

Evaluation of the anticoccidial effect of traditional medicine plant *Calotropis procera* against *Eimeria tenella* oocysts and Sporozoites infecting broiler

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[Avaliação do efeito anticoccidiano da planta medicinal tradicional *Calotropis procera* contra oocistos de *Eimeria tenella* e esporozoítos que infectam frangos de corte]

M. Murshed , J. AL-Tamimi , O.B. Mohammed , S. Al-Quraishy 

Department of Zoology, College of Science, King Saud University, P.O. Box 2455,
Riyadh 11451, Kingdom of Saudi Arabia

ABSTRACT

Broiler production is important for boosting wealth and covering the needs of human white meat. However, common parasite infestations pose a threat to survival. The study aimed to assess the efficiency of *Calotropis procera* extract on *Eimeria tenella* oocyst inhibition and sporozoite viability. Distributed oocysts were given seven different treatments: a control solution of 2.5% potassium dichromate; five different concentrations of extract (5, 10, 25, 50, and 100 100mg/mL); and 25 25µg/mL of amprolium, as standard medicine. Assessed the mixture's oocysticidal properties after 24, 48, 72, and 96 hours of exposure. In addition, there are five concentrations (125, 250, 500, 750, and 1000 µg/mL) for the anti-sporozoite activities test. After 72 and 96 hours, the extract was able to block the *E. tenella* oocysts at 50% and 100% doses by roughly 76% and 87%, respectively. The relationship between sporulation and concentration was highly negatively associated at 72 and 96. *C. procera* showed the largest suppression of 85% of the viability of *E. tenella* sporozoite at 1000 g/mL. The results that the extract can prevent the oocyst of *Eimeria tenella* from sporulation. This shows that these extracts might have anticoccidial properties.

Keywords: anticoccidial, eimeria, oocysticidal, inhibitory, destroyed

RESUMO

A produção de frangos de corte é importante para aumentar a riqueza e suprir as necessidades de carne branca humana. Entretanto, infestações comuns de parasitas representam uma ameaça à sobrevivência. O objetivo do estudo foi avaliar a eficiência do extrato de *Calotropis procera* na inibição de oocistos de *Eimeria tenella* e na viabilidade de esporozoítos. Eles receberam sete tratamentos diferentes: uma solução de controle de dicromato de potássio a 2,5%; cinco concentrações diferentes de extrato (5, 10, 25, 50 e 100 mg/mL); e 25µg/mL de amprolium, como medicamento padrão. Avaliamos as propriedades oocisticidas da mistura após 24, 48, 72 e 96 horas de exposição. Além disso, há cinco concentrações (125, 250, 500, 750 e 1000µg/mL) para atividades antiesporozoítas. Após 72 e 96 horas, o extrato foi capaz de bloquear os oocistos de *E. tenella* em doses de 50% e 100% em cerca de 76% e 87%, respectivamente. A relação entre a esporulação e a concentração foi altamente associada negativamente às 72 e 96 horas. A *C. procera* apresentou a maior supressão de 85% da viabilidade do esporozoíto de *E. tenella* a 1000 g/mL. Os resultados mostram que o extrato pode impedir a esporulação do oocisto de *Eimeria tenella*. Isso mostra que esses extratos podem ter propriedades anticoccidianas.

Palavras-chave: anticoccidiano, eimeria, oocisticida, inibitório, destruído

INTRODUCTION

Coccidiosis is a protozoan infection that impairs the health and performance of most vertebrates. It is caused by coccidia, which can be described as *Eimeria* spp. in birds and livestock or *Isospora* in cats and dogs (Jones and Garcia 2021). According to Al-Quraishy *et al.*, 2020, the overall prevalence of coccidiosis in home-reared chicks in Riyadh, Saudi Arabia, was 80% in a single year. According to Dubey *et al.* (2019), broiler chickens frequently include *Eimeria acervulina*, *Eimeria maxima*, *Eimeria tenella*, and *Eimeria praecox*. These four species of *Eimeria* are commonly recognized.

To prevent and control avian coccidiosis in the poultry industry, we used a wide range of chemical anticoccidials (Felici *et al.*, 2021). In contrast to its role as a coccidial medicine, salinomycin, which is sold under the brand name Sacox®, is a monovalent ionophore that is administered in conjunction with food as a preventative agent or coccidiostat. According to Noack *et al.*, 2019, it disrupts ion gradients across the cell membrane of the parasite, which causes it to act earlier in the life cycle of the parasite. However, the evolution of resistance to antiparasitic agents has resulted in greater treatment doses and residues in chicken products. Additionally, the desire of consumers for meat that is free of residue has prompted several researchers to propose and develop alternative agents (Upadhaya *et al.*, 2019).

In the most recent decades, it has been demonstrated that certain nature supplements help prevent hens from damage caused by coccidia, but they do not have a favorable effect on performance (Galli *et al.*, 2021). Furthermore, the organic acid mixtures were able to inhibit in vitro and in vivo pathogens, such as *E. tenella* and *Eimeria bovis* parasites, by reducing the number of *E. tenella* oocysts, reducing the amount of oxidative stress that was caused by inflammation, enhancing the immune response by reducing reactive oxygen species, increasing manganese superoxide dismutase levels, and short-chain fatty acid contents (Balta *et al.*, 2021).

When shed from birds in feces, oocysts are unsporulated and thus non-infectious. Sporulation, an oxygen-intensive process, is the exogenous

mechanism by which oocysts mature in their environment. Only live, sporulated oocysts will cause infection when consumed. Temperature, humidity, and oxygen availability all have an impact on sporulation efficiency (Conway and Mckenzie, 2007). *Eimeria* oocyst degradation and sporulation tests are procedures for investigating the effect of substances or circumstances on integrity or exogenous development. However, their goals and methods differ. *Eimeria* oocyst degradation assays are used to assess the ability of disinfectants or other treatments to destroy the oocyst wall. In these assays, oocysts are treated with a chemical or physical agent, and the proportion of disintegrated oocysts is counted using a microscope. This assay is designed to assess a treatment's efficacy in killing or degrading *Eimeria* oocysts (Laverty, 2023).

As an anticoccidial feed additive, mixtures of thymol eugenol, piperine, and vitamin D3 can be utilized in broilers infected with coccidian bacteria (Upadhaya *et al.*, 2019). Although Upadhaya *et al.* (2019) discovered that cumulative BWG was enhanced and FCR was lowered, little research has been conducted on the synergistic benefits of natural herbs in lowering coccidial infection while simultaneously enhancing growth performance. Barbour *et al.* (2015) conducted research to determine whether medicinal plants and their derivatives may be used as alternatives to coccidiostats in the chicken industry. According to Alhotan and Abudabos (2019), these herbal components create bioactive phytochemicals that are efficient in preventing chicken coccidiosis and being safe for meat consumers.

Calotropis procera is a perennial shrub that grows upright and has softwood. Its extensive application in the ancient medical systems of Sudan, India, and the Arab world for the treatment of a wide range of illnesses has been used for centuries in a variety of human cases, as the parts of CPLE and its metabolites contain bioactive constituents that have diverse biological, medicinal, and pharmacological activities (Wadhwani *et al.*, 2021).

Previous reviews of *Calotropis procera* have covered its phytochemistry, ethnobotany, and medicinal potential (Chan *et al.*, 2017; Bairagi *et al.*, 2018). The medicinal significance of

Calotropis species has been discussed in a review by Pradeepkumar *et al.* (2017), which provided an overview of *Calotropis procera*'s phytochemistry, pharmacology, commercial features, traditional medicinal uses, toxicity, and contemporary findings. To determine *C. procera*'s many biological activities and mechanism of action, its future range has also been confirmed. Consequently, this experiment studied the efficacy of CPLE dosages as a means of controlling coccidia with *E. tenella* oocysts, and sporozoites, and compared it with a synthetic drug.

MATERIALS AND METHODS

The study followed Saudi Arabia's animal use ethics (Ethics approbation ID: KSU-SE-21-86).

C. procera leaves were collected from a desert in Saudi Arabia. The plant was identified by a specialist in botany at the Faculty of Sciences, Riyadh, King Saud University. Subsequently, the leaf parts of the plant were separated and dried for three days, in an oven set at 47 °C. Accurately dried, finely ground leaves (0.5 kg) were obtained, from *C. procera*. The powdered leaves in 80 % methanol were macerated under stirring at room temperature for three days. After filtration (Whatman sheets), the extracts were evaporated to dryness under vacuum (Yamato RE300, Tokyo, Japan) at 40 °C., the extract was kept at -20 °C until they were employed in an experiment. To dissolve the powder for the various experiments distilled water was used (Zhang *et al.*, 2018).

The experiment was conducted at the King Saud University Parasite Laboratory, Zoology. Samples of rabbit feces were collected and examined for *Eimeria* infection. Using flotation, oocysts were cleaned and concentrated before experimental use. The McMaster method was used to examine stool for *Eimeria* spp. oocytes. More than one type was obtained. The oocysts were distinguished based on their morphological characteristics. 10 oocysts were separated for each species, and *E. tenella* injected was into rabbits free of *Eimeria* to obtain pure oocysts. Both the parasite oocysts field isolates were kept alive by undergoing periodic transmission (passage) through young rabbits, according to the method Schito *et al.* (1996).

After obtaining pure non-sporulated *E. tenella* oocysts. A test was conducted on the oocyst's sporulation in the solution of 2.5% Potassium dichromate $K_2Cr_2O_7$ (10^4 oocysts/mL) with 3 mg/mL of *C. procera* dissolved in distilled water at 23°C for 96 h. Used 2.5% potassium dichromate solution as the non-treated control; five different extract concentrations (5, 10, 25, 50, and 100mg/mL); In addition, 25mg/mL Amprolium as the reference medicine. The mixture's oocysticidal properties were assessed after 24, 48, 72, and 96 hours. The percentage of sporulated oocysts was assessed by microscopic observation and by counting the number of sporulated oocysts in a total of 100 oocysts.

Oocysts decrease % = $\frac{(\text{Mean oocyst number of control} - \text{Mean oocysts number of treatment})}{(\text{Mean oocyst number of control})} \times 100$

Phosphate-buffered saline (PBS), at pH 7.4 was used to clean oocyst samples kept in potassium dichromate. The centrifuge was utilized in Falcon tubes with 10mL suspended at 1008 g for around 15 minutes, repeated 3-6 times until the $K_2Cr_2O_7$ solution was eliminated. The oocysts were incubated for 1 hour in a water bath at 42°C by moving the tubes in a semicircular manner. The parasite suspension of 1000 sporozoites was divided into 125, 250, 500, and 1,000µg/mL of extract. The components were then placed in a 3mL well-Petri plate with 24 wells. To enable comparability, a reference treatment of Amprolium 30µg/mL was used. In addition, a potassium dichromate solution was used as a control. Each treatment was administered in 3 replicates. The findings were recorded after 6, 12, 18, and 24 hours to assess the number of viabilities sporozoites. A McMaster chamber was used to count sporozoites from a total of 100 using the following mathematical law:

Inhibition of viability (Vi)% = $\frac{((\text{Vi\% control} - \text{Vi\% bile}))}{(\text{Vi\% control})} \times 100$

The SPSS software, namely IBM SPSS Version 23, was utilized for statistical analysis, and the data was analyzed using one-way ANOVA and provided as mean ± SD of three replications. The levels of significance were set at $P \leq 0.05$.

RESULTS

Figure 1 illustrates the results of microscopic analysis and enumeration of oocysts treated with *C. procera* leaf extract at several dosages. The picture showed inhibitory, sporulatory, and lysis oocysts, which contained oocysts with serrated walls that were deformed and degraded (Fig. 1).

Figure 2 illustrates the results of microscopic analysis and shapes of oocysts treated with *C. procera* leaf extract at several dosages during incubation periods. Incubation at 24-48 hours, oocysts Irregular sporont composed of enlarged granules. Incubation at 48-72 hours, not fully formed sporocysts containing discernible sporozoites. Incubation at 72 - 96 hours for oocysts sporulation fully (Fig. 2).

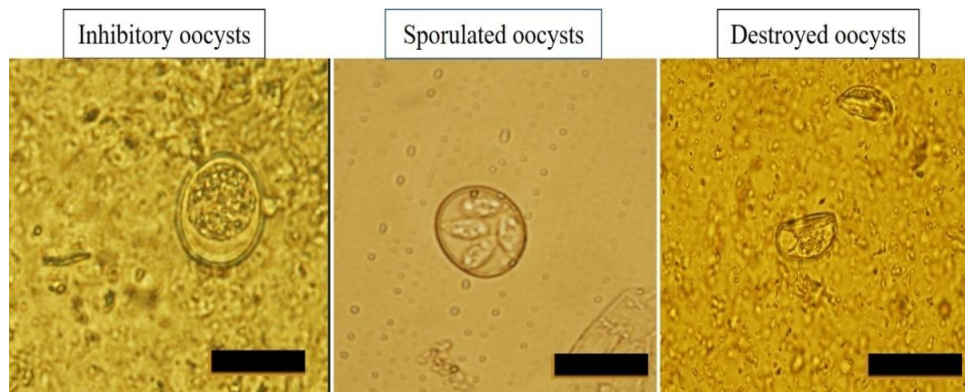


Figure 1. The morphological characteristics of *E. tenella* oocysts that were treated with *C. procera* at varying doses. a) Inhibitory oocysts. b) sporulatory oocysts. c) and destroyed oocysts. Scale-bar = 10µm.

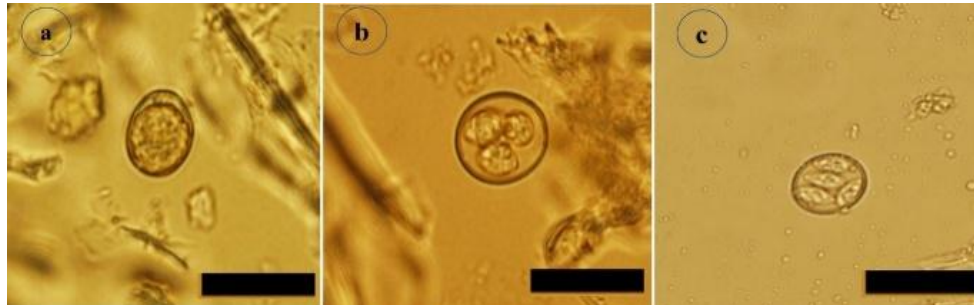


Figure 2. Stages of oocyte development during the Incubation periods. a) at 24-48 h, Irregular sporont composed of enlarged granules. b) at 48-72 h, oocyst containing discernible sporozoites. c) at 72 - 96 h for oocysts sporulation fully. Scale-bar = 10µm.

Incubation of *E. tenella* oocysts during four periods (24, 48, 72, and 96 hours) and at different concentrations (5, 10, 25, 50, and 100mg/mL), and comparing them with the control on the one hand and with the reference treatment (amprolium) At the best dose. The results showed low sporulation as the concentration increased compared to the control, in which sporulation increased as the incubation time increased, and the sporulation rate reached 43% within 24 hours, but most of it was irregular spores consisting of enlarged granules. However,

sporulation was observed at a concentration of 100mg/mL, which is less than 4%. While different levels of sporulation were observed at concentrations of 10, 25, 5, 10, and 25mg/mL during the experiment. We note that there are statistically significant differences between the treatments with all CPLE concentrations and the reference treatment and potassium dichromate (2.5%), although the control group, which consisted of a 2.5% potassium dichromate solution, did not develop spores throughout the group (Fig. 3).

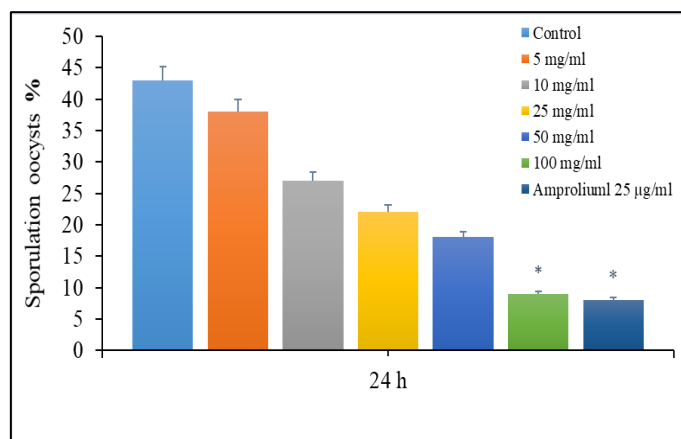


Figure 3. Impact of CPLE on the sporulation of *E. tenella* oocysts at 24 h. Significance is with the group that served as a control ($p < 0.05$). Significance (*): p -value ≤ 0.05 . (CPLE): *Calotropis procera* leaf extract. (h): hours.

The results showed that examining oocysts after 48 h increased oocyst sporulation in low concentrations compared to the control group, in which sporulation increased as the incubation time increased, and the sporulation rate reached 57%, but it was not fully formed sporocysts containing discernible sporozoites. Sporulation inhibition continued at a concentration of 100 mg/mL and the reference treatment. However, different levels of sporulation were observed at concentrations of 5, 10, 25, and 50 mg/mL during the experiment but it was low compared to incubation with 24 h. We note that there are statistically significant differences between the treatments with 100 mg/mL of CPLE and amprolium concentrations compared with the

potassium dichromate (2.5%) control group (Fig. 4).

The results showed sporulation inhibition as the incubation time increased after 72 h compared to the control group, the sporulation rate reached 80%, followed by the 5mg/mL concentration of 71%, most of it was Incubation at 72 - 96 hours for oocysts sporulation fully. The rate of inhibition varied in concentrations (10, 25, and 50%) after 72 hours of incubation (44, 31, and 15%), respectively. We note that there are statistically significant differences between the treatments with 100 mg/mL of CPLE and amprolium concentrations compared with the potassium dichromate (2.5%) control group (Fig. 5).

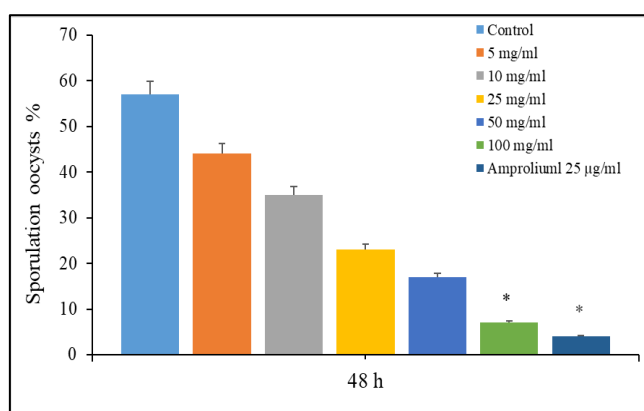


Figure 4. Impact of CPLE on the sporulation of *E. tenella* oocysts at 48 h. Significance is with the group that served as a control ($p < 0.05$). Significance (*): p -value ≤ 0.05 . (CPLE): *Calotropis procera* leaf extract. (h): hours.

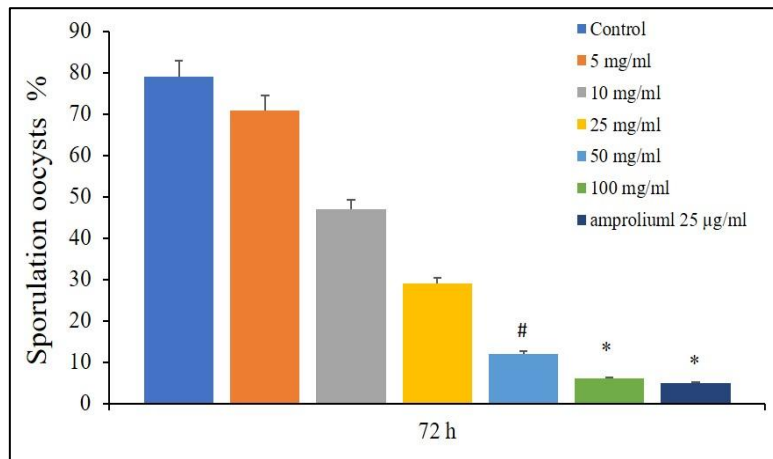


Figure 5. Impact of CPLE on the sporulation of *E. tenella* oocysts at 72 h. Significance is with the group that served as a control. Significance (*): p -value ≤ 0.01 . (#) p -value ≤ 0.05 . (CPLE): *Calotropis procera* leaf extract. (h): hours.

The results showed sporulation inhibition at 94 % as the incubation time increased after 96 h compared to the control group, the sporulation rate reached 96%, followed by the 5 mg/ml concentration of 81%, most of it was Incubation at 96 hours for oocysts sporulation fully. The rate of inhibition varied in concentrations (10, 25,

and 50%) after 96 hours of incubation (43, 38, and 10%), respectively. We note that there are statistically significant differences between the treatments with 100 mg/mL of CPLE and amprolium concentrations compared with the potassium dichromate (2.5%) control group (Fig. 6).

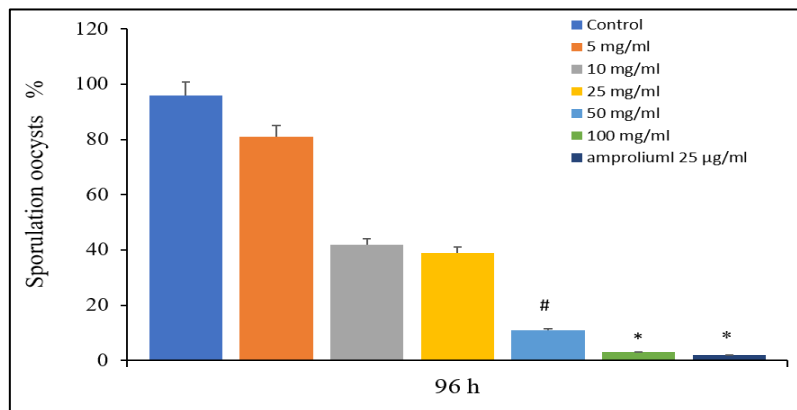


Figure 6. Impact of CPLE on the sporulation of *E. tenella* oocysts at 96 h. Significance is with the group that served as a control. Significance (*): $p \leq 0.01$. (#): $p \leq 0.05$. (CPLE): *Calotropis procera* leaf extract. (h): hours.

The dose of 100mg/kg of CPLE suspension, and the reference treatment (Amprolium 25µg/mL) were the most effective in inhibiting sporulation compared to the control group (Figure 7, A). In general, the experimental groups had a significant effect on inhibition rates ($p \leq 0.05$).

Concentrations (50 and 100) mg/mL of CPLE suspension had the highest inhibition rates and the lowest sporulation rates. Concentrations of 5, 10, and 25mg/mL of CPLE suspension had higher sporulation rates and lower sporulation inhibition ($p \geq 0.05$) (Figure 7, B).

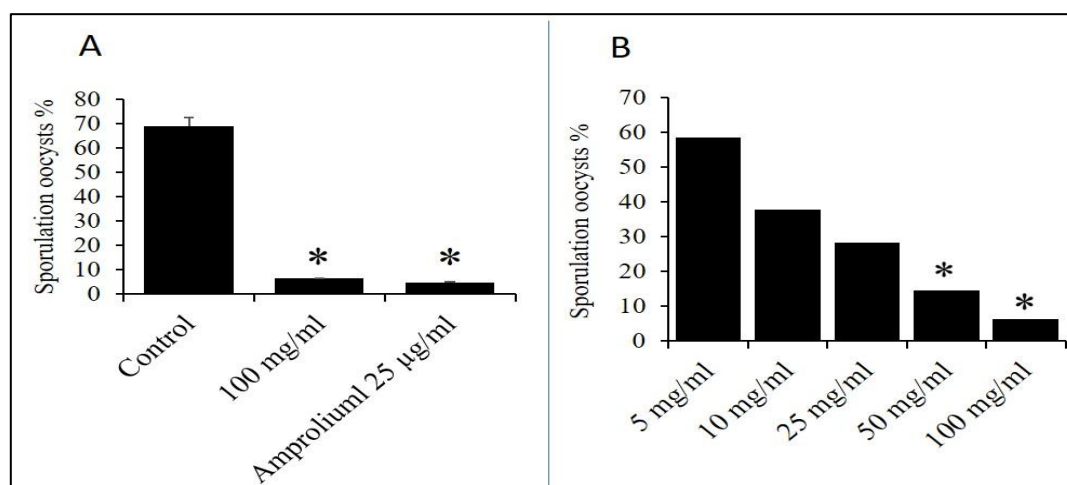


Figure 7. The effect of different doses of CPLE suspension on oocysts inhibition, A) 100mg/kg, and Amprolium 25 µg/mL, compared with control. B) Obtaining the best concentration affects the inhibition of oocysts. * Significance is in comparison to the infected group ($p \leq 0.05$).

The inhibition rate for the control after 96h was 94 ± 3.64 ; 95.5 ± 1.98 . At the doses of 100mg/mL CPLE and Amprolium 2 µg/mL ($P \leq 0.05$), CPLE significantly reduced sporulation rate ($P \leq 0.05$) and it stopped at a dose of 100 mg/mL, symmetric with reference drug. The sporulation inhibition rates of CPLE relative to control were 100%, $47 \% \pm 5.76$; $30 \% \pm 4.56$; $18 \% \pm 1.77$; and $11 \% \pm 2.36$ for 100, 50, 25, 10,

and 5 mg/mL doses, respectively (Table 1). Also, the oocysticidal effect was confirmed by the higher ratio of degenerated oocysts 93.45 ± 5.45 ; 29.33 ± 5.65 ; 19.43 ± 4.32 ; 12.21 ± 2.35 ; 4.57 ± 3.24 ; 94.6 ± 5.13 for 100, 50, 25, 10, 5mg/mL of CPLE doses and Amprolium 25µg/mL, respectively compared with control.

Table 1. Effect of different doses of *C. Procera* on sporulation and destructive rates of *E. tenella* oocysts,

Concentration Mg/mL	Inhibition oocysts%	Destructive oocysts%
Control + 2.5% DPS	00	00
100mg/mL CPLE	$94 \pm 3.64^*$	$93.45 \pm 5.45^*$
50mg/mL CPLE	47 ± 5.76	29.33 ± 5.65
25mg/mL CPLE	30 ± 4.56	19.43 ± 4.32
10mg/mL CPLE	18 ± 1.77	12.21 ± 2.35^{cd}
5mg/mL CPLE	11 ± 2.36	4.57 ± 3.24
Amprolium 25µg/mL	$95.5 \pm 1.98^*$	$94.6 \pm 5.13^*$

At a significance level of $P < 0.05$, the means that have distinct superscripts within a column are found to be significantly different. The linear equation showed the highest coefficient of determination (R^2) values ($P \leq 0.001$) for inhibition at 96 compared with (72, 48, 24 h), (Fig. 8).

It was observed that the viability rate of *E. tenella* sporozoites was significantly different ($p < 0.05$) when exposed to 1000µg/mL of *C.*

procera and 25µg/mL of amprolium. This was compared to the control group ($K_2Cr_2O_7$) at 12 and 24 hours. On the other hand, the viability rate of *E. tenella* sporozoites was found to be different at 500, 250, and 125µg/mL, exhibiting varying degrees of vitality. It was observed that these sporozoites were viable at low concentrations of *C. Procera* (Fig. 9).

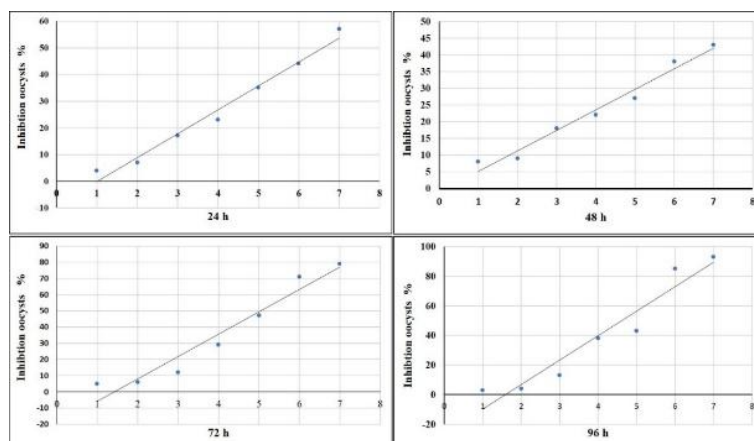


Figure 8. Linear correlation between sporulated and concentration of CPLC after 24, 48, 72, and 96 h of incubation with control and amprolium groups. $P \leq 0.001$.

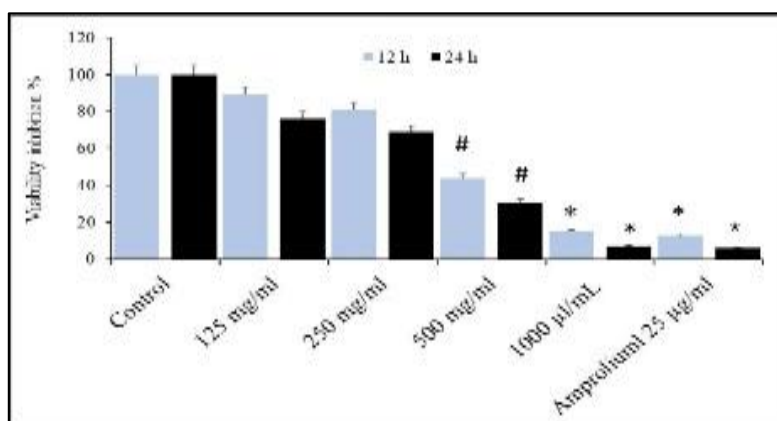


Figure 9. Effect of *C. procera* extracts on the in vitro vitality inhibition of *E. tenella* sporozoites after 12 and 24 hours. *significant differences compared to the control $p < 0.01$. # *significant differences compared to the control ($p < 0.05$; $p < 0.01$), $n = 3$.

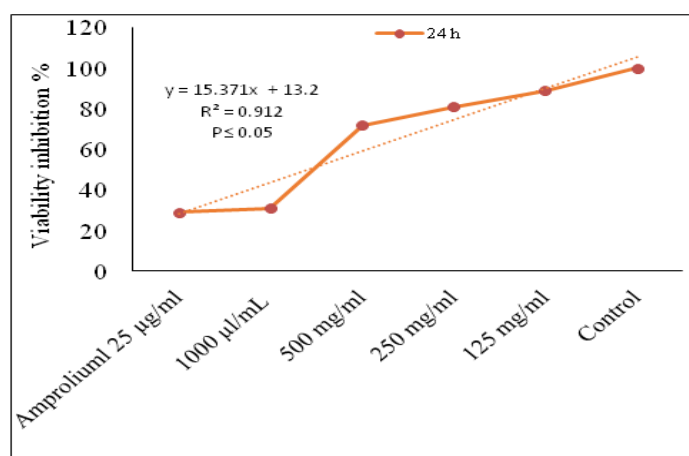


Figure 10. Linear correlation between viability rate to sporozoites oocysts, and concentrations of CPLC after 12 and 24 h of incubation with control and amprolium groups.

The liner equation showed the highest coefficient of determination (R^2) values ($P \leq 0.001$) for sporozoite viability inhibition at 12 and 24, (Fig. 10). The P-value is .003085. The result is significant at $p < 0.05$. 0.954 at 12 h, The P-Value is .011786. The result is significant at $p < 0.05$. 0.91 at 24 h.

DISCUSSION

It is estimated that *Eimeria tenella*, a pathogenic species that lives in the intestine, is responsible for large economic losses in several countries spread around the globe. The fecal samples that were analyzed and found to contain oocysts were found to contain it. The in vitro anticoccidial capabilities of *C. procera* extracts were evaluated based on the quantity and percentage of *Eimeria* oocysts that were destroyed, in addition to the number of oocysts that were inhibited. This was done to determine where the extracts were most effective. In the process of sporozoites being released from sporulated oocysts that have been consumed, they can enter the hepatic cells. Once inside, they rapidly multiply and produce infections that are caused by *E. tenella* before the formation of oocysts (Hernández-Velasco *et al.*, 2014). The oocysts are the location where the progeny develops outside of the host, and it is the sporozoites that are responsible for the transmission of the infection. Multiple investigations (Zhang *et al.*, 2018) have demonstrated that eimeriosis is resistant to the drug that is used to treat it. As a result of this, there is an increasing interest in the utilization of natural products as antiparasitic agents. This is because, in comparison to conventional chemical agents, natural products are more effective, less toxic, and have less adverse side effects (Wunderlich *et al.*, 2014).

The use of a variety of plant extracts has become increasingly common in recent years to prevent and treat infectious diseases in livestock and poultry, such as coccidiosis. Several investigations (Pieri *et al.*, 2014; Xiao *et al.*, 2014) have demonstrated that the methanolic extract of the Madar plant contains anticoccidial properties. *Calotropis procera* extracts were tested for anticoccidial activity in vitro by measuring the quantity and percentage of damaged *Eimeria* oocysts, as well as sporulation inhibition. Eimeriosis has caused significant economic losses in several nations because it affects the majority of animals (Andrews, 2013).

According to the previously described problems, CPLE has a high oocysticidal activity due to their hydrophobic properties, low molecular weight, and bioactive chemicals, which can destroy parasites including oocysts and sporozoites (Jitviriyanon *et al.*, 2016).

In this topic, *C. procera* against *E. tenella* infection was investigated. Different doses of CPLE (5, 10, 25, 50, 100mg/kg) have been tested, and compared with reference drugs. In terms of anticoccidial activity, CPLE might impede *E. tenella* sporulation in the oocyst's sporulation test in vitro, with the highest concentration (100mg/kg) being the most potent.

Following the findings of our investigation, 25µL/ mL of amprolium and concentrations of 100mg/mL demonstrated effectiveness against oocysts. The sporulation inhibitory actions that it possesses are demonstrated to be at their highest, and it is observed to be more efficient against *E. tenella*. Almost no research has been done on the effectiveness of CPE against *Eimeria* oocysts. To the best of our knowledge, these are some of the few papers that have been seen in Saudi Arabia that use CP extracts to prevent the oocyst sporulation of the coccidiosis parasite *E. tenella*. In vitro sporulation suppression was detected using *Psidium guajava* leaf extracts in four different species of *Eimeria* (Cedric *et al.*, 2018), showing results that are comparable to the present findings. Since condensed grape tannins have been shown to inhibit endogenous enzyme activities (such as mannitol-1 phosphate dehydrogenase, mannitol-1 phosphatase, mannitol dehydrogenase, and hexokinase), it is possible that CPLE reduced the rate of sporulation by inhibiting or inactivating the enzymes responsible for the sporulation process as in helminth eggs (Molan *et al.*, 2009). According to Molan *et al.* (2009), one of the most prominent criteria used to evaluate anticoccidial characteristics is known as sporulation inhibition. Nevertheless, most of the researchers made use of the cell culture approach to evaluate the in vitro effect (Burt *et al.*, 2013), as well as the influence on oocyst shedding following the administration of anticoccidials (Zaman *et al.*, 2012). The approach that was taken in this investigation, on the other hand, was both economical and time efficient. The evaluation of the effectiveness of inactivation procedures

carried out for botanical and synthetic agents to disinfect the environment is also a relevant application of this technology. When it came to the sporulation of oocysts, this strategy was predicated on the effect that some herbal extracts had. As a result of the phytochemicals' interference with the physiological processes that are essential for the sporulation process, such as restricting access to oxygen and blocking the enzyme that is responsible for sporulation, it is possible to speculate that the phytochemicals demonstrated an anti-sporulation impact (Nahed et al., 2022). The results of the botanicals may have been affected by several different circumstances, possibly resulting in some minor discrepancies. In the case of herbs that greatly inhibit or stop the sporulation process, for instance, the phytochemicals' capacity to pass through the resistant oocyst wall and the variance in molecular size may be smaller.

CONCLUSIONS

The findings of the current study showed that *C. procera* plant extract was influential in controlling coccidiosis due to the *E. tenella* infection. It has promising efficacy as an effective alternative medicine against coccidiosis. The usage of plant extract could be helpful in the control of coccidiosis; however, this needs to experience more experimental studies to demarcate its effectiveness. However, these findings need to be additionally investigated in the other species of coccidia that influence chickens.

ACKNOWLEDGMENTS

The King Saud University Researchers Supporting Project (RSP2024R94) funded this research.

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