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In vitro Anticoccidial Activity of Methanolic Leaves Extract of *Lannea schimperi* Against Oocysts of *Eimeria tenella*

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Abstract: *Eimeria belonging to the phylum apicomplexan protozoa is recognized as the the major parasitic disease of poultry. Caecal coccidioisis caused by Eimeria tenella is a major threat to poultry production. The drugs which can inhibit sporulation process are best choice as preventive measures. Anticoccidial drugs have played a major role in the effective control of avian coccidiosis, but their extensive use has resulted in the emergence of drug resistant coccidian strains which necessitates sourcing of better alternative drugs. The present study is aimed at evaluating the in vitro anticoccidial potential of the methanolic leaves extract of Lannea schimperi. Eimeria tenella isolated from infected chicks was used in this study. The isolated oocysts were divided into two; the first portion was used for testing the efficacy of the different drug solutions on unsporulated oocysts while the second portion was allowed to sporulate in 2.5 % potassium dichromate solution at room temperature (27 ^{O}C) which was used for testing the efficacy of the different drug solutions on sporulated oocysts. The extract was tested at concentrations of 25, 50 and 100 mg/ml. Amprolium at concentration of 1 mg/ml was used as a positive control, while 2.5 % potassium dichromate was used as negative control. The data obtained in this study showed that the methanolic leaves extract of Lannea schimperi possess anticoccidial activity against unsporulated and sporulated oocysts of Eimeria tenella in a dose dependent manner, the extract at concentration of 100 mg/ml inhibited oocyst sporulation (98 %) and inhibited the viability of sporulated oocysts (97 %) similar to that recorded by the standard drug aprolium after 72 hours of incubation at room temperature (27 ^{O}C). The negative control recorded 4 and 2 % efficacy for unsporulated and sporulated oocysts respectively with 96 and 98 % sporulated oocysts. Therefore the present study revealed that methanolic leaves extract of Lannea schimperi possesses anticoccidial principle that may require further scientific elucidations.*

Key words: *Anticoccidial activity, Eimeria tenella oocysts, Lannea schimperi, methanolic leaves extract.*

I. Introduction

Caecal coccidioisis, caused by *Eimeria tenella*, is a major threat to poultry production. Heaping of litter near the poultry farms is customary [1] especially in the developing countries. *E. tenella* oocysts remain infective for nine months in litter [2] and, therefore, become a source of disease for other farms through air current dispersion. The drugs which can inhibit sporulation process are best choice as preventive measures. Ammonia, methyl bromide, carbon disulfide and some phenolic products are commonly used as disinfectants on soil and litter but are related to several pitfalls and public concerns [3]. Coccidiosis is recognized as the major parasitic disease of poultry and is caused by the apicomplexan protozoan Eimeria. In the past, conventional disease control strategies have depended mainly on anticoccidial drugs and, to a certain extent, live vaccines [4]. *Lannea schimperi* is a small tree up to10-15 m tall; bole short, sometimes stunted and low-branching. It is distributed from Togo, northern Nigeria and Cameroon eastward to Ethiopia, southward through Uganda, Kenya, eastern Congo, Rwanda, Burundi, Tanzania and Malawi to Zimbabwe and Mozambique [5]. It is a deciduous shrub or tree with a spreading crown. It can grow from 2-15 m tall. The tree is valued locally for its edible fruit which is commonly harvested from the wild. It also has local medicinal uses and is a source of wood and fibre [5].

*Lannea schimperi*is used in folk medicine to treat a number of illnesses. The potential pharmacologic activities of the extract from the fruit, leaf, bark and roots includes antimicrobial, anti-cough, anti-diabetic, anti-diarrhoeic, anti-vomitting and anti-fungal activities in vivo and/or in animal model [6]. *Lannea schimperi* has been used in various areas. The bark is used to make string and rope. The wood is occasionally used in carpentry, as fuel and is valued for charcoal production. The fruit is eaten fresh by children in Nigeria during rainy season and throughout East Africa. The seed is also eaten. The flowers are probably a source of nectar for honey bees. Branchlets and leaves are eaten by domestic animals [5].

Anticoccidial drugs have played a major role in the effective control of avian coccidiosis, but, their extensive use has resulted in the emergence of drug resistant coccidian strains. In such situations, new drugs should be available to replace the older ones against which resistance has developed, however it takes a long time to develop any new compounds. Because of the high cost of developing new drugs and vaccines, development of drug resistance and concerns over drug residues associated with the continuous use of these chemicals, there is a renewed interest in the use of botanicals for safe, effective and cheap control of avian coccidiosis [4]. Herbal anticoccidials open new perspectives and proved suitable to serve as alternatives to conventional treatment, particularly in countries with limited economic potential [7]. The present study is aimed at evaluating the in vitro anticoccidial potential of the methanolic leaves extract of *Lannea schimperi*.

II. Methodology

Plant material

The fresh plant was collected from Gurfata, Ibwa ward of Gwagwalada area council, Federal Capital Territory (F.C.T), Abuja, Nigeria in the month of February, 2015. Botanical identification and authentication was done by U.S Gallah of National Research Institute of Chemical Technology (NARICT) Zaria, Kaduna State, Nigeria and a voucher specimen numbered 0512 was deposited at the departmental herbarium.

Processing and Extraction

The fresh leaves of *Lannea schimperi* were carefully separated from the other morphological parts of the plant and washed clean with water, air dried under shade for two weeks, pounded with pestle and mortar mechanically into fine particles. Fifty grams (500 g) of the pounded dried plants materials were weighed and extracted by maceration for 72 h in 100% methanol. The methanolic extracts were filtered and evaporated to dryness *in vacuo* and stored in capped bottles inside the refrigerator at 4°C until required.

Preparation of drug and chemical solutions

The dried extract was weighed and reconstituted in distilled water just before use during the experiment, a solution of 25, 50 and 100 mg/ml respectively were prepared by dissolving 2.5, 5 and 10 g of the dried methanolic leaves extract of *Lannea schimperi* into 100 ml each of distilled water. Amprolium 250 WSP (KEPRO B.V./ Holland) was used, 1 g of amprolium was dissolved in 1 litre of water to produce a drug solution of 1 mg/ml, 5 g of poatasium dichromate (**$K_2Cr_2O_7$)** was dissolved in 200 ml of distilled water to make a 2.5 % solution.

***Eimeria tenella* isolate**

Eimeria tenella isolated from infected chicks was used in this study. Coccidial oocysts of *E. tenella* were obtained from the caeca of naturally infected chicks. Sufficient oocysts were recovered after propagation from the caeca of infected chicks using the centrifugal floatation technique [8]. The isolated oocysts were divided into two; the first portion was used for testing the efficacy of the different drug solutions on unsporulated oocysts while the second portion was allowed to sporulate in 2.5 % potassium dichromate solution at room temperature which was used for testing the efficacy of the different drug solutions on sporulated oocysts.

In vitro* anticoccidial effects of methanolic leaves extract of *Lannea schimperi* on oocysts of *Eimeria tenella

Ten millilitres of different concentrations of drug solutions (25, 50, 100 mg/ml of extracts and 1 mg/ml of amprolium) were placed in petridishes labelled A to D, a negative control petridish containing 2.5 % potassium dichromate labelled E was included. Approximately 10 x 10^1 suspensions of unsporulated oocysts were dispensed in each of the petri-dishes A-E. The set up were allowed to stay for 72 hours at room temperature (27^o C), the content of each petri-dish was transferred to a test tube, the oocysts were washed from the test solutions by mixing with distilled water, and it was centrifuged at 200 *g* for 5 minutes 3 times. The anticoccidial activity was determined by counting the proportion of lysed and unsporulated oocysts to that of the sporulated oocysts in wet film preparations using light microscopy at x 40 magnification.

In vitro* anticoccidial effects of methanolic leaves extract of *Lannea schimperi* on oocysts of *Eimeria tenella

The experiment was repeated with the exposure of similar amount of sporulated oocysts of *Eimeria tenella* in petri-dishes containing same concentration the drug solutions (25, 50, 100 mg/ml of extracts and 1 mg/ml of amprolium) and 2.5 % potassium dichromate as control for 72 hours at room temperature (27^o C). The anticoccidial activity was determined by counting the proportion of lysed sporulated oocysts and that of the sporulated unlysed oocysts in wet film preparations using light microscopy at x 40 magnification.

Conflict of Interests
Authors have declared no conflict of interest

III. Results

The data obtained in this study showed that the methanolic leaves extract of *Lannea schimperi* possess anticoccidial activity against unsporulated oocysts of *Eimeria tenella* in a dose dependent manner, the extract at concentration of 100 mg/ml is having the highest activity similar to that of standard drug aprolium with efficacy of 98% (Table I) and percentage inhibition of 97.92 % (Table II) after 72 hours of incubation at room temperature (27 ^{O}C). The extract at concentrations of 25 and 50 mg/ml possess anticoccidial activity with efficacy of 68 and 89 % (Table I) and percentage inhibition of 66.67 and 88.54 % (Table II) respectively. The negative control with 2.5 % potassium dichromate had 96 % of the oocysts sporulated, only 4 % of the oocysts (Table I) were found unsporulated after 72 hours of incubation at room temperature (27 ^{O}C).

The result from this study also showed that the plant extract possess anticoccidial activity against the sporulated oocyst of *Eimeria tenella* in a dose dependent manner, the extract at the highest concentration of 100 mg/ml is having 97 % efficacy (Table III), close to 99% produced by the positive control containing the standard drug amprolium after 72 hours of incubation at room temperature (27 ^{O}C). The extract at concentrations of 25 and 50 mg/ml possess anticoccidial activity with efficacy of 62 and 86 % (Table III) and percentage inhibition of 61.22 and 81.75 % (Table IV) respectively. The negative control with 2.5 % potassium dichromate had 98 % of the oocysts sporulated, only 2 % of the oocysts (Table III) were found unsporulated after 72 hours of incubation at room temperature (27 ^{O}C).

Table I: *In vitro* efficacy of methanolic leaves extract of *Lannea schimperi* on unsporulated oocysts of *Eimeria tenella*

Different concentrations of tested solutions	Oocysts proportion			
	Sporulated	Unsporulated/lysed	Total	Efficacy (%)
25 mg/ml	**32 (32%)**	**68 (68%)**	**100**	**68**
50 mg/ml	**11 (11%)**	**89 (89%)**	**100**	**89**
100 mg/ml	**02 (2%)**	**98 (98%)**	**100**	**98**
2.5 % $K_2Cr_2O_7$	**96 (96%)**	**04 (4%)**	**100**	**4**
1 mg/ml amprolium	**02 (2%)**	**98 (98%)**	**100**	**98**

Table II: Percentage inhibition to oocysts sporulation

Different concentrations of tested solutions	% Inhibition to oocysts sporulation	
25 mg/ml	66.67	≈ 67
50 mg/ml	88.54	≈ 89
100 mg/ml	97.92	≈ 98
2.5 % $K_2Cr_2O_7$	0	
1 mg/ml amprolium	97.92	≈ 98

Table III: *In vitro* efficacy of methanolic leaves extract of *Lannea schimperi* on sporulated oocysts of *Eimeria tenella*

Different concentrations of tested solutions	Oocysts proportion			
	Sporulated	Unsporulated/lysed	Total	Efficacy (%)
25 mg/ml	**38 (38%)**	**62 (62%)**	**100**	**62**
50 mg/ml	**14 (14%)**	**86 (86%)**	**100**	**86**
100 mg/ml	**03 (3%)**	**97 (97%)**	**100**	**97**
2.5 % $K_2Cr_2O_7$	**98(98%)**	**02 (2%)**	**100**	**2**
1 mg/ml amprolium	**01 (1%)**	**99 (99%)**	**100**	**99**

Table IV: Percentage inhibition to sporulated oocycsts viability

Different concentrations of tested solutions	% Inhibition to sporulated oocystsviabilty	
25 mg/ml	61.22	≈ 61
50 mg/ml	85.71	≈ 86
100 mg/ml	96.94	≈ 97
2.5 % $K_2Cr_2O_7$	0	
1 mg/ml amprolium	98.98	≈ 99

IV. Discussion

The drugs which can inhibit sporulation process are best choice as preventive measures against coccidiosis [3].Oocysts of coccidia are very resistant to physical and chemical treatment because of the two proteinous layers of its walls derived from the coalescence of wall-forming bodies found in the macrogamete stage of the parasite [9]. The result of this study showed that the methanolic leaves extract of *Lannea schimperi*

at different concentarations were able to dose dependently weakened the oocysts wall with subsequent lysis of the oocyst which agrees with reports by Guimaraes *et al*.,[10] and Jatau *et al.,* [11]. This ability of the extract to lyse the oocysts inhibited the sporulation of the oocysts as well as the oocysts viability, some of the oocysts were found completely missing in the sample showing that the extract affects the oocysts viability. As reported by Ferguson *et al*.,[12] the oocysts wall of Eimeria is primarily made up of tyrosine rich protein (90%) with relatively small amount of lipid and carbohydrate. The dityrosine crosslinking proteins which subsequently becomes dehydrated ("tanned") and hardened is responsible for the notorious resilience of oocysts[9]. Ferguson *et al.,*[12] reported that the composition of this protein in the walls of sporulated oocysts is reported to be higher than that of unsporulated oocysts of Eimeria. The different concentrations of the plant extracts in this study were able to break and lyse the thicker proteinous walls of the sporulated oocysts as oppose to reports by Jatau *et al*., [11] which shows some disinfectants inability to lyse the walls of sporulated oocysts. Our finding is also in line with the report by Abbas *et a*l.,[4] which shows some plants to possess anticoccidial activity. The result of this study shows the anticoccidial potential of the plant extract.

V. Conclusion

The data generated in this experiment revealed the anticoccidial potential of the methanolic leaves extract of *Lannea schimperi*. However, efficacy *in vitro* atimes does not mean efficacy *in vivo* due certain metabolic processes that occur in the body. Therefore, there is the need to further evaluate the activity of the plant extract *in vivo*. There is also the need to isolate the active anticoccidial principle contained in the plant extract.

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