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EFFECT OF AQUEOUS EXTRACTS OF *Sacoglottis gabonensis* STEM BARK AND LEAVES OF *Azadirachta indica* ON THE SPORULATION OF OOCYSTS OF *Eimeria tenella* AND *Eimeria maxima*

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ABSTRACT

The anticocidial efficacy of aqueous extracts of the stem bark of Sacoglottis gabonensis (SG), leaves of Azadirachta indica (AI) and their equal (w/w) mixture (AI + SG) was evaluated through their effect on the sporulation of a mixture of Eimeria tenella (80%) and Eimeria maxima (20%) oocysts in an in vitro study. Oocyst sporulation was 100% and 78% respectively in 2% potassium dichromate solution and plane water. At their highest concentrations (2,000 mg/ml), the percentage sporulation of the oocysts was 0%, 12%, 0%, 6% and 10% respectively in SG, sulphaquinoxaline (Embazin-forte), AI + SG, AI and amprolium. Using oocyst sporulation in 2% potassium dichromate solution as the standard with 0% percent inhibition of sporulation, the maximum inhibition of oocyst sporulation was 100 % for S. gabonensis, 94 % for A. indica and 100 % for the mixture of the two extracts at their highest concentrations (2,000 mg/kg). These percentage reductions in oocyst sporulation were similar to those due to amprolium (90%) and sulphaquinoxaline (embazin-forte) (88%) at their highest concentrations (1.0 mg/ml and 1.2 mg/ml respectively). In all cases, the inhibition in oocyst sporulation was concentration dependent as the highest percentage inhibition occurred at the highest concentration of the extracts, their mixture or the drugs and vice versa. The results of this study suggest that the crude aqueous extracts of the leaves of A. indica and stem bark of S. gabonensis have anticoccidial activity exhibited through the inhibition of the sporulation of Eimeria oocysts and thus suggest their possible usefulness in the control of coccidiosis in animals.

Keywords**:** *Sacoglottis gabonensis*, *Azadirachta indica*, aqueous extract, efficacy, *Eimeria*

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INTRODUCTION

Avian coccidiosis caused by intracellular protozoan parasites of the genus *Eimeria* constitutes a serious health problem in poultry production [1,2,3]. The disease is ubiquitous and cosmopolitan in poultry establishments, especially those maintained on a deep litter system where pathogenic populations of the

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causative agent easily build up [4]. Coccidiosis is common in Nigeria [5] and the disease is usually characterized by enormous economic losses as a result of the associated high morbidity in the form of enteritis, diarrhea which may be bloody, reduced weight gain and feed conversion efficiency as well as poor performance, mortality and cost of treatment and control programs [6,7,8].

Avian coccidiosis is usually controlled by hygiene and the use of chemical anticoccidial agents among which the synthetic anticocidials such as amprolium and sulphaquinoxaline (sulphanamides) are the most common [9,10]. Treatment of the infection with the synthetic drugs is usually targeted at the developmental stages *in vivo* and this has been associated with the development of resistance against the agents by the parasites. However, the difficulty associated with the discovery of new active anticoccidial substances has necessitated many options including the need to return to older products that have been discontinued for a long time, the introduction of combinations of two or more products and the alternating use of anticoccidials in rotation and shuttle programs [10,11]. Similarly, scientific efforts are concentrating on the use of ancient medicinal systems to find beneficial herbs and plants that may be medicinally useful against coccidiosis [12].

Presently, several plants and their extracts used in traditional veterinary practice have been claimed to have medicinal value in the treatment of various ailments of man and animals in Nigeria [13,14,15]. Although the efficacy and safety of most of these plants and their extracts have remain doubtful, many plants including *Azadirachta indica* (AI) and *Sacoglottis gabonensis* (SG) have been proved scientifically to have medicinal value in the traditional treatment of certain ailments of man and animals [16,17,18,19,20,21,22,23].

In this study, the aqueous extracts of AI, SG and their 50:50 mixture w/w (AI + SG) were tested for efficacy against the *in vitro* sporulation of oocysts of *Eimeria tenella* and *Eimeria maxima*.

MATERIALS AND METHODS

Study Area

The study was conducted at the Parasitology Laboratory of the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Nigeria.

Collection and Preparation of *Eimeria* Oocysts

The intact caeca of local chickens diagnosed to have suffered natural clinical coccidiosis were dissected out at post mortem. The caeca were sliced open and washed into a beaker using tap water. The contents of the beaker were centrifuged after which the sediment was re-mixed with saturated sodium chloride solution to float out the oocysts [24]. Traces of salt and colouring matter were removed by washing the floated oocysts five times with water through the process of centrifugation. The sediment was examined microscopically for presence of oocysts after which the harvested oocysts were routinely sporulated, passaged and multiplied in four chicks (aged 4 weeks) through oral infection.

The birds were monitored daily for the development of clinical coccidiosis and their faeces checked for presence of *Eimeria* oocysts. Ten days post infection two chicks were humanely sacrificed for gross and histological examinations for lesions. Based on the morphological characteristics and sporulation times of the oocysts as well as the manifested clinical signs and pathological lesions observed in the infected birds, the oocysts were identified as a mixture of *Eimeria tenella* (80%) and *Eimeria maxima* (20%) [24].

Collection and Processing of Plant Materials

The stem bark of *Sacoglottis gabonensis* was collected from the tree at Abawsi in Abia State while the leaves of *Azadirachta indica* were collected from a neem tree in the Botanical Garden, University of Nigeria, Nsukka, Enugu State, Nigeria. The identification of the plants was confirmed by a botanist in the

Department of Botany, University of Nigeria, Nsukka, where voucher specimens of the plants were deposited.

The stem bark and leaves of the plants were separately dried under shade for 10 days at 8 hours per day and then ground into powder using a pestle and mortar. The powdered extracts were sieved to remove excess coarse plant materials and one kilogram of each plant material was measured and individually soxhlet extracted with water for 8 hours at 60^{o}C [25,26]. The soluble extract was then concentrated in a conical flask placed in a water bath maintained overnight at 60^{o}C. Thereafter, the concentrated extract, in gel form was collected, weighed and stored at 4^{o}C for later use in this study.

Experimental Drugs and Plant Extracts

Embazin-forte (containing Sulphaquinoxalin B.P 9.94 and Diaveridine B.P., v -0.98 g fortified with Vitamin K 0.053 g, Turner Wright Ltd., Lagos, Nigeria) and Amprolium 300 ws (containing per gram Amprolium HCL 300 mg, Interchemie Werken B.V- Holland) which are commercially available and commonly used anticoccidial drugs for the routine treatment of avian coccidiosis in Nigeria were used to compare the anticoccidial effects of the plant extracts. The normal dosages and double of the normal dosages of amprolium (0.5 mg/ml and 1 mg/ml) and embazin-forte (0.6 mg/ml and 1.2 mg/ml) were used for the *in vitro* antisporulation study.

One gram of the respective extracts of SG and AI and their mixture (SG + AI) were each dissolved in two milliliters of distilled water and made up to ten milliliters with distilled water to achieve the various concentrations used in the study.

Experimental Procedure

Fresh faecal samples collected from the remaining two infected birds were used in this study. Faecal oocyst counts were determined by the modified McMaster technique using saturated solution of sodium chloride as the floating medium [27]. The recovered oocysts were adjusted to 100 oocysts/ml and the 100 oocysts added to separate Petri dishes containing the various concentrations of AI, SG and AI + SG (100, 500, 1000, 1500 and 2000 mg/ml) as well as amprolium (0.5mg/ml and 1.0 mg/ml) and sulphaquinoxaline (Embazin-forte) (0.6 mg/ml and 1.2 mg/ml). Untreated oocysts were also added to separate Petri dishes containing water and 2% potassium dichromate solution as controls. The samples were incubated at ambient temperature (29 ± 2°C) and monitored every 6 hours over a 72 hours period for sporulation of the oocysts.

The proportion of unsporulated oocysts at each dilution of the extracts, drugs or water was calculated by relating the number of sporulated oocysts to the number of oocysts cultured using the rate of sporulation in potassium dichromate solution as the standard.

Statistical Analysis of Data

The data generated during the study were summarized as percentages while differences in the proportion of sporulated oocysts cultured in the various, extracts, drugs, water and potassium dichromate were determined using the paired student's 't' test at 5% confidence interval [28].

RESULTS

The *in-vitro* anti-sporulation effects of various concentrations of AI, SG, AI + SG, amprolium and sulphaquinoxaline (embazin-forte) on the sporulation of a mixture of *Eimeria tenella* and *E. maxima* oocysts are shown in Table 1. There was 100% sporulation of *Eimeria* oocysts in 2% potassium dichromate solution whereas in plane water the level of oocyst sporulation was 78%. At their highest concentrations, the percentage sporulation of the oocysts were 0%, 12%, 0%, 6% and 10% respectively in SG, sulphaquinoxaline (embazin-forte), AI + SG, AI and amprolium.

When potassium dichromate solution was used as the standard with zero percent inhibition of sporulation, there was increasing percentage reduction in the inhibition of oocyst sporulation in the various extracts and drugs tested. The maximum inhibition of oocyst sporulation was 90 % for amprolium and 88 % for sulphaquinoxaline (embazin-forte) at their highest concentrations (1.0 mg/ml and 1.2 mg/ml respectively). Similarly, at their highest concentrations (2000 mg/ml), the percentage reductions in oocyst sporulation were 100 % for *S. gabonensis*, 94 % for *A. indica* and 100 % for the mixture of the two extracts. These percentage reductions were similar to those due to amprolium or sulphaquinoxaline (embazin-forte) at the concentrations used in this study. In all cases, the inhibition in oocyst sporulation was concentration dependent as the highest percentage inhibition occurred at the highest concentration of the extracts, their mixture or the drugs and vice versa.

Table 1. Percentage inhibition of sporulation of *Eimeria tenella* and *E. maxima* oocysts by the extracts and routine drugs.

Extract/Drug (mg/ml)	No (%) sporulation	% inhibition of sporulation
2% Potassium dichromate	100	0*
Water	78 (78)	22
S. gabonensis		
100	80 (80)	20
500	30 (30)	70
1000	24 (24)	76
1500	8 (8)	92
2000	0	100
A.indica		
100	42 (42)	58
500	28 (28)	72
1000	20 (20)	80
1500	8 (8)	92
2000	6 (6)	94
Mixture		
100	60 (60)	40
500	32 (32)	68
1000	20 (20)	80
1500	0	100
2000	0	100
Amprolium		
0.5	36 (36)	64
1.0	10	90
Embazin-forte		
0.6	66 (66)	34
1.2	12 (12)	88

*Percentage oocyst sporulation in 2 % potassium dichromate solution was used as the standard (i.e. zero percent inhibition of sporulation).

DISCUSSION

The *in vitro* anti-sporulation effect produced by *S. gabonensis, A. indica* and their mixture was greater than that due to amprolium and embazin-forte at the doses tested during the study. The observation may be due to the fact that amprolium and sulphonamides (embazin-forte) have their anti-coccidial effect usually on the second schizogonic stage of the life cycle and not on the matured oocysts, [10,29].

Amprolium and sulphonamides (embazin-forte) respectively antagonize thiamine (vitamin B_1) and folic acid which are needed for schizogony.

The results of the study revealed that the crude aqueous extract of the stem bark of SG and leaves of AI have antisporulation effect against the oocysts of *Eimeria tenella* and *E. maxima*. At the various dilutions used, the efficacy was concentration dependent as the extracts showed graded antisporulation efficacy which was highest at the 2,000 mg/ml dilution. At this concentration, the extracts and their mixture showed comparable antisporulation activity (100%, 94% and 100% respectively for SG, AI and AI + SG) with amprolium (90%) and embazin-forte (88%) that are commercial anticocidials presently in use for the routine medication of infected birds. The results, therefore, suggest that the crude aqueous extracts of the stem bark of SG and leaves of AI may have great promise as antisporulation agents since even at their lowest concentrations, they were still effective in restricting oocyst sporulation *in vitro*.

In conclusion therefore, the results of this study have shown the crude aqueous extracts of the leaves of *A. indica* and stem bark of *S. gabonensis* to have anticoccidial activity through the inhibition of the sporulation of *Eimeria* oocysts and thus suggest their possible usefulness in the control of coccidiosis in animals. However, further studies, especially *in vivo* studies, in animals are however necessary to confirm the present observations.

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