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In vitro evaluation of the inhibitory effects and antioxidant properties of hydro-ethanolic extracts from the roots of *Sarcocephalus latifolius* against *Eimeria* spp. sporozoites

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ABSTRACT

Introduction: Coccidiosis is a severe and lethal parasitic disease affecting birds globally, caused by protozoans from the genus *Eimeria*, posing significant economic challenges. Africa native tree *Sarcocephalus latifolius* is traditionally used to treat diarrhea, gastrointestinal disorders and parasitic diseases. The present *in vitro* study aimed to evaluate the impact of a processed hydro-ethanolic extract of *S. latifolius* root on the survival of poultry *Eimeria* sporozoites.
Methods: Spectrophotometric methods were used to evaluate antioxidant activity, specifically the DPPH and ferric-reducing antioxidant power (FRAP) assays. The total phenolic (TP) and flavonoid (TF) levels were also measured using the Folin-Ciocalteu and aluminum chloride techniques, respectively. The extract was tested in vitro for oocysticidal sporulation suppression at five different concentrations (25, 50, 75, 100, and 150 mg/mL). The reference controls were K2Cr2O7 (2.5%) and Amprolium (20%) (negative and positive, respectively).
Results: The ascorbic acid's antioxidant activity (97.37 ± 0.86 µM Fe(II)/g) and that of the *S. latifolius* root extract (86.21 ± 4.28 µM Fe(II)/g) were comparable ($P > 0.05$). *S. latifolius* root extract ability to trap free radicals was tested similar ($P > 0.05$) to that of ascorbic acid. Compared to the untreated control, *S. latifolius* root extract significantly reduced ($P < 0.001$) the count of sporulated *Eimeria maxima* oocysts to a level comparable to that of Amprolium 20% ($P > 0.05$).
Conclusion: *S. latifolius* root extract seems to have the potential properties as an alternative to conventional anticoccidial drugs in controlling of poultry coccidiosis.

Implication for health policy/practice/research/medical education:
Sarcocephalus latifolius root extract has the capacity to prevent *Eimeria* oocyst sporulation while also scavenging free radicals. These results may indicate its potential benefits in controlling poultry coccidiosis. However, further *in vivo* investigations of its properties are required.
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Introduction

Coccidiosis is a major poultry disease caused by single-celled protozoa from the genus *Eimeria*, which includes species such as *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria maxima*, *Eimeria mitis*, *Eimeria necatrix*, *Eimeria praecox*, and *Eimeria tenella* (1-3). This severe condition induces a significant inflammatory response in the gastrointestinal tract (4), leading to oxidative stress in tissues, damage from the oxidization of lipids, hemorrhagic diarrhea, an adverse feed-to-gain ratio,

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reduced profitability, heightened susceptibility to other diseases, and potentially mortality (5).

Global annual losses attributed to *Eimeria* infection are estimated to surpass £10.36 billion (6). This parasite undergoes a complex life cycle that begins with the accidental ingestion of sporulated oocysts from the litter excreted in several hundreds of thousands to millions of non-sporulated oocysts in excreta per day by infested chickens. Mechanical friction in the gizzard causes sporocysts to be discharged once sporulated oocysts have entered the host. Under the influence of trypsin and bile, sporozoites escape from the sporocysts and penetrate the epithelial cells of the intestinal mucosa, where they undergo gametogony and schizogony (7). One of the primary objectives for anticoccidial medications is intestinal mucosal epithelial cell invasion by sporozoites, which is necessary for coccidiosis to develop (8). Therefore, disrupting the sporulation process is one of the most important methods for managing this parasite. In modern poultry production, anticoccidial drugs have traditionally been the primary means of controlling avian coccidiosis. Although this approach is both economical and effective, the emergence of drug resistance and the public's demand for residue-free meat have led to the investigation of alternative control strategies (9). These elements, together with medication resistance, highlight the need for substitute therapies for coccidiosis and other poultry infections. Since plant extracts are natural items that may include novel therapeutic chemicals that have not yet acquired resistance, using them as medicinal choices may help address these problems. In this sense, anticoccidials made from medicinal plants might be used in place of traditional therapies, especially in underdeveloped nations (10).

Physiologically active compounds (natural products) such as flavonoids and tannins found in herbal products have been demonstrated in several studies to function as curative anticoccidial substances and boost the body's defenses to protect the gut lining against pathogenic infiltration (1,11). They are believed to have minimal or no adverse effects compared to synthetic antimicrobials (12). The Rubiaceae family, which includes *Sarcocephalus latifolius*, comprises tropical evergreen trees or shrubs (13). Native to Africa and Asia (14), these plants are widely found in the forests and tropical areas of West Africa, including countries such as Benin, Togo, Burkina Faso, Cameroon, the Democratic Republic of Congo, Ghana, and Nigeria (15). *Sarcocephalus latifolius* (Sm.) E.A.Bruce is a small, multi-stemmed tree characterized by glossy, rounded-ovate leaves, unique flowers, and large red, ball-shaped fruits (16). Commonly known as the "Pin cushion tree" in English, it is also referred to as "African peach" and "Scille maritime" in French (17). In Togo, it is called "Degangande", "Alo Kubasa Kaio", and "Bunangim" by the Bassari, Ewe, and Konkomba tribes, respectively (18). This plant species has been observed to have various therapeutic effects and as such been utilized in the treatments of different diseases such as hypertension, tooth decay, indigestion, wounds, diabetes, fever, malaria, constipation, wounds, cough, gonorrhea, leprosy, debility, septic mouth, diarrhea (13,19-21).

Despite the various applications of *S. latifolius* in managing human diseases, particularly those caused by parasites, the documentation of *S. latifolius* extracts as an anticoccidial agent in poultry is limited. This study aims to provide a scientific foundation for the use of *S. latifolius* as an anticoccidial and antioxidant remedy, paving the way for its adoption as an innovative strategy for controlling parasitic diseases in poultry farms.

Materials and Methods

Chemicals and reagents used

Reagents for preparing phosphate-buffered saline (PBS) including 2.5% potassium dichromate (K2Cr2O7) and all other materials for the oocyst sporulation were sourced from the Faculty of Health Science, University of Lome. A 2.5% solution of potassium dichromate (K2Cr2O7) was utilized as the culture medium.

- To prepare 1 L of PBS solution, 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na2HPO4, and 0.24 g of KH2PO4 were added to 800 mL of distilled water. The mixture was then adjusted with distilled water to reach a final volume of 1000 mL at a pH of 7.4 (22).
- The ferric reducing antioxidant power (FRAP) reagents required a 300-mL acetate buffer solution, which was made by dissolving 3.10 g of sodium acetate trihydrate and adding 16 milliliters of glacial acetic acid (23). After adjusting the pH to 3.6, distilled deionized H2O was used to get the final volume down to 1 L.

Collection of plants and preparation of extract

Sarcocephalus latifolius roots were collected from a market called Gbossime, Lome, Togo. Dr Komlan Edjèdu Sodjinou, a botanist, authenticated the plant material, and the voucher samples (TOGO07535) were safely stored at the botanical laboratory of the Faculty of Sciences. After being cleaned, the plants were dried at 20 °C in a climate-controlled environment before being ground into powder using an electric grinder. The process outlined in (22) was used to create the hydro-ethanolic extract. Overall, 3 L of a 70:30 ethanol/water mixture was used to submerge 300 g of plant powder for 72 hours while being continuously stirred with the SM-30 CONTROL stirrer. After that, the mixtures were filtered twice using Whatman filter paper and three times with hydrophilic cotton. The filtrate resulting from each plant was evaporated at 45 °C using a BUCHI R-210 rotary evaporator.

Plant extract yields

Following the evaporation of the solvent, the plant extract yield was determined using the following formula (23)

and the extracts were kept in bottles in the refrigerator at 4 °C until they were needed.

$$Yield = \frac{Mass\ of\ extract\,(g)}{Mass\ of\ dried\ plant\ powder\,(g)} \times 100$$

Qualitative screening of phytochemicals

Table 1 shows the tests carried out to evaluate the presence or absence of secondary metabolites using the standard assay protocols previously defined (24,25).

Determination of total polyphenolic content

The total polyphenolic content of the extract was determined using the Folin-Ciocalteu method (26) with minor modifications. In short, 1000 µL of a 10% diluted Folin-Ciocalteu solution was combined with 200 µL of the extract, and the combination was let to stand at room temperature for two minutes. Next came the addition of 800 µL of sodium carbonate (75 $g.L^{-1}$). The final mixture was thoroughly combined. After a 30-minute incubation at room temperature, protected from light, the absorbance at 765 nm was measured using a spectrophotometer. A calibration curve was created using gallic acid standards with dosages between 0 and 100 µg/mL. Three replicates of each extract were prepared, and the total phenolic content was expressed as gallic acid equivalents (GAE) per gram of dry weight (DW).

Total flavonoids determination

The method adhered to the guidelines outlined in (27). A total of 2.0 mL of 2% (w/v) AlCl3 was combined with 1 mg/mL of the extract in methanol to produce pure methanol. After allowing the mixture to sit in the dark for 10 minutes, the absorbance at 415 nm was measured using a spectrophotometer, with quercetin serving as the reference material. The results from three replicates of each extract were reported in milligrams of quercetin equivalent (mg EqQ) per milligram. The same conditions were applied for quercetin in the 0–100 mg/L range, which is standard.

Antioxidant tests *in vitro*

DPPH test

In accordance with the methodology described in reference (27), the antioxidant capacity of the extracts to scavenge free radicals was evaluated utilizing the DPPH assay, which employs a stable free radical. The antioxidant activity of the herbal extract was determined by combining 100 microliters of each extract, diluted in methanol to a concentration of 1 mg/mL, and 2 mL of a methanolic DPPH solution (0.004%). The mixture was strongly vortexed to ensure homogeneity, and the absorbance was subsequently measured using a spectrophotometer following a 30-minute incubation in the dark at a wavelength of 517 nm. Each sample was subjected to three independent trials. Quercetin served as a positive control, prepared at concentrations ranging from 0 to 100 mg/mL, with absorbance assessed under the same experimental conditions. The results were quantified in terms of milligrams of quercetin equivalents per milligram of extract (mg EqQ/mg sample), based on the calibration curve equation.

Table 1. Phytochemical screening of *Sarcocephalus latifolius* root extract to evaluate the presence or absence of secondary metabolites

Phytoconstituents	Test/reagents
Total phenols	Ferric chloride test
Tannins	Stiasny test
Saponin	Frothing test
Flavonoids	Shinoda test
Alkaloids	Dragendorff 's test
Reducing sugar	Fehling's solution
Coumarins	Ammonium hydroxide
Triterpenes	Acetic anhydride, chloroform, sulphuric acid
Steroids	

FRAP assay

As mentioned, a slightly reviewed method of Benzie and Strain (28) was used to perform the FRAP test. Based on the sample's capacity to change the ferric tripyridyl-triazine (Fe(III)-TPTZ) complex into ferrous tripyridyl-triazine (Fe(II)-TPTZ) at low pH values, this method was used. At 593 nm, the intense blue hue that results from the synthesis of Fe(II)-TPTZ can be seen. Two milliliters of freshly prepared solution were mixed with 100 µL of extracts to create the FRAP solution. The mixture included 2.5 mL of 10 mM 2,4,6-tripyridyls-triazine (TPTZ) in 40 mM hydrochloric acid, 25 mL of 300 mM acetate buffer at pH 3.6, 2.5 mL of water for the standard FRAP, and a 20 mM ferric chloride (FeCl3·6H2O) solution for the sample FRAP. After sitting in the dark for 30 minutes, absorbance was measured at 593 nm. The standard curve was linear between 0 and 100 µM FeSO4·7H2O. Results were expressed as µM Fe(II)/g dry plant material and were compared to ascorbic acid values.

Determination of anticoccidial activity

Isolation and purification of Eimeria oocysts

Fresh fecal samples were collected from naturally infected broiler chicken farms in Togo's Maritime Region. The oocysts were collected using a concentration technique, as reported in (29). A vortex mixer was used to homogenize the fecal samples, which were then filtered through a mesh screen after being diluted five times in PBS (pH 7.4). For ten minutes, the filtrate was spun at 1000 × g in a polypropylene centrifuge container. After being re-suspended with PBS, the oocyst-containing sediment was centrifuged twice (1000 × g, 10 minutes). Sporulation was undertaken for 48 hours in an aqueous solution of potassium dichromate 2.5% (W/V) (30). The silt was

then resuspended in tiny Petri plates with 2 mL of a 2.5% potassium dichromate solution. This was done to provide adequate moisture while also eliminating other bacteria in the samples that competed with the oocysts for oxygen and nutrients. The samples were incubated at a temperature of 28 °C for a duration of 1-3 days at with aeration to promote sporulation. Oocyst sporulation was observed using a microscope (Olympus compound) and a digital camera (IX73, 40× magnification). Photographs were obtained for identification purposes. The main morphological traits employed were characterized based on the key provided by McDougald (31). In poultry farms in southern Togo, morphological analysis revealed the presence of *E. maxima* (54.17%), *E. brunetti* (33.33%), *E. tenella* (25%), *E. acervulina* (8.33%), *E. praecox* (8.33%), and *E. mitis* (4.17%) (32).

Effect of Sarcocephalus latifolius roots on oocysts sporulation

The *in vitro* anticoccidial activity of a crude hydro-ethanol extract of *S. latifolius* was assessed by observing how it affected the sporulation time of *E. maxima* oocysts. Oocysts count was obtained using the McMaster counting machine. Following the approach indicated in (33), 21-well petri dishes were used to examine the extract's in vitro activity. A Petri plate was filled with 2 mL of each extract concentration (25, 50, 75, 100, and 150 mg/mL). A negative control (2 ml of 2.5% potassium dichromate) was used as described by 46 and 47 while a positive control (60 mg/L of amprolium 20%) were used. Each Petri dish received a 2-mL suspension of freshly unsporulated *E. maxima* oocysts, with triplicates prepared for each concentration. All groups' 21-well Petri dishes were incubated at 27-29 °C, 65-75% relative humidity, and semi-covered (34). After 24 and 48 hours, 1 mL suspension from the Petri plate was transferred into the test tubes.

PBS was used to rinse the oocysts from the test solutions before centrifuging them three times at 200 g for 5 minutes. Each group was examined with a light microscope (IX73, OLYMPUS, Tokyo, Japan) with a magnification of 40X. The viability of the oocysts (including those with sporocysts, deformed walls, and inhibitory oocysts) was assessed with a light microscope equipped with a McMaster chamber. The percentages of sporocysts and inhibitory oocysts were calculated using the following formulas described by Murshed et al (35):

$$Sporulation\,(\%) = \frac{Number\ of\ sporulated\ oocysts}{Total\ number\ of\ oocysts} \times 100$$

$$The\ inhibition\ of\ sporulation\,(SI\%) = \frac{Sporulation\ of\ control - sporulation\ of\ treated}{Sporulation\ of\ control} \times 100$$

Statistical analysis

To evaluate whether factors differed between treatments, data were statistically analyzed using the Statistical Package for Social Science (SPSS) for Windows, version 22, Chicago, USA. To establish mean differences, data were examined using ANOVA and Duncan's multiple range test. The percentage inhibition was determined using two-way ANOVA with SPSS's General Linear Models tool. The following model was also used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

Yijk signifies individual observations, μ is the mean generated, αi denotes the influence of ith treatment, βj designates the effect of the jth period of oocysts incubation (24 and 48 hours), (αβ)ij is the interactive treatments effect incubation time, and εijk shows the random error. GraphPad software was used to construct each graph. The results are shown as means ± SE. *P* values at 5% were considered significant.

Results

Extracts yield and screening phytochemical assessment

The evaporation process yielded 3.41% dry extract. The phytochemical examination revealed that the hydroalcoholic root extract of *S. latifolius* included phenolic compounds, alkaloids, flavonoids, polyphenols, tannins, saponin, triterpenes, and steroids.

Quantitative phytochemical analysis and extract antioxidant activity

Table 2 displays the linear regression curves for the amounts of phenol and total flavonoids, represented by the equations y = 0.02910x + 0.1172 (R^2 = 0.9766) and y = 0.01527x + 0.05453 (R^2 = 0.9946), respectively. The polyphenol concentration was 56.11 ± 0.33 mg gallic acid equivalents (GAE)/g, with flavonoid content of 36.65 ± 1.85 mg quercetin equivalent (QE)/g. The linear regression equation (y= -0.01043x + 1.286) was used to measure the capacity to scavenge free radicals (DPPH), with a R^2 value of 0.9933. *S. latifolius* root extract showed promising antioxidant activity (76.25 ± 0.53 mg/mL for quercetin) (Table 2). We measured a FRAP of 86.21±4.28 μM Fe(II)/g using the FRAP method, using the linear regression equation y = 0.04919x + 0.9782 with a R^2 of

Table 2. Antioxidant activity, total phenolic and flavonoid contents of hydro-ethanolic extract of *Sarcocephalus latifolius*

Phytochemicals content and antioxidant analysis	Measured values
TPC (mg GAE/g)	56.11 ± 0.33
TFC (mg EQ/g)	36.65 ± 1.85
Antioxidant capacity (DPPH in mg EQ/g)	76.25 ± 0.53
FRAP (μM Fe(II)/g)	86.21±4.28

Abbreviations: GAE/g: Gallic acid equivalent per gram; QE/g: Quercetin equivalent per gram; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power, TPC: Total phenol content, TFC: Total flavonoid content.

0.9203. Ascorbic acid, the positive control, had a Fe(II) concentration of 97.37±0.86 µM/g.

In vitro oocysticidal activities of plant extracts

The preliminary in vitro effects of hydro-ethanolic extracts of *S. latifolius* roots at various doses were investigated for suppressing *Eimeria maxima* oocysts. Oocyst sporulation was investigated at various time intervals (24 and 48 hours) following incubation, and Figure 1 depicts the sporulated and unsporulated oocysts. After 48 hours of incubation, the sporulated *Eimeria* spp. were oval-shaped, with outer and inner layers of oocyst walls each containing four sporocysts and two sporozoites.

The crude extract at 150 mg/mL concentration exhibited the highest sporulation inhibition efficacy ($P<0.0001$) of 77.28 ± 1.10% after 24 hours. However, it was not statistically significant ($P>0.05$) when compared to the positive control (Amprolium 20%). After incubation for 24 hours, the highest oocyst sporulation was found with *S. latifolius* root extract at 25 mg/mL and $K_2Cr_2O_7$, which was dose-dependent (Table 3).

After 48 hours of incubation, the similar suppression of sporulation by *S. latifolius* root extract was found. Figure 2 demonstrates that the plant considerably prevented the sporulation of *E. maxima* oocysts when compared to the control groups ($P<0.05$) as the rate of inhibition varied according to the concentration. With an increase in plant treatment concentrations, fewer sporulated oocysts were observed. The negative control had the highest sporulation (84.71±3.23%), while the oocysts treated with the extract at 150 mg/mL had the lowest (15.52±3.23%) and the positive group had the highest sporulation (12.44±3.23%) (Figure 2a). The plant extract at a dosage of 150 mg/mL and the synthetic anticoccidial had the greatest ability to suppress sporulation (Figure 2b).

Figure 3 depicts the effect of incubation time on the sporulation of *E. maxima*. The inhibition percentage rose dramatically with extended incubation time. The highest efficiency of studied plant extracts was obtained after 48 hours following inoculation ($P<0.0001$), which varied based on extract quantities.

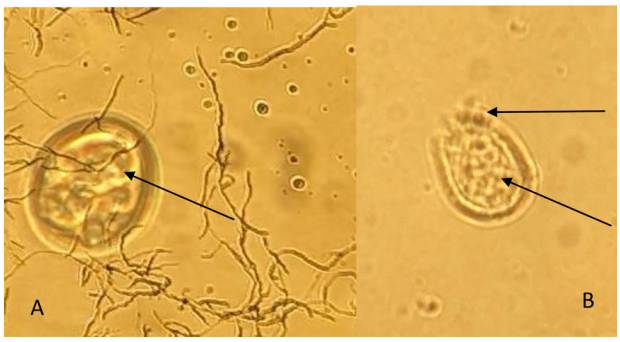

Figure 1. Microscopic examination of the modifications that occurred on oocysts (*Eimeria maxima*). A illustrates the transformation of the oocyst into spores and sporozoites, while B shows an unsporulated, destroyed oocyst by the arrows.

Table 3. Effect of *in vitro* herbal plant extracts on *Eimeria maxima* oocysts sporulation and inhibition after 24 hours of incubation

Groups	Sporulated oocysts (%)	Unsporulated oocysts (% inhibition)
150	22.38 ± 1.10^{ab}	77.28 ± 1.10^{ab}
100	28.10 ± 1.28^{b}	70.58 ± 1.28^{b}
75	40.80 ± 0.76^{c}	59.87 ± 0.76 ^{c}
50	39.61 ± 2.52^{c}	58.06 ± 2.52^{c}
25	40.56 ± 2.15^{c}	57.19 ±2.15^{c}
$K_2Cr_2O_7$	87.51 ± 0.14^{d}	12.82 ± 0.14^{d}
Amprolium 20%	16.95 ±0.95^{a}	83.72 ± 0.95^{a}
P value*	< 0.0001	< 0.0001

$K_2Cr_2O_7$: potassium dichromate solution (2.5%)
* a-d values within a column with different letters differ significantly ($P<0.05$).

Sarcocephalus latifolius roots extract at 150 mg/mL had the best sporulation suppression efficiency of more than 82% but was similar when compared to the positive control (Amprolium 20%) ($P>0.05$). In comparison, oocysts treated with 2.5% K2Cr2O7 (negative control) had a

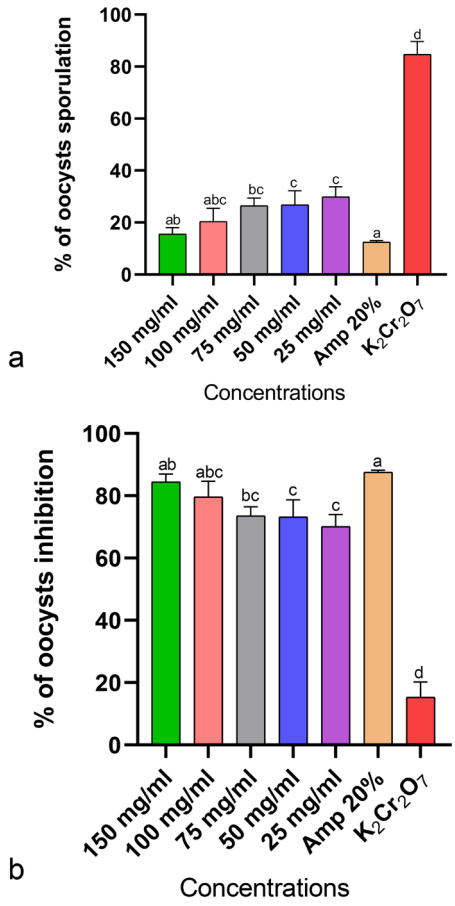

Figure 2. Effect of *Sarcocephalus latifolius* root extracts on the sporulation (a) and inhibition (b) of *Eimeria maxima* oocysts after 48 h of incubation. Amprolium 20%: Positive control; $K_2Cr_2O_7$: 2.5% Potassium dichromate solution (negative control). a-d values with various letters in a graph show significantly different ($P < 0.05$).

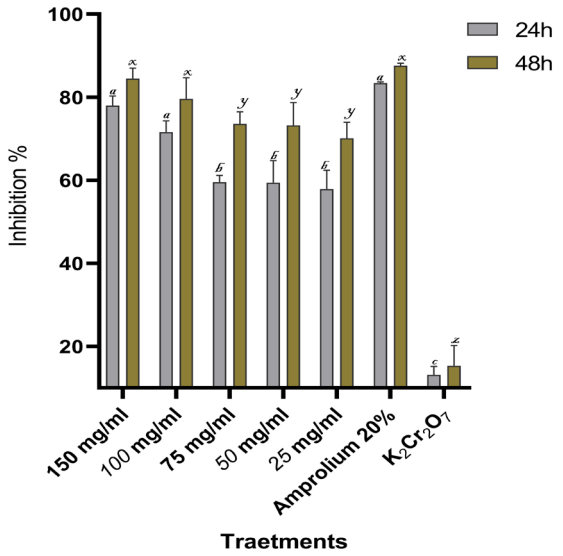

Figure 3. *In vitro* inhibitory effect of *Sarcocephalus latifolius* root extracts on *Eimeria maxima* oocyst concentration at 24 and 48 hours. Amprolium 20%: positive control; K2Cr2O7: 2.5% Potassium dichromate solution (negative control). A graph with different letters indicates statistically significant differences ($P < 0.05$).

sporulation inhibition of 15.30%, whereas the amprolium 20% group had a substantial inhibition of 87.56% after 48 hours of incubation (Figure 2b).

Discussion

This research aimed to evaluate the bioactive components of plant extracts derived from the roots of *S. latifolius*, a herb traditionally utilized for its antiparasitic properties in Togolese poultry farms. We assessed the antioxidant efficacy and anti-sporulation effects on *E. maxima* oocysts. The results indicated that the yield of the extract was 3.41%. Previous investigations have yielded varying results. Previous studies showed ethanol extracts of *S. latifolius* yielding 3.99% (36) and 3.54% (21). However, one study showed a yield of 37.7% with the aqueous extract of *S. latifolius*, while another study reported a yield of 9.28% with the same aqueous extract (21). This could be explained by the fact that several parameters influence the extraction procedure, including the chemical form of the compounds studied, the extraction method, the size of the particles sampled, the plant parts used, the solvent polarity, the drying conditions, and the extraction time or climatic circumstances in the region where the plant samples were taken (37,38).

The phytochemical screening indicated the presence of all of the bioactive components required. The phenolic compounds found in this plant have been reported to have oocysts inhibitory potential *in vitro* (39). Anticoccidial properties of this plant were assessed through *Eimeria maxima* oocysts sporulation inhibition in this study. In this investigation, five *in vitro* concentrations of *S. latifolius* root extract (150, 100, 75, 50, and 25 mg/mL) were tested as a potential natural product against coccidia. The hydro-ethanolic extract of *S. latifolius* root exhibited significantly a high anti-coccidial efficacy ($P < 0.05$) in a concentration-dependent manner. Among all the concentrations examined, 150 mg/mL was found to be the most effective. The current findings illustrate the effectiveness of *S. latifolius* root extract at 150 mg/mL in inhibiting the oocyst's sporulation process. This is congruent with the findings of Arlette et al (40) who discovered that ethanolic extracts of *Calotropis procera* leaf showed higher anticoccidial action against *E. tenella in vitro*, with the anti-sporulation effect ascribed to the presence of several secondary metabolites. Plant extracts with phenolic components have been shown to have inhibitory effects in a number of *in vitro* and *in vivo* investigations.

Natural polyphenolic compounds obtained from therapeutic plants have been found to prevent *E. tenella* sporozoite cell invasion *in vitro* (41). These researchers also proposed that extracts containing polyphenolic compounds may have the ability to inhibit enzymes involved in the sporulation process of coccidian oocysts by weakening the oocyst wall, resulting in subsequent lysis and sporulation inhibition (8). Additionally, some oocysts exhibited abnormal sporocysts due to a reduction of the calcium ATPase in the macrogamete endoplasmic reticulum, which likely resulted in abnormal oocyst formation and an increased number of nonviable oocysts (42,43). The presence of tannins found in this study could also inhibit the life cycle of coccidia as evidenced by the reduction in sporulation of the oocysts. This corroborates the findings of (8) who suggested that the presence of tannins in *Annona reticulate* leaves extracts might account for the inhibition of sporulation in *E. tenella* oocysts. The mechanism of action of tannins might involve their ability to penetrate the oocyst wall, leading to damage to the cytoplasm, as tannins have the potential to deactivate endogenous enzymes responsible for the sporulation process (30).

The ability of *S. latifolius* root extract to scavenge free radicals, comparable to ascorbic acid as an antioxidant, may indicate the potential of the extracts to inhibit the sporulation of *E. maxima* oocysts. Several studies have shown that secondary metabolites such as polyphenols, flavonoids, tannins, and polysaccharides found in plants can disrupt the balance of oxidants and antioxidants on both sides of oocyst membranes, resulting in oxidative stress, cytoplasm penetration, and cell cycle interference, all of which impede *Eimeria* replication (30,44,45). As a result, it is possible that the complex combination of bioactive compounds in *S. latifolius* root, such as polyphenols, flavonoids, and tannins, may act synergistically to cause an antioxidant imbalance on the sporozoite membrane, disrupt internal homeostasis, and ultimately lead to sporozoite collapse.

Conclusion

The findings of the present in vitro study indicate that the root extract of *S. latifolius* effectively inhibits poultry Eimeria spp. during the oocyst sporulation stage. This suggests that *S. latifolius* may serve as a viable alternative to anticoccidial antibiotics. However, since amprolium 20% can inhibit the *Eimeria* life cycle at various stages including sporulation, sporozoites, merozoites, and trophozoites, it would be advantageous to investigate whether the extract of *S. latifolius* exhibits similar inhibitory effects on these additional stages of the *Eimeria* life cycle. Furthermore, further research including feeding trials are required to elucidate the mode of action of the secondary metabolites of the herbal extract and to evaluate their potential toxicity.

Authors' Contribution

Conceptualization: Ferdinand Grégoire Tchodo, Hervé Brice Dakpogan, and Batomayena Bakoma
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Methodology: Ferdinand Grégoire Tchodo, Sabrina Sanvee, Benjamin Adjei-Mensah and Banfitebiyi Gambogou.
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Conflict of interests

The authors declare no conflict of interest concerning this work.

Ethical considerations

This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Experimental Animals of the University of Lome, Togo. The protocol was approved by the Ethics of Animal Experimentation Committee of the same University (Permit no. 019/23.05.2023).

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