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ORIGINAL ARTICLE

Effects of N-Butanol and Aqueous Fractions of *Khaya senegalensis*, *Guiera senegalensis* and *Tamarindus Indica* Leaves Extracts on *Eimeria tenella* Oocyst Sporulation *in Vitro*

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SUMMARY

The *in vitro* anti coccidial activities of n-butanol and aqueous fractions of *Khaya senegalensis*, *Gueira senegalensis* and *Tamarindus indica* leaves extracts on *Eimeria tenella* parasite was studied by observing the effects of the plant extracts on the sporulation of the *Eimeria tenella* oocysts. Drug resistance and consumer demanding decrease in the use of drugs in animals have generated interest in alternative strategies to control the avian disease one of which is herbal intervention studies. Fresh faecal samples were collected from infected birds and their oocysts load determined. Dilutions of the extracts (100mg, 250mg, 400mg, 550mg, 700mg and 850mg per ml) in distilled water were prepared and placed in separate well labelled petri dishes. 100 oocysts were added to each petri dish and the set up was left at ambient temperature on the laboratory table and monitored twice daily (9.00am and 3.00pm) to observe the sporulation of the oocysts over a 72 hours period. Laboratory sporulation medium (2.5% Potassium dichromate) and Amprolium were used for comparison. The phytochemical result implied that the active ingredients were present mainly in the n-butanol and crude extract fractions with the n-butanol fraction of the *Khaya senegalensis* extract containing phenols and flavonoids which have antioxidant effects. The *in vitro* study showed that the n-butanol fraction of *K senegalensis* (100mg/ml) and the aqueous fraction of *Tamarindus indica* (100mg/ml) extracts had similar effects to those obtained using Amprolium which was considered as the standard by inhibiting the sporulation of *Eimeria tenella* oocysts. It is therefore recommended that more work needs to be done to determine the anti coccidial activities of these extracts *in vivo*.

Key words: *Eimeria tenella*, sporulation, *in vitro*, extracts

INTRODUCTION

Coccidiosis is one of the most lethal and detrimental management diseases of poultry and is often characterised by marked morbidity, mortalities and reductions in productivity and feed conversion efficiency of affected chickens (Anosa and Okoro, 2011). Avian coccidiosis is caused by the infection of chickens with extremely well adapted *Eimeria* specie (Chapman, 2001). Amongst the seven species of *Eimeria* commonly detected in infected chicken (Patra *et al*., 2010), *Eimeria tenella* is the most virulent causing caecal coccidosis (Chandrakesan *et al*., 2009). The disease which causes considerable economic losses in both layers and broilers industries, has been reported to have current expenses for preventive medication exceeding 90 million dollars in the United States and more than 300 million worldwide (Al-Fifi, 2007; Chandrakesan *et al*., 2009). Although coccidiosis has remained the most important poultry disease in Nigeria due to its endemicity, records of losses due to disease at clinical and sub clinical levels and the cost of control is appreciable and reprehensible (Obasi *et al*., 2006; Mikail *et al*., 2007).

Anti coccidial drugs have been used extensively to control the disease (Chapman,2001) but the increase of resistant parasite population necessitates the need to find alternative targets and drugs (Chandrakesan *et al*., 2009). Also prophylactic drug usage creates anxiety in a consuming public already concerned with chemical residues in food (Alawa *et al*., 2003). Consequently the past two decades have witnessed great interest in alternative strategies to control the avian disease (Alawa *et al*., 2003; Tipu *et al*., 2006). 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* used to treat coccidiosis, helminthosis and diarrhoea in poultry (Gefu *et al*., 2000). In Nigeria, the bark of *Khaya senegalensis* is used to treat coccidiosis, helminthosis, amoebic dysentery and diarrhoea in poultry (Gefu *et al*., 2000). It has also been reported to have some therapeutic effects against *Trypanosoma* species (Otu *et al*., 2009). Anti oxidants and flavonoids are possible candidates in natural products which occur naturally (Taheri *et al*., 2005) with many medicinal plants been good sources (Emami, 2007) and are stored in different parts of some plants (Bhakuni *et al*., 2001; Brisibe *et al*., 2009). 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). Oxidants can be produced at elevated rates under patho-physiological conditions (Meskerem and Boonkaewwan, 2013). Phenolic compounds could be a major determinant of antioxidant potentials of foods and are herefore a natural source of antioxidants (Aberoumand and Deokule, 2008). Similarly, *Tamarindus indica* works as a purgative, diaphorectic and an antihelminthic and is also said to have anti trypanosomal activity (McCorkle *et al*., 1996; Gefu *et al*., 2000). *Gueira senegalensis* is widely used in traditional medicine in West Africa (Males *et al.,* 1998)

and is known to have antimicrobial effects (Sanogor *et al.*, 1997). However, the effect of leaf extracts of the n-butanol and aqueous fractions of these plants *in vitro* on *Eimeria tenella*, another important protozoan parasite are not known. The present study is therefore conceived to bridge the gap of this knowledge and probably contribute towards finding solutions to effective treatment and control of avian coccidiosis in Nigeria.

MATERIALS AND METHODS

Collection and Preparation of Plant materials for Extraction: Fresh leaves of *Khaya senegalensis* (KS), *Gueira senegalensis* (GS) and *Tamarindus indica* (TI) were collected from the environs of Ahmadu Bello University in the months of March and April due to their high bioactive content at these months (Ademola and Eloff, 2010) and dried under shade. They were identified as those of *K. senegalensis, G. senegalensis* and *T. indica* by staff of the Botany section of the Department of Biological Science, Ahmadu Bello University, Zaria, with the following voucher numbers 900181, 900141 and 900265 respectively (Otu *et al*.,2009). Ten kilogramme (10kg) each of the dried leaves of *Khaya senegalensis*, *Gueira senegalensis* and *Tamarindus indica* were ground to powder using mortar and pestle and sieved with two kilogramme (2kg) each of the powder for KS, GS and TI each respectively were defatted with 5 litres of Petroleum ether in a Soxhlet apparatus (Quick fit corning Ltd; Stafford, England) at 70^0C (Youn and Noh 2001). The leaves were air dried at room temperature and then extracted by maceration method for 72 hours using 70% methanol. The crude extracts were evaporated to dryness on a water bath at a temperature of 90^0C 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) each of the dried crude methanol extract for KS, GS and TI each were suspended in 300ml of water and each 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 90^0C for 6 hours and the portion tested for *in vitro* anticoccidial activities. The dried fractions were stored in a dessicator in well labelled containers until when needed.

Phytochemical analysis: The phytochemical components of the separated portions of the extracts were then eludated by Thin Layer Chromatography (TLC) according to the method of Agrawal and Paridhavi, (2007) and Brain and Turner, (1975). Silica gel coated thin layer chromatography plates were marked with a pencil drawn at 1.5cm (centimeter) to identify the origin. Using a microhaematocrit capillary tube(non heparinised), the fractions to be tested

(which were as follow; Pet-ether, chloroform, n-butanol and the crude extract of *Khaya senegalensis* which was dissolved in methanol) were drawn into the tube and separate spots made on the 1cm mark dot on the plate. The spots were allowed to air dry for 15 minutes and were then placed in a TLC jar containing the test solvent system which in this case was n-butanol: acetic acid: water at the ratio of 6:1:2 (Plate 1). The plates placed in the TLC jar were allowed to saturate for 15minutes before the set up was allowed to run while being monitored (Plate 1). The plates were removed and a pencil was used to mark the end point. Pictures were taken before and after the detecting agents were sprayed on the plate .The following detecting agents were used to spray the plates; Vanillin in 10% hydrogen sulphate, 10% hydrogen sulphate and Ferric chloride.

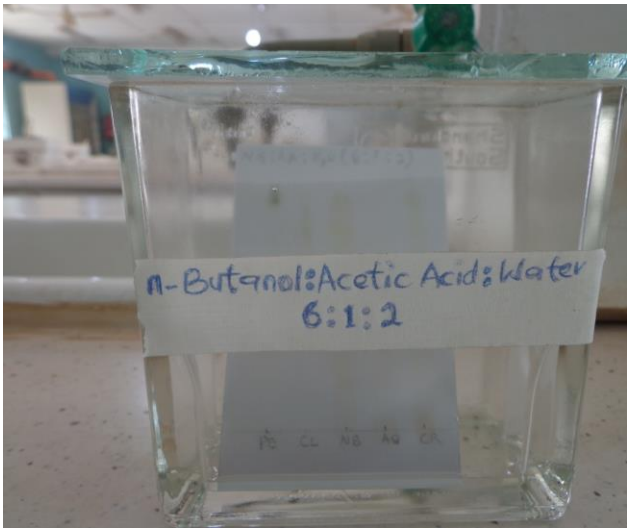

Plate 1: Thin Layer Chromatography Tank containing TLC plate eluted in the solvent system of n-butanol, acetic acid and water (6:1:2)

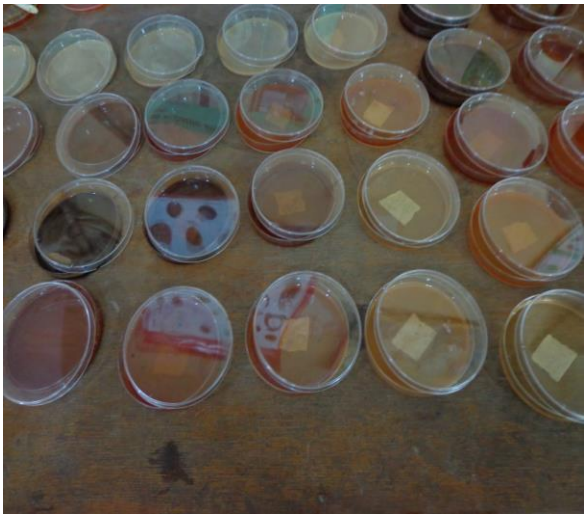

Plate 2: *In vitro* experiment setup with the suspension of the n-butanol fractions of the three Plant Extracts (*Khaya senegalensis, Guiera senegalensis* and *Tamarindus indica*) and *Eimeria tenella* oocysts

Inoculum: *Eimeria tenella* oocysts were obtained locally from the intestinal scrapings of infected broiler birds and were sporulated using 2.5% potassium dichromate. The sporulated oocysts were filtered using a sieve, centrifuged (450g) for 10 minutes to remove the sporulating agent and diluted with distilled water (Reck and McQuistion, 1994). The sporulated oocysts were kept in the refrigerator at 4^0C until when they were needed.

Experimental birds: Twenty broiler chicks brooded under standard practice aged three weeks, were sourced from the National Animal Production Research Institute (NAPRI) and used for the propagation of the *Eimeria tenella.*

Infection of Experimental Birds: The chicks were infected orally at 4 weeks of age

with 3×10^3 sporulated oocysts /0.1ml / bird using insulin syringe (Reck and McQuistion, 1994).

Anticoccidial: Amprolium (Amprolium 200 NTCOX 20%, SAM pharmaceuticals LTD, Nigeria) which is routinely used as an anticcocidial for the treatment of coccidiosis was used to compare the anticoccidial effects of the three plant extracts.

***In vitro* Study:** The method described by Nwosu *et al*., 2011 was used. The *in vitro* anti coccidial efficacy trials was conducted by observing the effect of the plant extracts on the sporulation of the *Eimeria tenella* oocysts. Fresh faecal samples were collected from infected birds and the oocysts load determined. Using the method described by Nwosu *et al*., (2011) dilutions of the extracts (100mg, 250mg, 400mg, 550mg, 700mg and 850mg per ml) in distilled water were prepared and placed in separate petri dishes labelled appropriately. One hundred oocysts were added to each petri dish and the set up was left at ambient temperature on the laboratory table and monitored twice daily (9.00am and 3.00pm) to observe the sporulation of the oocysts over a 72 hours period. The percentage sporulation of the oocysts was determined and recorded. The findings were used to determine percentage inhibition of oocysts sporulation. A parallel experiment using laboratory sporulation medium 2.5% Potassium dichromate and Amprolium were used for comparison (Plate 2).

RESULTS

Extraction of plant material and partitioning of crude extracts: The extraction of 2kg of the leaves of *Khaya senegalensis* (KS), *Guiera senegalensis* (GS) and *Tamarindus indica* (TI) with 70% methanol as solvent gave a yield of 436.42g (21%), 496.41g (24.82%) and 394.20g (19.71%) respectively. The colour of the extract was dark brown for both KS and GS while TI had a reddish brown colour (Table 1). The extractive yield output of the partitioned portions of the crude methanol extracts of *Khaya senegalensis, Guiera senegalensis* and *Tamarindus indica* 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%) for KS, 0.03g (0.1%), 0.12g (0.4%), 6.83g (22.77%) for GS and 0g (O%), 0.33g (1.1%), 4.5g (15%) for TI. The aqueous yield were as follows; 13.47g (44.9%), 8.65g (28.83%), and 8.58g (28.6%) respectively for KS, GS and TI. The petroleum ether portion was greenish in colour for KS and GS and very faint colour of green for TI was. The chloroform, n-butanol and aqueous methanol portions were brown, reddish brown and brown respectively for KS and GS and reddish brown for TI (Table 2).

Phytochemical Analysis: The thin layer chromatography plate took 90 minutes to run in the Thin Layer Chromatograpy (TLC) tank. The retention factor was determined. The petroleum ether spot had a retention factor 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 which gave greenish coloured spots which signified the presence of phenolic compounds. The greenish colourations were mainly seen in the n-butanol and crude extract fractions of the plant (Plate 2). All the other detecting agents used did not yield any positive colouration.

Table 1: Crude extractive yield of the leaves of *Khaya senegalensis, Guiera senegalensis* and *Tamarindus indica* used in the study

Plant	*Khaya senegalensis*	*Guiera senegalensis*	*Tamarindus Indica*
Plant part collected	Leaves	Leaves	Leaves
Quantity of Plant material collected	10kg	10kg	10kg
Quantity of Plant sample extracted	2kg	2kg	2kg
Output of crude extract/Percentage Percentage yield after Extraction	436.42g (21%)	496.41g (24.82%)	394.20g (19.71%)
Colour of Extract	Dark brown	Dark brown	Reddish brown

Table 2: Fractional yield output of *K. senegalensis, G senegalensis* and *Tamarindus indica*

Plant	**Fractionated Portions**			
	Petroleum ether/ Colour	**Chloroform/ Colour**	**n-butanol/ Colour**	**Aqueous Fraction/ Colour**
Khaya senegalensis	0.01g (0.03%) Greenish	0.25g (0.83%) Brown	6.73g (22.43%) Brown	*13.47 (44.9%) Brown
Guiera senegalensis	0.03 (0.1%) Greenish	0.12 (0.4%) Brown	*6.83 (22.77%) Brown	8.65 (28.83%) Brown
Tamarindus indica	0 (O %) Faint Green	0.33 (1.1%) Brown	4.5 (15%) Reddish Brown	8.58 (28.6%) Reddish Brown

Table 3: 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- centimeter

$$Sof = 7.6cm$$

$$Spa = 3.5cm = \frac{3.5}{7.6} = 0.46cm$$

$$Spb = 4.6cm = \frac{4.6}{7.6} = 0.61cm$$

$$Spc = 5.4cm = \frac{5.4}{7.6} = 0.71cm$$

$$Spd = 6.4cm = \frac{6.4}{7.6} = 0.84cm$$

$$Spe = 6.9cm = \frac{6.9}{7.6} = 0.91cm$$

***In vitro* Study:** The n-butanol suspension for *Khaya senegalensis* investigated showed that no sporulation occurred in any of the concentrations (100mg/ml, 250mg/ml, 400mg/ml, 550mg/ml, 700mg/ml and 850mg/ml) while the aqueous fraction had sporulated oocyst present in all the concentrations except the 100mg/ml group (Plates 4 and 5). The n-butanol suspension for *Guiera senegalensis* (400mg/ml, 550mg/ml, and 700mg/ml) and *Tamarindus indica* (250mg/ml and 400mg/ml) as well as the aqueous fraction of *Guiera senegalensis* did not inhibit the sporulations of the *E tenella* oocysts (Plates 6 to 7 and Table 4). However, the aqueous fraction of *Tamarindus indica* had inhibited sporulation. The sporulation medium (2.5% Potassium dichromate) did enhance sporulation of the oocysts (Plate 8). The n-

butanol fraction of the *Khaya senegalensis* had the most anticoccidial activity among the fractions tested. The extract inhibited sporulation by 100% while *Guiera senegalensis* had the least anti coccidial activity with 49% sporulation inhibition. Amongst aqueous fractions, *Tamarindus indica* showed the most anticoccidial activity with a sporulation inhibition of 100% while *Khaya senegalensis* and *Guiera senegalensis* had the least. The n-butanol fraction of *K senegalensis* and the aqueous fraction of *Tamarindus indica* extracts had similar effects to those obtained using amprolium which was considered as the standard. Amprolium completely inhibited the sporulation of the oocysts in this study (Table 5).

Plate 3: Thin Layer Chromatography 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.

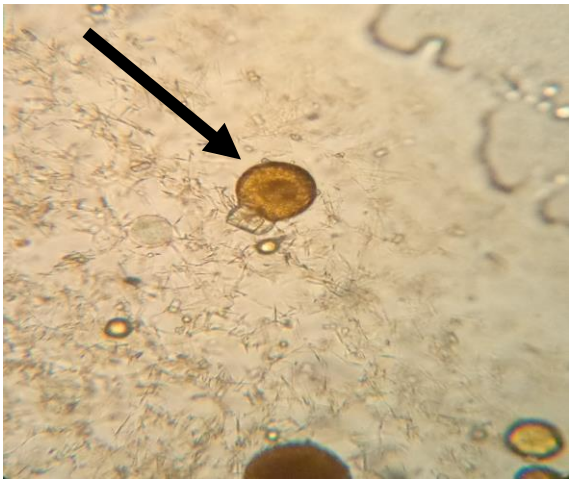

Plate 4: Sporulating *Eimeria tenella* oocyst (arrow) in aqueous fraction of *Khaya senegalensis* extract (700mg/ml) at × 40 magnification).

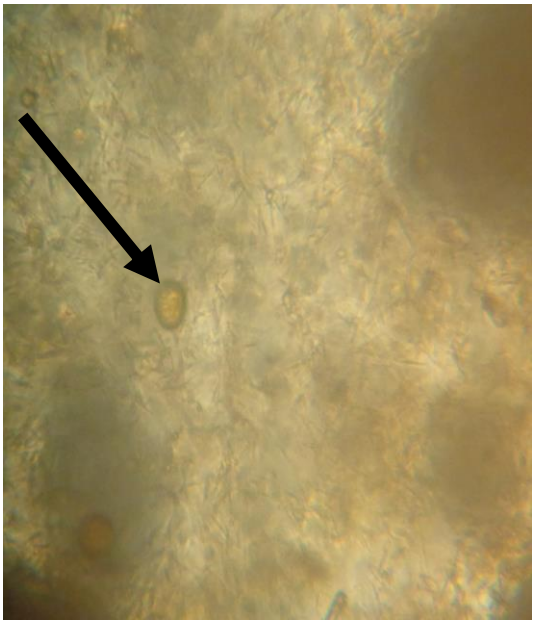

Plate 5: Unsporulated *Eimeria tenella* oocyst (arrow) in n- butanol fraction of *Khaya senegalensis* extract (850mg/ml) at × 40 magnification

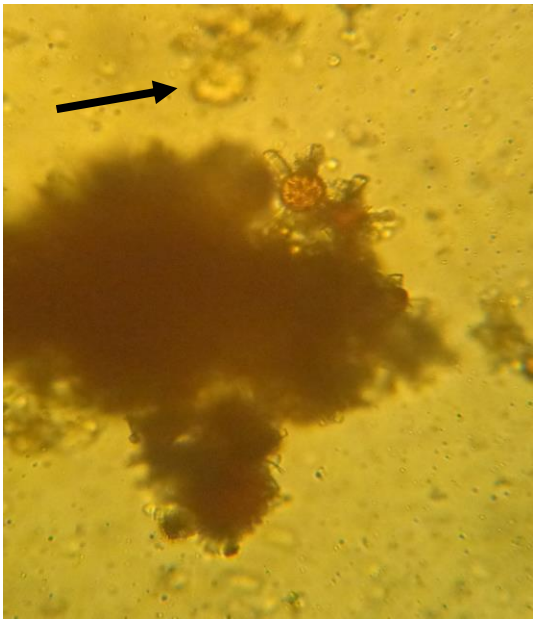

Plate 7: Sporulating *Eimeria tenella* oocyst in n-butanol fraction of *Guiera senegalensis* leaf extract (850mg/ml) at × 40 magnification

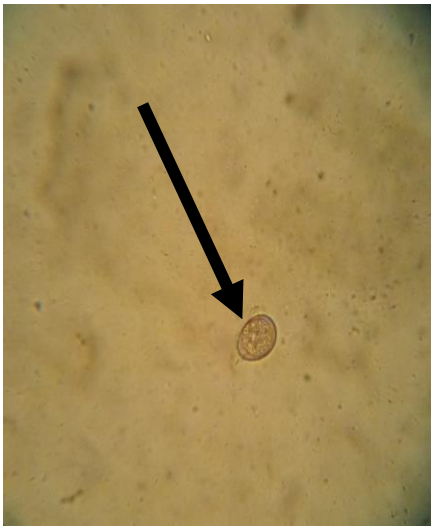

Plate 6: Sporulating *Eimeria tenella* oocyst in aqueous fraction of *Guiera senegalensis* leaf extract (250mg/ml) at × 40 magnification

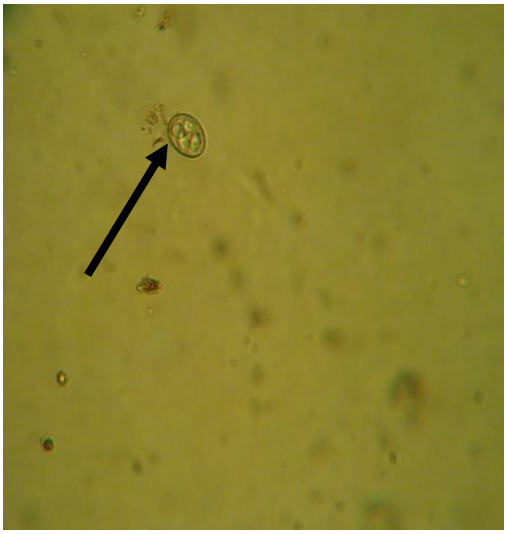

Plate 8: Sporulated *Eimeria tenella* oocyst (arrow) in 2.5% Potassium dichromate sporulation medium at × 40 magnification

Table 4: The effects of n-butanol and aqueous leaf extracts of *Khaya senegalensis, Guiera senegalensis and Tamarindus indica* extracts on sporulation of the *Eimeria tenella* oocysts

Extract	Concentration(mg/ml)	Percentage Sporulation (%)	
		n-butanol Fraction	Aqueous Fraction
Khaya senegalensis	100	0	0
	250	0	17
	400	0	17
	550	0	17
	700	0	17
	850	0	17
Guiera senegalensis	100	0	17
	250	0	17
	400	17	17
	550	17	17
	700	17	17
	850	0	0
Tamarindus indica	100	0	0
	250	17	0
	400	17	0
	550	0	0
	700	0	0
Amprolium	1.2mg/ml	0	
Potassium dichromate	2.5%	100	

Table 5: Percentage Sporulation Inhibition of n-butanol and aqueous leaf extracts of *Khaya senegalensis, Guiera senegalensis* and *Tamarindus indica*

Drug/Extract	Fractions	Percentage (%)		Total
		Sporulation	Sporulation Inhibition	
Khaya senegalensis	n-butanol	0	100	100
	Aqueous	85	15	100
Guiera senegalensis	n-butanol	51	49	100
	Aqueous	85	15	100
Tamarindus indica	n-butanol	34	66	100
	Aqueous	0	100	100
Amprolium	-	0	100	100
2.5% Pottassium dichromate	-	100	0	100

DISCUSSION

The phytochemical results 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, anticarcinogenicity 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 oxidizeable 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 via 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, they 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 (Omoregie *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 and Ogunwenmo (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 antioxidant activity of *Khaya senegalensis is* not only dependent on the phenolic content but also

the phenolic acid component (Horax and Islam, 2005). Polyphenolics possess hydroxyl and carboxyl groups able to bind to metal ions bearing strong positive charges such as iron III and copper II (Aude and Edwards-Levy, 2011) and this may directly contribute to the antioxidant activity and is a key determinant of their scavenging and metal chelating activities (Elmastas *et al*.,, 2007). 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 aqueous extract of *Khaya senegalensis* had the least anti sporulation activity due to the presence of sporulated oocysts as against the n-butanol suspension for *K senegalensis.* This suggests that the anticoccidial activity was absent in the aqueous fraction of the plant leaves extract. Traditionally *Tamarindus indica* has been said to have anti protozoal activities Gefu *et al*. (2000), however, the sporulation medium (2.5% Potassium dichromate) did enhance sporulation of the oocysts. Lack of inhibition of sporulation by the n-butanal fractions of *Guiera senegalensis* and *Tamarindus indica* as seen in these studies may be attributable to the absence of anticoccidial factor in their extracts. The n-butanol fraction of *K senegalensis* and the aqueous fraction of *Tamarindus indica* extracts had similar effects to those obtained using Amprolium which was considered as the standard. Amprolium which has been proved to reduce oocyst sporulation (Arakawa *et al*., 1981) also completely inhibited the sporulation of the oocysts in this study. Consequently the mechanism of action of n-butanol of *K. senegalensis,* aqueous fractions of *T indica* and Amprolium may show some similarities.

The n-butanol suspension for *Khaya senegalensis* investigated showed that no sporulation occurred in it while the aqueous fraction had sporulated oocyst present in all the concentrations except the 100mg/ml group. This is in agreement with Nwosu *et al*., (2011) who observed that the aqueous extract of *Khaya senegalensis* had the least anti sporulation activity. The n-butanol suspension for *Guiera senegalensis* (400mg/ml, 550mg/ml, and 700mg/ml) and *Tamarindus indica* (250mg/ml and 400mg/ml) as well as the aqueous fraction of *Guiera senegalensis* did not inhibit the sporulations of the *Eimeria tenella* oocysts. However, the aqueous fraction of *Tamarindus indica* recorded inhibited sporulation. Traditionally, *Tamarindus indica* has been said to have anti protozoal activities (Gefu *et al*., 2000). The sporulation medium (2.5% Potassium dichromate) did enhance sporulation of the oocysts (Table 4).

Khaya senegalensis n-butanol fraction had the most anticoccidial activity in which there

was total sporulation inhibition while *Guiera senegalensis* had the least anti coccidial activity with 49% sporulation inhibition. The higher anticoccidial activity recorded with n- butanol might indicate that the active ingredients are more soluble in this solvent than in the aqueous fractions. Amongst aqueous fractions, *Tamarindus indica* showed the most anticoccidial activity with a sporulation inhibition of 100% while *Khaya senegalensis* and *Guiera senegalensis* had the least. The n-butanol fraction of *K senegalensis* and the aqueous fraction of *Tamarindus indica* extracts had similar effects to those obtained using Amprolium which was considered as the standard. Amprolium which has been proved to reduce oocyst sporulation (Arakawa *et al*., 1981) also completely inhibited the sporulation of the oocysts in this study (Table 5).

CONCLUSION

The n-butanol fractions in this study had higher anti coccidial activities than the aqueous fractions generally. *Khaya senegalensis* (n-butanol fraction) and *Tamarindus indica* (aqueous fraction) had the most anti coccidial activity which were comparable to Amprolium. The result of the phytochemistry also implied the active ingredients were present mainly in the n-butanol and crude extract fractions and the n-butanol fraction of the *Khaya senegalensis* extract contains phenols and flavonoids which have antioxidant effects. It is therefore recommended that more work needs to be done to determine the anti coccidial activities of these extracts *in vivo*.

Conflict of Interest

The authors declare no conflict of interest.

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