice daily for 5 days, group D was given Amprolium while E and F were each given 0.2ml water. Faecal samples were collected daily for 4 weeks into clean well labelled polythene bags and analysed in the laboratory for oocyst count using the McMaster counting chamber and were expressed as oocysts count per gramme of faeces. The birds were observed for pathological lesions grossly and histopathologically and the survival rates were determined. Data collected were analysed using analysis of variance and chi square. Results from the Phytochemical studies showed the presence of phenolic compounds in *Khaya senegalensis*. Post-infection faecal examination revealed oocyst load of +++ in all the infected pens (A-E) on the 6th day. Comparison of the groups with time showed statistical significance ($P < 0.05$). High

mean oocyst production (A; 156060 ± 67020 , B; 261590 ± 144310 , C; 211620 ± 114280 , D; 276930 ± 233650 and E; 159230 ± 100970) among the infected groups one week post infection as well as irregular oocyst production were observed in the course of this study. The higher mean oocysts count obtained in the infected untreated group (1748849 ± 40869) than the extract treated groups in the first week post treatment indicated that the extract had some inhibitory effects on oocyst production. This however, was dose dependent. Among the extract treated group, the 99mg/kg had lower mean oocyst production 2 weeks post treatment (9720 ± 3180) and this was comparable to the group treated with normal dose of the conventional drug Amprolium (8600 ± 40). This was therefore seen as the effective dose. Grossly, the extract had a beneficial effect in alleviating the damages to the caecal epithelium of the infected treated groups compared to the shrunken caeca of the infected untreated groups. The survival percentage was higher in the treated groups compared to the infected un treated group (55%) though Amprolium was more efficacious in the *in vivo* study with the highest survival rate of 90%. The histopathological lesions observed in the infected birds in this study were consistent with those associated with *E tenella* infection in which the parasite induced very severe lesions including severe villous atrophy and fusion. The anticoccidial efficacy of *Khaya senegalensis* promises greater areas for research as it relates to drug development and it is recommended that *Khaya senegalensis* should be exploited further for its anticoccidial properties using other parts of the plant.

Key words: Broiler chickens, *Eimeria tenella*, oocyst production, *Khaya senegalensis*, *in vivo*

INTRODUCTION

Avian coccidiosis is one of the most common and important diseases in poultry production caused by protozoan parasites of the *Eimeria* genus (Perez – Carbajal *et al.*, 2010). Seven species of *Eimeria* have been recognized to specifically parasitize the domestic chicken *Gallus gallus* with differing levels of pathogenicity and specific areas of localization within the digestive tract (Fornace *et al.*, 2013). The disease is characterized by enteritis and diarrhoea which can become bloody with certain *Eimeria* species (Dakpogan and Salifou, 2013). The most common and pathogenic species are *Eimeria tenella* which causes caecal coccidiosis and *Eimeria necatrix* (Gyorke *et al.*, 2013), while *Eimeria*

acervulina and *Eimeria maxima* cause chronic intestinal coccidiosis (Chandrakesan *et al.*, 2009). *Eimeria tenella* is one of the most ubiquitous parasites (Patra *et al.*, 2010) and most pathogenic (Abbas *et al.*, 2008). It is an intracellular obligate protozoan (Abbas *et al.*, 2008) having a complex life cycle of seven day (Cacho *et al.*, 2014) during which it under goes intracellular development and proliferates through characteristic intracellular stages confined to the caecal epithelium (Cacho *et al.*, 2014). The disease is associated with haemorrhagic diarrhoea, emaciation, growth retardation and sometimes death (Takagi *et al.*, 2006). Birds of any age may be affected but problems are not common in chickens under two weeks of age (Wright, 2005). Coccidiosis has remained the most important poultry disease

in Nigeria (Obasi *et al.*, 2006). Lawal *et al.* (2008) showed that the infection can occur in both local chickens and exotic birds with the former serving mainly as reservoir hosts. The disease causes considerable economic losses in both the layer and broiler industries (Al-Quraishy *et al.*, 2009). Anticoccidial drugs have been in use for more than 50 years and presently almost all commercial broilers are reared with the agents in their feeds (Chandrakesan *et al.*, 2009). The development of drug-resistant field strains and the necessity for withdrawal period for these drugs prior to slaughter prompted the exploitation of alternative methods for controlling coccidiosis (Zulpo *et al.*, 2007). There is an increasing use of medicinal plants and plant products as alternatives to orthodox medicine especially in developing nations (Mikail *et al.*, 2007). Plants native to Nigeria have been experimented and shown to have some anti-coccidial activities (Anosa and Okoro, 2011) with the bark of *Khaya senegalensis* a well-known medicinal plant (Sale *et al.*, 2008; Ndjonka, *et al.*, 2011; Kolawole, *et al.*, 2012; Ibrahim *et al.*, 2013) being used to treat coccidiosis, helminthosis and diarrhoea in poultry (Gefu *et al.*, 2000). Yaun *et al.* (2013) and Nakatani *et al.* (2002) have described the active compounds of *Khaya senegalensis* and the identification of phenolic compounds in *Khaya senegalensis* which are also antioxidants has been indicated to have anticoccidial activities (Naidoo *et al.*, 2008; Meskerem and Boonkaewwan, 2013). Phenolic compounds could be a major determinant of antioxidant potentials of many medicinal plants (Emami, 2007) and are therefore a natural source of antioxidants ((Taheri *et al.*,

2005; Aberoumand and Deokule, 2008). This study was carried out to ascertain the effectiveness of leaves extract of *Khaya senegalensis* in reducing the oocyst output of experimentally infected broilers.

MATERIALS AND METHODS

Collection and Preparation of Plant materials for Extraction: Fresh leaves of *Khaya senegalensis* (KS) was collected from the environs of Ahmadu Bello University between the months of March and April due to their high bioactive content at these months (Ademola and Eloff, 2010) and dried under shade away from sunlight.

Crude Plant Extraction by Maceration Method

Ten kilogramme (10kg) of the dried leaves of *Khaya senegalensis* KS with voucher numbers 900181 (Otu *et al.*, 2009) was ground to powder using mortar and pestle and sieved. Two kilogramme (2kg) of the powder for KS was defatted with 5 litres of Petroleum ether in a Soxhlet apparatus (Quick fit corning Ltd; Stafford, England) at 70°C (Youn and Noh 2001). The leaves were air dried at room temperature and then extracted by maceration method for 72 hours using 70% absolute methanol (WHO, 1998). The crude extract was evaporated to dryness on a water bath at a temperature of 90°C for 6 hours.

Fractionation of Crude Methanol Extract: The method of Brain and Turner (1975) was adopted for the partial purification of the dried crude methanol extracts. Thirty grammes (30g) of the dried crude methanol extract for KS was suspended in 300ml of water and partitioned

with three portions of 300ml petroleum ether respectively using separate funnels. The petroleum ether portions were each carefully separated into 3 labelled clean 1000ml beakers. Subsequently, the aqueous methanol portions were partitioned with three portions (each 300ml) of chloroform; followed by three equal volume of N-butanol. The portions were then referred to as petroleum ether, chloroform and N-butanol and aqueous portions respectively. The solvents were evaporated in a hot water bath 90°C for 6 hours. The dried fractions were stored in a dessicator in well labelled containers until when needed.

Experimental Birds: One hundred and twenty (120) four weeks old birds were obtained from NAPRI, Shika, Zaria. The birds were raised under standard management practice at the Faculty of Veterinary Medicine A.B.U. Zaria for a period of 4 weeks and were on Broiler finisher ration. The birds were randomly divided into six groups namely A, B, C, D, E and F of 20 birds each. Each group were further subdivided into two replicates each having 10 birds. The birds were provided standard anticoccidial-free feed. The feed and water were provided *ad libitum* during the study period. All the birds were number tagged to maintain their identity.

Inoculum: Infective sporulated *Eimeria tenella* oocysts were obtained locally from infected broiler birds. The oocysts from the intestinal scrapings were sporulated using 2.5% potassium dichromate. The sporulated oocysts were filtered using a sieve, centrifuged (750g) for 10 minutes to remove the potassium dichromate and diluted with

distilled water (Reck and McQuiston, 1994). The sporulated oocysts were kept in the refrigerator (4°C) until when they were needed.

Infection of birds with the sporulated oocysts: The birds were infected orally at 4 weeks of age with 1.0×10^5 sporulated oocysts / ml / bird using insulin syringe (Reck and McQuiston, 1994). Group F was only given 0.2ml of distilled water.

Administration / Treatment of the experimentally infected birds: Three dose levels namely 11mg/kg, 33mg/kg and 99mg/kg were selected from the pilot toxicity experiment (Otu et al., 2016) that did not produce toxic symptoms. These were given to groups A, B and C respectively. Group D was given Amprolium (1.2gm/L) while Group E was infected but not treated. Group F served as the negative control and was not infected or treated but was given 0.2ml of distilled water twice daily for 5 days.

Dosages of n-butanol Plant Extracts: Groups A, B and C were given calculated dosages of the n-butanol methanol plant leaves extracts 6 days post infection twice daily for 5 days, while Group D was given Amprolium. However, Groups E (positive control) and F (negative control) were given 0.2mls of distilled water twice daily respectively.

Anticoccidial: Amprolium (Amprolium-20S VMD nvlsa Hoge Mauw 900-2370, Arendonk, Belgium) was used for the treatment of coccidiosis and to compare the anticoccidial efficacy of the plant extract.

Collection and Examination of Faecal Samples: Faecal samples were collected from the birds in each group daily using clean polythene bags from 4 to 8 weeks of age and examined for the presence of *Coccidia* oocysts. *Eimeria tenella* oocyst output was determined and expressed as per gram of faeces using McMaster counting chamber (Sloss *et al.*, 1994).

Clinical Signs: The birds will be observed daily for signs of bloody faeces, depression, decreased feed intake and mortalities.

Post Mortem Examinations

i Gross Pathology: Two birds from each of the 6 groups were selected at random 7 days post treatment and the end of the experiment and sacrificed humanely using cardiac air pumped. Birds were also sacrificed humanely from the positive and negative control groups for post mortem examination. The carcasses were examined for pathological lesions and samples such as the intestines and caeca were taken for histopathology. They were stored in 10% formalin. Appropriate photographs of the gross lesions were also taken.

ii: Histopathology: Slides for histopathologic examination were prepared at the Histotechnique laboratory of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Tissues were processed routinely and embedded in paraffin wax. Paraffin sections of about 5 μ m-6 μ m were prepared. Deparaffinization in xylene and dehydration in graded series of ethanol was followed by staining with Haematoxylin and

Eosin stain. Appropriate photographs of the histopathologic lesions were also taken.

Pre and Post Microscopic Examination of Intestinal Scraping: The birds that were humanely sacrificed for the gross pathology examination also had samples taken from them for microscopic examination of the intestinal scraping.

Assessment of Response to the Experiment Included: (i) Oocysts count, (ii) clinical signs, (iii) mortality and survival rate, (iv) gross pathology, pre and post treatment microscopic examination of intestinal scraping and histopathology.

Statistical Analysis: Data obtained from the experiment were analyzed with Analysis of Variance (ANOVA) using SAS statistical package of General linear model and Chi square. P values of < 0.05 were considered to be statistically significant.

RESULTS

Extraction of Plant Material and Partitioning of Crude Extract: The extraction of 2kg of the leaves of *Khaya senegalensis* (KS) with 70% methanol as solvent gave a yield of 436.42g (21%) with the colour of the extract being dark brown (Table 1). The extractive yield output of the partitioned portions of the crude methanol extract of *Khaya senegalensis* when petroleum ether, chloroform and n-butanol were used as partitioning solvents for the partitioning of 30g of crude methanolic gave the following; 0.01g (0.03%), 0.25g (0.83%), 6.73g (22.43%) respectively while the aqueous yield was 13.47g (44.9%). The petroleum ether portion was greenish in

colour while the chloroform, n-butanol and aqueous methanol portions were brown, reddish brown and brown in colour respectively (Table 2).

Phytochemical Analysis: The thin layer chromatography (TLC) plate took 90 minutes to run in the TLC tank eluted in the solvent system of n-butanol, acetic acid and water at the ratio of 6:1:2. The retention factor was determined. The petroleum ether spot had a retention factor (R_f) of 0.47cm,

chloroform R_f : 0.61, n-butanol; R_f : 0.70 and the crude extract of *K senegalensis* R_f : 0.84 (Table 3). The TLC plate was sprayed with ferric chloride as the detecting agent and this gave greenish coloured spots which signified the presence of phenols and flavonoids. The greenish colourations were mainly seen in the n-butanol and crude extract fractions of the plant (Plate 1). All the other detecting agents used did not yield any positive colouration.

Table I: Crude Extractive yield of the leaves of *Khaya senegalensis* used in the study

Plant	<i>Khaya senegalensis</i>
Plant part collected	Leaves
Quantity of Plant material collected	10kg
Quantity of Plant sample extracted	2kg
Output of crude extract/Percentage yield after Extraction	436.42g (21%)
Colour of Extract	Dark brown

Table II: Fractional Yield Output of Partitioned Portions of Crude Methanolic Extract of *K. senegalensis*

Fractionated Portions	Fractional yield Output (grammes/ %)	Colour
Petroleum ether	0.01g (0.03%)	Greenish
Chloroform	0.25g (0.83%)	Brown
N-butanol	6.73g (22.43%)	Reddish brown
Aqueous Fraction	13.47 (44.9%)	Brown

Table III: Retention factors for the compounds present in the n- butanol fractions of *Khaya senegalensis* extracts.

Fractions	Distance travelled by Spot (S) in cm	Distance travelled by Solvent Front (S _{of}) in cm	Retention factor (R _f) in cm
Petroleum ether	3.5	7.6	0.46
Chloroform	4.6	7.6	0.61
N-butanol	5.4	7.6	0.70
Aqueous	6.4	7.6	0.84
Crude extract	6.9	7.6	0.91

Keys: cm- centimetre



Plate 1: TLC plate after spraying the various fractions eluted in the solvent system (6:1:2) with Ferric chloride. Note the greenish spots indicating the presence of phenols and flavonoids.

Clinical Signs: Pre-treatment clinical signs such as bloody diarrhoea, bloody perineum, feet and feathers, depression and anorexia were observed. No signs were seen prior to the shedding of the oocysts. However, faecal examination revealed oocyst load of +++ in all the infected pens (A-E). Mortalities were recorded in all the infected birds 6 days post infection.

Gross Lesions: The gross lesions seen 7 days post- infection lesions were enlarged bloody caeca, friable liver and pale carcass (Plates 2 and 3). Two days post-treatment, dead birds showed moderate caecal lesions and the flesh was moderately pale. There were no gross lesions seen in all the sacrificed birds (12 birds comprising of 2 from each group A-F) 13 days post-infection. However, the caeca of the infected untreated group was shrunken in size when compared to those of the treated groups (Plates 4, 5,6,7,8 and 9).

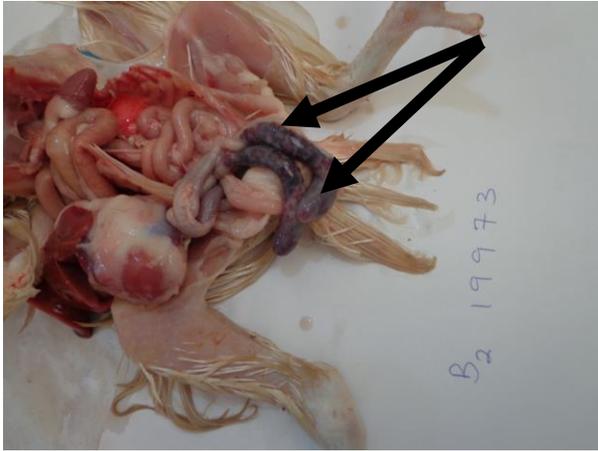


Plate 2: *In situ* appearance of *Eimeria tenella* infected broiler caeca distended with blood six days post infection (arrows)



Plate 3: Bloody caecal contents of infected boiler chicken with *Eimeria tenella* six days post infection (arrow)



Plate 4: Caeca of an *Eimeria tenella* infected bird one week post treatment with n-butanol fraction of the extract of *Khaya senegalensis* (11 mg/kg) filled with faecal contents (arrows)

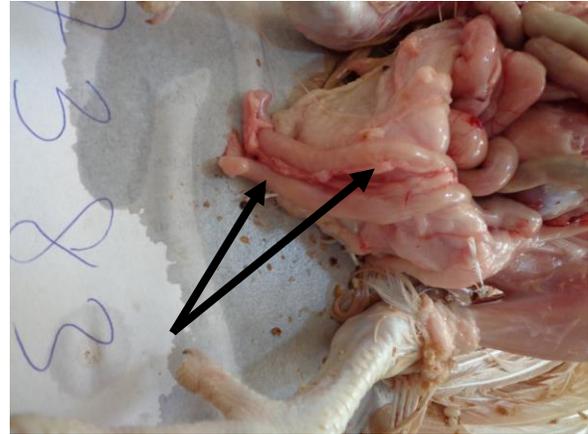


Plate 5: Caeca of an *Eimeria tenella* infected bird one week post treatment with n-butanol fraction of the extract of *Khaya senegalensis* (33 mg/kg) (arrows)

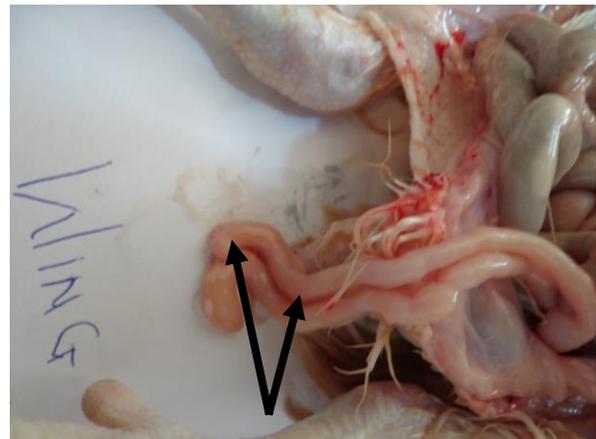


Plate 6: Caeca of an *Eimeria tenella* infected bird one week post treatment with n-butanol fraction of the extract of *Khaya senegalensis* (99 mg/kg) (arrows)



Plate 7: Caeca of an *Eimeria tenella* infected bird one week post treatment with Amprolium (1.2g/l) (arrows)



Plate 8: Shrunken caeca of *Eimeria tenella* infected non treated bird (positive control) one week post treatment with water (0.2 ml/ bird) (arrows)

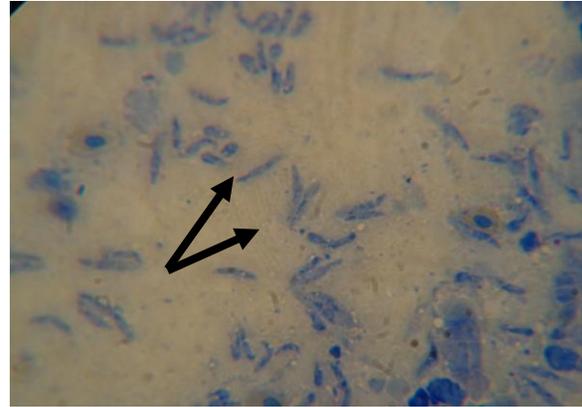


Plate 10: Photomicrograph of merozoites from caecal scrappings of infected un treated birds (arrows). Giemsa stain $\times 100$ magnification



Plate 9: Caeca of a non infected broiler chicken (negative control) one week post treatment with water (0.2 ml/ bird) filled with faecal contents (arrows)

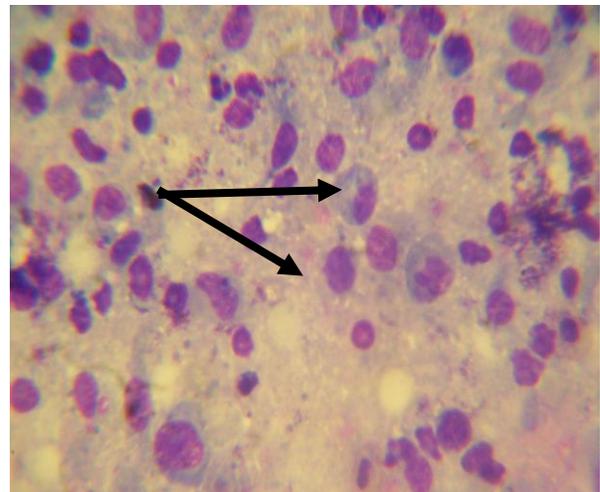


Plate 11: Photomicrograph showing schizonts from caecal scrappings of infected untreated bird (arrows). Giemsa stain $\times 100$ magnification

Pre and Post Infection Microscopic Examination of the Intestinal Scrappings:

Pre-infection microscopic examination of intestinal scrappings revealed no parasitic stages. However, at 7 days post treatment, the untreated infected group (group E) had massive schizonts seen (Plates 10 and 11). The group infected and treated with Amprolium (Group D) had few merozoites and schizonts seen and this was similar to what was obtained in the groups treated with 11mg/kg (group A) and the 99mg/kg (group C) (Plates 12). The un infected and untreated (group F) had no parasites seen in them.



Plate 12: Photomicrograph showing scanty schizonts from caecal scrappings of infected and Amprolium treated bird (arrows). Giemsa stain $\times 100$ magnification

Observation of Oocyst Load and Shedding by *Eimeria tenella* Infected and Extract Treated Birds

Post infection faecal examination revealed oocyst load of +++ in all the infected pens (A-E) on the 6th day. Comparison between the groups with One Way Analysis of Variance (ANOVA) showed no statistical significance ($P > 0.05$). However, when groups were compared based on weeks they were statistically significant ($P < 0.05$). Numerically there were variations in the mean oocyst count with the highest oocyst production observed among the Amprolium treated group first week post infection (276930 ± 233650). Among the extract treated birds infected with *Eimeria tenella* one week post infection, the highest mean oocyst output was recorded in the 33mg/kg group (261590 ± 144310) while the least was in the 11mg/kg treated group (156060 ± 67020). One week post treatment, the mean oocysts count of the excreted oocysts was higher in the infected untreated group (1748849 ± 40869) than the treated groups which indicated that the extract had some

anticoccidial effect on oocyst production. Group A given extracts at 11mg/kg showed the lowest mean oocysts counts of 1389510 ± 479250 while the 99mg/kg extract treated group had the highest (1680820 ± 52880). A varied decrease of the mean oocysts count was observed with the Amprolium treated birds one week post treatment with the lowest mean oocysts output of 340600 ± 184780 . However, the situation changed two weeks post treatment among the extract treated groups with the 99mg/kg giving the lowest oocysts counts of 9720 ± 3180 which was comparable to the Amprolium treated group (8600 ± 40). By the third week post treatment, the 99mg/kg treated group had an outstanding reduction in the mean oocysts counts of 1920 ± 680 and this was the lowest oocysts counts among the infected treated groups. The positive control maintained the highest mean oocysts output from the second week post infection 1748849 ± 40869 rising to 29570 ± 9290 by the end of the experiment. The uninfected birds had no oocysts counts (0 ± 0) first week post infection (Tables IV and V).

TABLE IV: Mean Experimental *Eimeria tenella* oocysts production one week post infection

Group	Doses	Means (\pm SEM) Oocysts production/gramme of faeces
A (Extract)	11mg/kg	156060 ± 67020
B (Extract)	33mg/kg	261590 ± 144310
C (Extract)	99mg/kg	211620 ± 114280
D (Amprolium)	1.2g/L	276930 ± 233650
E (Positive Control)	0.2ml (water)	159230 ± 100970
F (Negative control)	0.2ml (water)	0 ± 0

Key: $P > 0.05$: Not statistically significant

TABLE V: Mean Weekly Experimental *Eimeria tenella* Oocysts Production Post treatment

Group	Doses	Means (\pm SEM) oocysts production/gramme of faeces		
		Week 1	Week 2	Week 3
A (Extract)	11mg/kg	1389510 \pm 479250	13840 \pm 1600	16580 \pm 13100
B (Extract)	33mg/kg	1521280 \pm 712960	10060 \pm 1840	7120 \pm 3200
C (Extract)	99mg/kg	1680820 \pm 52880	9720 \pm 3180	1920 \pm 680
D (Amprolium)	1.2g/L	340600 \pm 184780	8600 \pm 40	7000 \pm 4900
E (Positive control)	0.2ml (water)	1748849 \pm 40869	39420 \pm 28920	29570 \pm 9290
F Negative control)	0.2ml (water)	10 \pm 10	470 \pm 90	800 \pm 180

Key:Row factor (P=0.0233),Column factor (P=0.2592),P>0.05: Not statistically significant

Survival Rate of Experimentally Infected Birds

Analysis of the survival rate of the experimentally infected birds using Chi square showed that comparisons between the groups were not statistically significant (P>0.05). However, the survival rate was higher numerically (80%, 85%, 70% and 90%) in the medicated groups compared to the infected untreated group E (55%). Among the extract medicated groups, survival rate was numerically higher in the 33mg/kg treated group (85%) which was comparable to the untreated uninfected group (85%) while the least was in the 99 mg/kg treated group (70%). The conventional drug Amprolium treated group maintained the highest survival rate in the medicated group of 90% (Table VI).

Histopathological Lesions

The histopathological lesions observed were the sloughing of the mucosal surface into the intestinal lumen in the 11mg/kg treated group one week post infection (Plate 13) and desquamation of intestinal epithelium/villi with sloughing into the intestinal lumen in the infected untreated group one week post treatment (Plate 14). However, 4 weeks post treatment, there was reduction and desquamation of the intestinal villi with intact intestine in the 99mg/kg treated groups (Plate 15). No significant findings were seen in Amprolium, uninfected untreated groups as well as all the other groups.

TABLE VI: Survival rate of experimentally infected birds with *E tenella* and treated with different doses of *Khaya senegalensis*

GROUP	Doses	TOTAL	Alive	%	Dead	%	Chi-square	df	P value
A (Extract)	11mg/kg	20	16	80	4	20	9.606	5	0.0872
B (Extract)	33mg/kg	20	17	85	3	15			
C (Extract)	99mg/kg	20	14	70	6	30			
D (Amprolium)	1.2gm/L	20	18	90	2	10			
E (Positive control)	0.2ml(water)	20	11	55	9	45			
F (Negative control)	0.2ml(water)	20	17	85	3	15			

Key : df- Degree of freedom, P>0.05: Not statistically significant

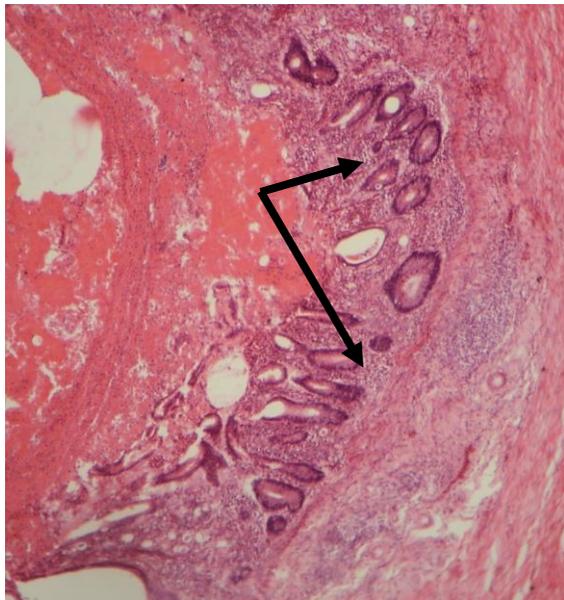


Plate 13: Sloughing of the mucosal surface into lumen of intestine of infected and extract treated (11mg/kg) group one week post infection (arrows) (H and E) × 40 magnification

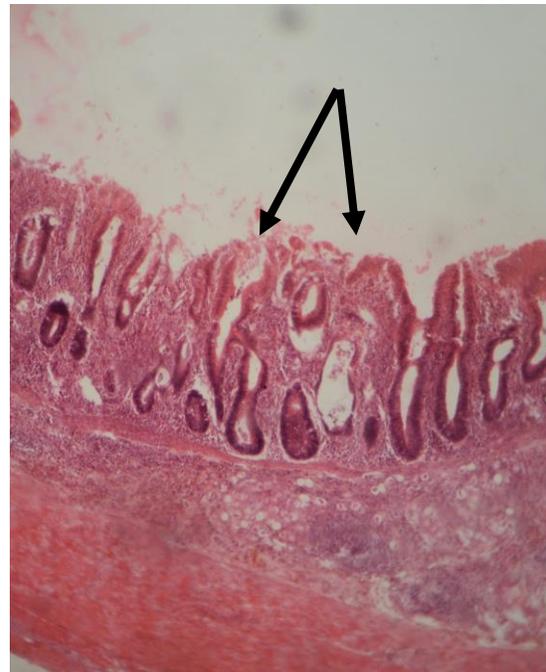


Plate 14: Desquamation of intestinal epithelium/villi with sloughing into intestinal lumen of Positive control (infected untreated group) one week post treatment (arrow). (H and E) × 40 magnification

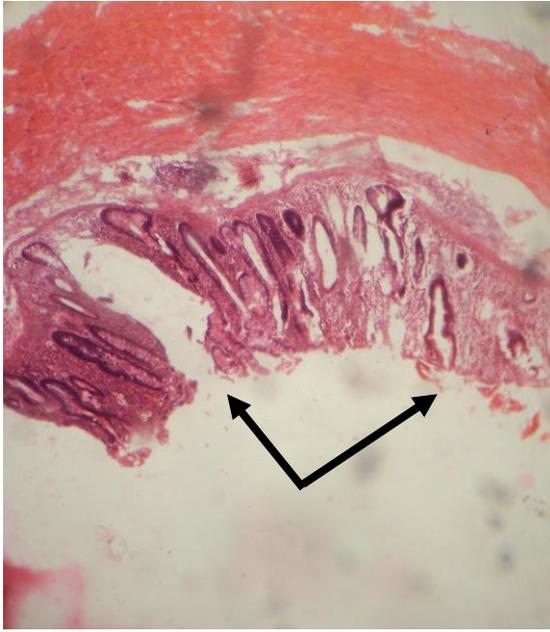


Plate 15: Reduced and desquamation of villi with intact intestines in infected and treated bird with 99 mg/kg four weeks post treatment (arrows). (H and E) × 40 magnification

DISCUSSION

The results of the chromatography showed that *Khaya senegalensis* has phenols and flavonoids which are secondary metabolites. Studies have shown that sources of natural antioxidants (Emami, 2007) are primarily plant phenolics such as flavonoids that exhibit antioxidant, antimicrobial, anti carcinogenicity and other biological activities (Aziman *et al.*, 2012) and recent pharmacological studies have proven that flavonoids possess antiviral, antibacterial and anti inflammatory activities *in vitro* (Liu *et al.*, 2015). They also have high antioxidant activity and may have anticoccidial effects (Ferreira *et al.*, 2010). Antioxidants which are defined as substances present at low concentration relative to the oxidizable substrate which significantly delay or prevent oxidation of the substrate (Anyasor and Ogunwenmo,

2010; Okugbo and Oriakhi, 2015). Free radicals are inevitably produced in biological systems and also exogenously when they are in excess cause damaging effects on cells and antioxidants combat free radicals by intervening at any one of the three major steps of the free radical mediated oxidative processes viz initiation, propagation and termination (Kedare and Singh, 2011). Studies show that the human body does not synthesize overwhelming amounts of anti oxidants (Halliwell, 1990) and this indirectly can be related to poultry to compensate for the damaging effects of reactive oxygen species (ROS) and oxygen free radicals (Anyasor and Ogunwenmo, 2010). The production of synthetic antioxidants such as butylated hydroxyl toluene, gallic acid esters and tertiary butylated hydroquinone which have the potential to neutralize free radicals, have been criticized due to possible toxic effects, low solubility along with moderate antioxidant activity (Kothari and Seshadri, 2010). Anti oxidant from plant sources are currently receiving increasing attention due to their potential health benefits, availability and affordability (Omoriegbe *et al.*, 2014). Also anti oxidant compounds such as flavonoids containing compounds which inhibit growth of pathogens and are least toxic to host cells are good candidates for new drug developments (Aziman, *et al.*, 2012). This can be applied to *Khaya senegalensis* which can have its natural antioxidant compounds harnessed for therapeutic purposes due to its availability and affordability as well as its beneficial health implications.

The greenish colouration obtained when Ferric chloride was sprayed on the plate is in agreement with Anyasor *et al.* (2010) in which he also obtained greenish colouration when ferric chloride was sprayed on flavonoids containing extracts. The result of the chromatography also implied the active ingredients were present mainly in the n-butanol and crude extract fractions. The high tendency of polyphenols to chelate metal ions may contribute to their antioxidant activity by preventing redox-active transition metals from catalyzing free radical formation and may be responsible for the greenish colouration seen when ferric chloride was sprayed on the extract (Aude and Edwards-Levy, 2011).

The large R_f values obtained indicate that non polar compounds were mainly present in the n-butanol, aqueous and crude extract fractions. This implies that the phenolic compounds present in *Khaya senegalensis* were non polar and so would not easily dissolve in polar solvents example of which is water. Also the speed with which the spots easily moved up the plate suggested they had less attraction for the stationary phase. This is in agreement with Cannell (1998) who reported that non-polar compounds have less affinity for the stationary phase and will move comparative very quickly up the plate and therefore have relatively larger R_f values.

The clinical signs and lesions observed in the birds challenged with *Eimeria tenella* such as bloody diarrhoea, high mortality and distended caeca with bloody faeces and mucous debris on the sixth day experimental infections are in agreement with the previous studies (Patra *et al.*, 2010; Zaman

et al., 2012; Meskerem *et al.*, 2013). The severity of the lesions observed in this study could be as a result of the challenge of 1×10^5 of sporulated oocysts of *Eimeria tenella* given to each of the experimental groups A-E respectively. This is similar to the results obtained by Conway *et al.* (1993) in a study in which he challenged birds with 1×10^4 of sporulated oocysts of *Eimeria maxima* and *Eimeria acervulina*. However, Zulpo *et al.* (2007) in a later study had lower severity of infection when he challenged birds with 2×10^4 of sporulated oocysts of *Eimeria tenella*, *Eimeria maxima* and *Eimeria acervulina*.

The high mean oocyst production (A; 156060 ± 67020 , B; 261590 ± 144310 , C; 211620 ± 114280 , D; 276930 ± 233650 and E; 159230 ± 100970) obtained among the infected groups one week post infection is in agreement with Myung-Jo. (2014) where the peak production of oocyst production for a comparative study on *Eimeria tenella*, *Eimeria acervulina* and *Eimeria maxima* showed that *Eimeria tenella* and *Eimeria maxima* both had peak productions on the 7th day. Also the irregular oocyst production observed in this study is in agreement with Myung-Jo. (2014) who had similar results. The higher mean oocysts count obtained in the infected untreated group (1748849 ± 40869) than the extract treated group in the first week post treatment indicated that the extract had some inhibitory effects on oocyst production. This however, depends on the dose of the extract administered. This is again in agreement with Myung-Jo. (2014) who stated that the inherent difference in reproductive potential is high for *Eimeria tenella* which are dependent on some factors

such as the potential to produce in a non-immune host, immunity or resistance of the host, nutrition and strain differences of the host. Among the extract medicated group, the high mean oocyst production by the 11mg/kg group (13840 ± 1600) 2 weeks post treatment suggests the dose was not adequate to prevent oocyst production in contrast to the 99mg/kg which had lower mean oocyst production (9720 ± 3180) and was comparable to the Amprolium treated group (8600 ± 40), thus was the effective dose. This was also in line with the statistical result which showed statistical significance ($P < 0.05$) when the groups were compared with time. Also the anticoccidial effect of the extract on the reproductive potentials of the parasite could be seen in the infected untreated group which had a mean oocyst output of 29570 ± 9290 at three weeks post treatment compared to the 99mg/kg treated with mean oocyst output of 1920 ± 680 . Amprolium, however, in this case had a mean oocyst output of 7000 ± 4900 at three weeks post treatment. This result is in agreement with Nwosu *et al.* (2011) who reported that in a study conducted, *K senegalensis* had some anticoccidial effect mediated by reduction in oocyst production.

The presence of schizonts and merozoites seen in caecal scrappings showed that reproductive activities of the parasites were not completely halted even after treatment. It also shows that the endogenous stages were not susceptible to the extracts at all doses although the population in the medicated infected group were lower than those seen in the infected un medicated groups. However, grossly the extract had a

beneficial effect in alleviating the damages to the caecal epithelium of the infected treated group. This is in agreement with Al-Fifi, (2007) who suggested the phenolic contents of extracts possessed anti inflammatory properties which might act in the caecal epithelium cell protection which is detrimental to the coccidia reproductive activities. The shrunken caeca of the infected untreated group compared to the intact caeca of the medicated and un medicated groups is in agreement with Jordan (1990) who associated this with the effect of the coccidia organism on the caecal epithelium. The uninfected untreated group had no parasite found in them one week post infection.

The survival percentage was higher in the treated groups compared to the infected un treated group (55%). Comparisons of the groups with each other yielded no statistical significance ($P > 0.05$), however, Amprolium was highly efficacious in the *in vivo* studies with the highest survival rate (90%) among the infected and treated groups. Among the extract treated groups, the survival rate was numerically higher in the 33mg/kg (85%) treated group which was comparable to the un infected untreated group (85%) while the least was in the 99mg/kg treated group (70%). The inverse trend in the survival rate and the dose of the extract can also not be easily explained as the expectation should be higher dose, higher survival rate. The higher survival rate at lower doses could be as a result of the therapeutic effect of the phenolic components of the plant (Otu *et al.*, 2016). These antioxidants could have played a role in the survival of the birds as they have been

identified in helping to block the action of/or inactivate lipid free radicals or by preventing the decomposition of hydroperoxides into free radicals which have been implicated in several stresses related to gastrointestinal injuries (Pourmorad *et al.*, 2006; Sani *et al.*, 2013). This potential mechanism makes the diverse group of phenolic compounds an interesting target in search for health beneficial phytochemicals (Akinpelu *et al.*, 2010).

The histopathological lesions observed in the infected birds in this study are consistent with those associated with *Eimeria tenella* infection in which the parasite induced very severe lesions including severe villous atrophy and fusion of the villi (McDougald and Reid, 1997; Khadim, 2014). The most pathogenic stage of *Eimeria tenella* is mainly due to the second generation schizonts which cause extensive tissue damage, bleeding, disruption of caecal glands and the destruction of the mucosa and the muscularis layer (McDougald and Fitz-Coy, 2008). The lesions still observed in the 99mg/kg group 4 weeks post treatment shows the irreversible damages done to the intestine by the *Eimeria tenella* parasite even though physically the birds appeared to have overcome the infection.

CONCLUSION

The anticoccidial efficacy of *Khaya senegalensis* in relation to oocyst shedding promises greater areas for research as it relates to drug development. This was demonstrated with some of the anticoccidial activity *in vivo* in which the n-butanol fraction of the extract at a dose of 99mg/kg reduced the mean oocysts counts of *Eimeria*

tenella to 9720 ± 3180 two weeks post treatment and this was comparable to the standard drug (Amprolium; 8600 ± 40). Also the *in vivo* model was found to be useful for quick and verifiable anticoccidial potency of plant preparations against *Eimeria* parasites. It is recommended that *Khaya senegalensis* should be exploited further for its anticoccidial properties *in vitro* and *in vivo* using other parts of the plant.

Conflict of Interest

The authors declare no conflict of interest.

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