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Anticoccidial and Cytotoxic Activities of *Moltkiopsis ciliata* Leaf Extract: *In vitro* Evaluation against *Eimeria tenella* Oocysts and MCF-7 Cells

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ABSTRACT

Background: *Moltkiopsis ciliata* is a medicinal desert plant rich in diverse secondary metabolites with potential antiparasitic and cytotoxic activities.

Methods: The present study aimed to investigate the in vitro anticoccidial activity of a 70% ethanolic leaf extract of *M. ciliata* against non-sporulated *Eimeria tenella* oocysts and to evaluate its cytotoxic activity against MCF-7 human breast cancer cells. Non-sporulated oocysts were incubated with various concentrations of the extract in 2.5% potassium dichromate, and inhibition of sporulation was determined at successive incubation intervals, with toltrazuril serving as the positive control. Cytotoxic activity was assessed using the MTT assay following exposure of MCF-7 cells to extract concentrations ranging from 15.6 to 1,000 µg/mL for 24 h.

Result: The extract inhibited oocyst sporulation in a concentration- and time-dependent manner. At the highest tested concentration (12.5 mg/mL), approximately 93% inhibition was maintained after 96 h, demonstrating an effect comparable to that of toltrazuril. Moderate inhibitory activity was observed at 6.25 mg/mL, whereas concentrations of 3.125 mg/mL and below primarily delayed sporulation without producing sustained inhibition. In MCF-7 cells, the extract induced a dose-dependent but moderate reduction in cell viability. At the highest tested concentration (1,000 µg/mL), cell viability decreased to approximately 66%, corresponding to about 34% growth inhibition; consequently, the IC_{50} was not reached within the tested concentration range. Collectively, these findings provide preliminary evidence for the promising anticoccidial activity of *M. ciliata* leaf extract against *E. tenella* oocysts, while indicating relatively limited cytotoxicity toward MCF-7 cells under the experimental conditions. Further bioassay-guided fractionation, chemical standardization, mechanistic investigations, and in vivo validation are warranted to identify the bioactive constituents and establish their efficacy and safety.

Key words: Anticoccidial activity, Cytotoxicity, *Eimeria tenella*, MCF-7, Medicinal plants, *Moltkiopsis ciliata*, Sporulation inhibition.

INTRODUCTION

Medicinal plants have long been regarded as important sources of structurally diverse bioactive compounds with potential therapeutic applications in infectious, inflammatory, metabolic and neoplastic diseases (Latif and Nawaz, 2026). Their pharmacological properties are primarily associated with a wide range of secondary metabolites, including flavonoids, phenolic acids, alkaloids, tannins, terpenoids, saponins and glycosides, many of which exhibit antioxidant, antimicrobial, antiparasitic, anti-inflammatory, immunomodulatory and cytotoxic activities (Ma *et al.*, 2026). Growing concerns regarding antimicrobial resistance, drug residues, environmental impact and adverse effects of conventional therapies have further stimulated interest in plant-derived extracts as potentially safer and more sustainable sources of therapeutic agents (Wang *et al.*, 2024). Owing to their chemically complex composition, medicinal plants may modulate multiple molecular and cellular pathways simultaneously, thereby offering opportunities for the discovery and development of novel pharmacologically active compounds (Latif and Nawaz, 2026). Nevertheless, rigorous scientific evaluation involving phytochemical characterization, *in vitro* biological screening, toxicity assessment and subsequent *in vivo* validation remains essential to establish their efficacy, safety and potential applications in veterinary and biomedical settings (Vaou *et al.*, 2022).

Avian coccidiosis remains one of the most economically important parasitic diseases affecting poultry production worldwide (Dubey *et al.*, 2026). It is caused by

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protozoan parasites of the genus *Eimeria*, which undergo intracellular development within the intestinal epithelium, resulting in epithelial damage, impaired nutrient absorption, intestinal lesions, reduced weight gain, poor feed conversion, morbidity and, in severe cases, mortality (Gao *et al.*, 2024; Mathis *et al.*, 2025). Among the *Eimeria* species known to infect chickens, *E. tenella* is regarded as one of the most pathogenic because of its marked tropism for the ceca, where infection can induce extensive mucosal damage, hemorrhagic lesions and substantial impairment of productive performance (Liao *et al.*, 2024; Ristanti *et al.*, 2024). Beyond the direct losses associated with mortality and impaired growth, coccidiosis imposes a substantial economic burden on the global poultry industry through expenditures related to prophylaxis, therapeutic intervention and disease-management programs (Blake *et al.*, 2020).

Current strategies for controlling avian coccidiosis rely primarily on anticoccidial drugs, ionophores, chemical disinfectants and live vaccines (Sharma *et al.*, 2025). Although these interventions have contributed substantially to disease control and improved poultry health, their long-term effectiveness is increasingly constrained by the emergence of drug-resistant *Eimeria* strains, limitations in vaccine coverage, production-associated costs and growing consumer preference for safer poultry products with minimal drug residues (Gao *et al.*, 2024; Ahmad *et al.*, 2024). These challenges have stimulated considerable interest in natural products, particularly medicinal plants and their secondary metabolites, as potential alternative or complementary anticoccidial agents (Al-Malki *et al.*, 2025). Plant-derived extracts and essential oils contain chemically diverse bioactive constituents, including phenolics, flavonoids, terpenoids, tannins, alkaloids, saponins and sterols, which may suppress parasite development through multiple mechanisms, such as disruption of cellular membranes, inhibition of key enzymes, modulation of oxidative stress, regulation of host immune responses and attenuation of parasite-associated inflammation (Ramdani *et al.*, 2023).

Inhibition of oocyst sporulation represents an important endpoint in anticoccidial screening because unsporulated *Eimeria* oocysts are non-infective (Nguyen *et al.*, 2026). Accordingly, suppressing or delaying sporulation may help disrupt environmental transmission and reduce the infectious burden within poultry production systems (Ristanti *et al.*, 2024). Increasing evidence indicates that plant-derived products can act directly on *E. tenella* oocysts and interfere with their development. For instance, Piper betle essential oil has demonstrated oocysticidal activity and inhibited *E. tenella* sporulation in vitro, whereas resveratrol has been reported to suppress oocyst sporulation while also improving growth performance, lesion scores, antioxidant status and intestinal barrier-associated responses in infected chickens (Ristanti *et al.*, 2024). Collectively, these observations provide a strong rationale for further investigation of locally available medicinal plants as potential sources of new anticoccidial agents.

M. ciliata (Forssk.) I.M. Johnst., a member of the Boraginaceae family, is a desert medicinal plant distributed in Saudi Arabia and other arid regions. Recent phytochemical characterization of *M. ciliata* using DART-ToF-MS and GC-MS revealed a chemically diverse profile comprising alkaloids, sterols, saponins, flavonoids, triterpenes, tannins, essential oils, lignans, vitamin E, campesterol, stigmasterol, γ-sitosterol, phytol, echinatine and heliotrine (Al Suliman *et al.*, 2025). Several of these phytochemical classes have been associated with antimicrobial, antioxidant, anti-inflammatory, antiparasitic and cytotoxic activities, highlighting *M. ciliata* as a potentially valuable yet comparatively underexplored source of bioactive metabolites (Moustafa *et al.*, 2025). Despite its ethnomedicinal importance and phytochemical diversity, however, the anticoccidial activity of *M. ciliata*, particularly against *E. tenella* oocysts, remains insufficiently investigated.

In addition to their antiparasitic properties, medicinal plants have attracted considerable attention as sources of compounds with potential cytotoxic and antiproliferative effects against cancer cells, including the human breast adenocarcinoma MCF-7 cell line (Fosu *et al.*, 2025). Plant-derived phytochemicals are particularly relevant to cancer research because they may influence multiple cellular and molecular processes involved in tumor progression, including oxidative stress, apoptosis, cell proliferation, inflammation, angiogenesis and metastasis-associated signaling pathways (Gunasekaran *et al.*, 2025; Devaraji *et al.*, 2025). The MTT assay is widely employed as an initial *in vitro* screening approach to assess the effects of crude plant extracts and isolated compounds on cell metabolic activity and viability. Recent studies have demonstrated that botanical extracts enriched in flavonoids, triterpenoids and polyphenolic compounds can reduce MCF-7 cell viability in a concentration- and time-dependent manner, supporting the utility of this cell model for the preliminary identification of plant-derived compounds with potential antiproliferative activity (Zulkipli *et al.*, 2024).

Based on this rationale, the present study aimed to evaluate the in vitro anticoccidial activity of ethanol *M. ciliata* leaf extract against unsporulated *E. tenella* oocysts and to assess its cytotoxic effects on MCF-7 human breast adenocarcinoma cells.

MATERIALS AND METHODS

Fresh specimens of *M. ciliata* were collected from Riyadh, Saudi Arabia. The plant material was taxonomically identified by a specialist at King Saud University and a voucher specimen was deposited under voucher number 24231. This experiment was completed between January 2026 and July 2026 in the Zoology Department of King Saud University's College of Science. The collected plant material was thoroughly washed with tap water, rinsed

several times with distilled water and air-dried at room temperature. The dried leaves were subsequently ground into a fine powder using an electric grinder. The powdered material was extracted with 70% ethanol by cold maceration according to Manikandan *et al.* (2008), with minor modifications. Briefly, the powdered leaves were suspended in 70% ethanol and incubated for 24 h at 4°C. Following filtration, the combined filtrates were concentrated under reduced pressure using a rotary evaporator at 50°C to remove the ethanol. The resulting crude extract was then lyophilized to obtain a dry extract and stored at -20°C until further analysis.

Assay of sporulation inhibition

The challenge inoculum was derived from a laboratory-maintained monospecific *E. tenella* line that had originally been characterized by expert parasitologists on the basis of oocyst morphology, size, shape index, wall characteristics and PCR analysis (Mares *et al.*, 2023). Unsporulated *E. tenella* oocysts were initially recovered from the feces of naturally infected domestic chickens (*Gallus gallus domesticus*) and purified by flotation. The purified oocysts were subsequently incubated in 2.5% potassium dichromate at 28°C under humid conditions to allow sporulation. For propagation and maintenance of the parasite line, five broiler chickens were orally inoculated with 1×10^3 sporulated oocysts. Oocysts recovered from cecal contents and fecal material at 7 days post-infection, washed thoroughly, concentrated and subsequently used for the in vitro sporulation-inhibition assay.

Sporulation Inhibition Assay. The *in vitro* sporulation inhibition assay was performed according to Zaman (2015), with modifications, using 24-well flat-bottom microtiter plates. Unsporulated *E. tenella* oocysts were suspended in 2.5% potassium dichromate ($K_2Cr_2O_7$; Veterinary Products Company [VAPCO], Amman, Jordan), which served as the negative-control medium and provided conditions suitable for oocyst viability and sporulation. The *M. ciliata* leaf extract was prepared in the same 2.5% $K_2Cr_2O_7$ medium at final concentrations of 12.5, 6.25, 3.125, 1.563, 0.781, 0.391 and 0.195 mg/mL. The use of an identical $K_2Cr_2O_7$ medium for both treated and negative-control groups minimized potential differences attributable to the incubation medium.

Each well contained 2 mL of the corresponding treatment solution and 1×10^3 unsporulated *E. tenella* oocysts. The plates were semi-covered and incubated at 28°C and 65% relative humidity, with gentle agitation at regular intervals to facilitate aeration and sporulation (Al-Quraishy *et al.*, 2026). Each treatment was evaluated in triplicate.

Oocyst sporulation was assessed at 24, 48 and 72 h after incubation. At each time point, a 10 µL aliquot was collected from each treatment and examined using a McMaster counting chamber under a light microscope (BX51TF; Olympus, Tokyo, Japan) at ×40 magnification. Sporulated and unsporulated oocysts were enumerated and sporulation in each treatment group was compared with that of the negative control (Murshed *et al.*, 2023). The percentages of sporulation and sporulation inhibition were calculated using the following equations:

$$\text{Sporulation} = \frac{\text{Sporulated oocysts}}{\text{Total oocysts}} \times 100$$

$$\text{Inhibition \%} = \frac{\text{Sporulation of control} - \text{Sporulation of medicine}}{\text{Sporulation of control}} \times 100$$

Cytotoxicity evaluation

Human breast adenocarcinoma MCF-7 cells were obtained from the American Type Culture Collection (ATCC). The cells were maintained in RPMI-1640 culture medium supplemented with 10% fetal bovine serum and 50 µg/mL gentamicin. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 and were routinely subcultured to maintain optimal growth conditions.

MTT assay

MTT Cytotoxicity Assay. The cytotoxic effect of the ethanolic *M. ciliata* extract on MCF-7 cells was evaluated using the MTT colorimetric assay according to Siddiqui *et al.* (2008), with appropriate modifications. Actively proliferating cells were detached using 0.25% trypsin–EDTA and seeded into 96-well plates at a density of 1×10^4 cells per well. The cells were then exposed to different concentrations of *M. ciliata* extract ranging from 15.6 to 1,000 µg/mL for 24 h. Following treatment, 10 µL of MTT solution (5 mg/mL) was added to each well and the plates were incubated for an additional 4 h. The culture medium was subsequently removed and the resulting formazan crystals were dissolved in 200 µL of dimethyl sulfoxide (DMSO). Absorbance was measured at 550 nm using a microplate spectrophotometer. Cell viability was calculated relative to the untreated control.

Statistical analysis

Statistical analyses were performed using SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA). Differences among experimental groups were evaluated using one-way analysis of variance (ANOVA). Data are expressed as the mean ± standard error of the mean (SEM) and differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

M. ciliata leaf extract exhibited a concentration-dependent reduction in MCF-7 cell viability and a concentration- and time-dependent inhibitory effect on *E. tenella* oocyst sporulation, with the greatest sporulation inhibition observed after 96 h of exposure.

Cytotoxicity against MCF-7 (Breast cancer)

The cytotoxicity results demonstrated a concentration-dependent reduction in MCF-7 cell viability following exposure to *M. ciliata* leaf extract (Fig 1). Cell viability

progressively decreased with increasing extract concentration, indicating a measurable but moderate cytotoxic effect under the tested conditions. The corresponding cellular observations further supported the MTT findings, showing treatment-associated alterations in MCF-7 cell growth and morphology compared with untreated cells (Fig 2). Collectively, these findings indicate that *M. ciliata* leaf extract affects MCF-7 cell viability in a concentration-dependent manner. However, because 50% inhibition of cell viability was not achieved within the tested concentration range, the IC_{50} could not be determined, suggesting that the extract exhibited only limited cytotoxic activity against MCF-7 cells under the experimental conditions.

The consistency of the observed response across experimental replicates supports the reproducibility of the cytotoxic effect. Overall, *M. ciliata* leaf extract produced a concentration-dependent reduction in MCF-7 cell viability, indicating the presence of bioactive constituents capable of affecting cellular metabolic activity and/or survival (Fig 3). However, the underlying mechanisms, including possible effects on cell proliferation, apoptosis, or metabolic pathways, require confirmation using dedicated mechanistic assays.

Anticoccidial activity

After 24 h of *in vitro* exposure, MCLE significantly affected the sporulation of *E. tenella* oocysts compared with the negative control, while its activity was also evaluated relative to the positive control, toltrazuril (Fig 4). The inhibitory effect observed at this early time point indicates that MCLE begins to interfere with oocyst development within the first 24 h of exposure. However, the present assay does not establish whether this effect results from structural damage, loss of oocyst viability, or other mechanisms.

After 48 h of exposure, MCLE produced a statistically significant inhibitory effect on *E. tenella* oocyst sporulation compared with the control group ($p<0.05$), indicating that the anticoccidial activity became more pronounced with prolonged exposure (Fig 5). At 72 h, MCLE continued to significantly suppress oocyst sporulation ($p<0.05$), demonstrating that the inhibitory effect was sustained over time (Fig 6). These findings support a time-dependent anticoccidial effect of MCLE against *E. tenella* oocyst development under the tested in vitro conditions.

At 96 h, MCLE produced the greatest inhibitory effect on *E. tenella* oocyst sporulation, with statistically significant differences relative to the control groups, $p<0.05$ (Fig 7). The enhanced inhibition observed at the longest incubation period demonstrates that the activity of MCLE was sustained and became more pronounced with increasing exposure time. These findings further support a time-dependent anticoccidial effect of the extract against *E. tenella* oocyst development under the tested *in vitro* conditions.

The present study demonstrates that *M. ciliata* leaf extract exhibits measurable biological activity in two distinct vitro systems, consistent with its previously reported phytochemical richness (Al Suliman *et al.*, 2025). The concentration-dependent reduction in MCF-7 cell viability, together with the progressive inhibition of *E. tenella* oocyst sporulation, suggests that the extract contains bioactive constituents capable of affecting both mammalian tumor cells and protozoan developmental stages. Such a broad spectrum of activity is plausible for plant extracts containing phenolics, flavonoids, sterols and related secondary metabolites, many of which have been associated with antioxidant, pro-oxidant, antiproliferative and antiparasitic properties (Sudha *et al.*, 2018; Murshed *et al.*, 2026). However, the present findings do not establish whether the two biological effects arise through shared or distinct molecular mechanisms.

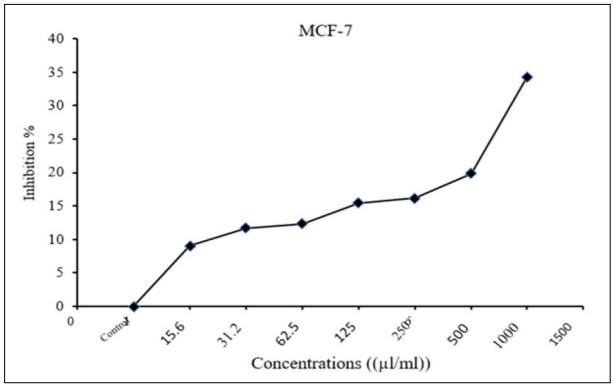


Fig 1: Cytotoxicity of *M. ciliata* plant extract against MCF-7.

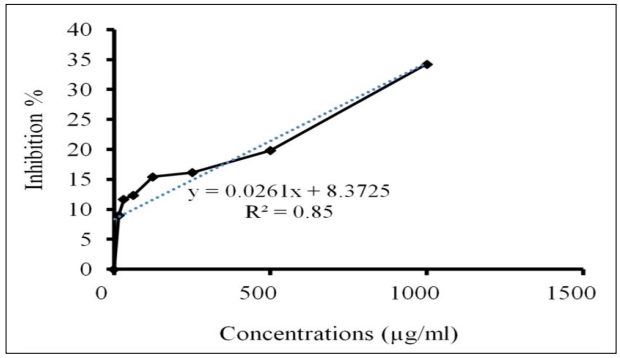


Fig 2: Effect of *M. ciliata* extract on MCF-7 breast cancer cells.

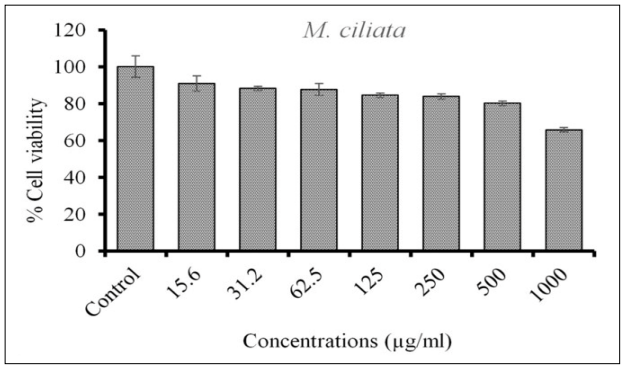
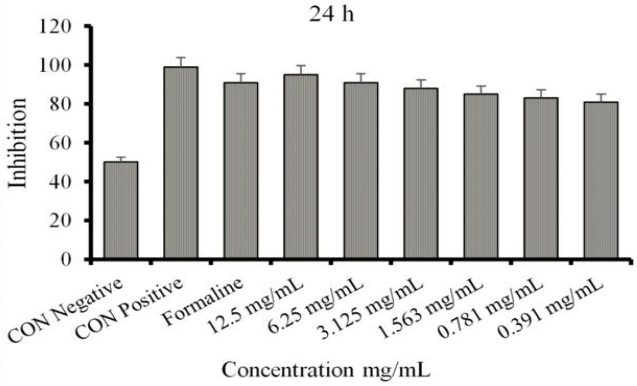


Fig 3: Cytotoxicity of *M. ciliata* plant extract against MCF-7.

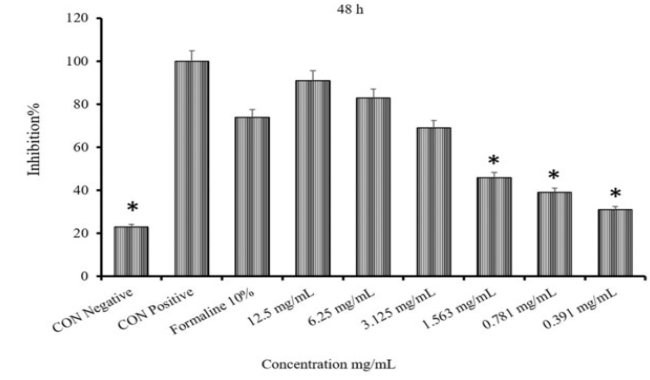
The reduction in MCF-7 cell viability observed following treatment with *M. ciliata* leaf extract is broadly consistent with previous studies reporting cytotoxic effects of plant-derived extracts and flavonoids in this cell model. Such phytochemicals have been shown in other experimental systems to modulate apoptosis-related pathways, alter the Bax/Bcl-2 balance, increase intracellular reactive oxygen species and impair mitochondrial function (Chihi *et al.*, 2021). The concentration-dependent decline in viability observed in the present study may therefore reflect the combined actions of multiple phytochemical constituents rather than interaction with a single molecular target. MCF-7 cells are widely used as an *in vitro* model of estrogen receptor-positive, hormone-responsive breast cancer, making them suitable for preliminary screening of plant-derived products with potential cytotoxic or antiproliferative activity (Kabala-Dzik *et al.*, 2018; Bagherian *et al.*, 2021). Nevertheless, because the IC_{50} was not reached within the tested concentration range and no mechanistic assays were performed, the present findings should be interpreted as evidence of limited cytotoxic activity rather than definitive anticancer efficacy.

The observed biological activity is also consistent with the reported phytochemical diversity of *M. ciliata*, for which recent chemical profiling identified more than 30 constituents, including vitamin E, campesterol, stigmasterol, γ-sitosterol, phytol and several alkaloid-related compounds (Al Suliman *et al.*, 2025). Several of these constituents or their broader phytochemical classes have previously been associated with antioxidant and cytotoxic activities, while phytol and phytosterols have been reported to influence cellular redox balance, membrane-



Oocysts incubated in 2.5% potassium dichromate served as the negative control (CON), whereas toltrazuril (25 mg/mL) was used as the positive control. Statistical significance was determined by comparison with the corresponding control groups.

Fig 4: Effect of *M. ciliata* leaf extract (MCLE) on *E. tenella* oocyst sporulation after 24 h of *in vitro* incubation.



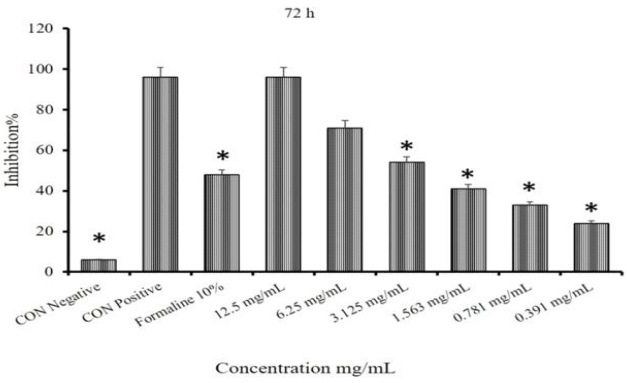
Oocysts incubated in 2.5% potassium dichromate served as the negative control (CON), whereas toltrazuril (25 mg/mL) was used as the positive control. Statistical significance was assessed relative to the corresponding control groups; (*): $p<0.05$ was considered significant.

Fig 5: Effect of *M. ciliata* leaf extract (MCLE) on *E. tenella* oocyst sporulation after 48 h of *in vitro* incubation.

associated processes and intracellular signaling pathways. Although the present study did not identify the constituent(s) responsible for the observed reduction in MCF-7 cell viability, the activity of the crude extract may reflect the combined contribution of multiple phytochemicals. Further bioassay-guided fractionation and mechanistic studies are required to determine the active compounds and establish whether additive, synergistic, or other interactions contribute to the observed effect.

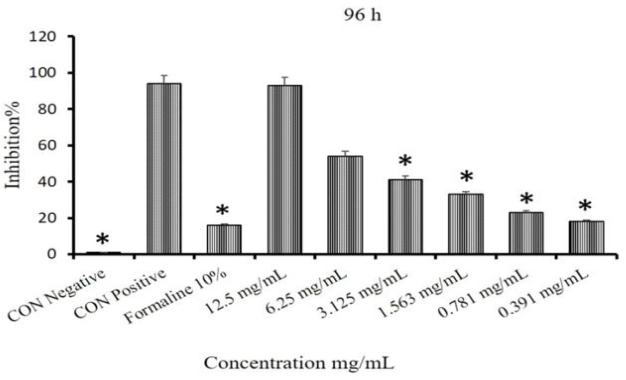
The anticoccidial activity of *M. ciliata* leaf extract against *E. tenella* oocysts became progressively more pronounced with increasing exposure time, with the greatest inhibition of sporulation observed at 96 h. This time-dependent response suggests that prolonged contact with the extract interferes with oocyst development and sporulation rather than producing an immediate effect. Similar studies have reported that plant-derived products can inhibit Eimeria development, reduce oocyst output and adversely affect parasite viability or structural integrity under experimental conditions (Habibi *et al.*, 2016; Felici *et al.*, 2023). These observations support further investigation of botanical extracts as potential complementary or alternative sources of anticoccidial agents.

The anticoccidial activity of *M. ciliata* may be associated with the actions of its phytochemical constituents on multiple parasite-related processes. Phenolic and related plant-derived compounds have been reported to affect membrane integrity, cellular redox balance, energy metabolism and enzymatic pathways involved in parasite development and survival (Han *et al.*, 2022). However, these mechanisms



Oocysts incubated in 2.5% potassium dichromate served as the negative control (CON), whereas toltrazuril (25 mg/mL) was used as the positive control. Statistical significance was assessed relative to the corresponding control groups; (*): p<0.05 was considered significant.

Fig 6: Effect of *M. ciliata* leaf extract (MCLE) on *E. tenella* oocyst sporulation after 72 h of *in vitro* incubation.



Oocysts incubated in 2.5% potassium dichromate served as the negative control (CON), whereas toltrazuril (25 mg/mL) was used as the positive control. Statistical significance was assessed relative to the corresponding control groups. p<0.05 was considered significant, Symbols denote the significance levels shown in the figure: p<0.05 (*).

Fig 7: Effect of *M. ciliata* leaf extract (MCLE) on *E. tenella* oocyst sporulation after 96 h of *in vitro* incubation.

were not directly investigated in the present study and therefore remain hypothetical for *M. ciliata*. Botanical extracts have attracted increasing interest as potential alternatives or adjuncts to ionophores and synthetic anticoccidial drugs because of concerns regarding drug resistance, chemical residues and the sustainability of conventional control strategies. Given the economic importance of *E. tenella* in poultry production, the observed inhibition of oocyst sporulation supports further investigation of *M. ciliata* as a potential source of naturally derived anticoccidial compounds (Murshed *et al.*, 2025).

Comparison with previously published anticoccidial studies provides additional context for the present findings. Herbal preparations derived from Sophora flavescens, Pulsatilla koreana and other medicinal plants have previously been reported to reduce intestinal lesion scores, oocyst shedding and clinical severity in chickens experimentally infected with *E. tenella* (Youn and Noh, 2001). More recent studies have also investigated botanical formulations containing compounds such as apigenin, eugenol and eucalyptus-derived constituents, reporting varying degrees of anticoccidial activity and acceptable tolerability in broilers (Geng *et al.*, 2024). Although direct comparison with these *in vivo* studies is limited by differences in experimental design, preparations and measured endpoints, the concentration- and time-dependent inhibition of *E. tenella* oocyst sporulation observed in the present study supports further investigation of *M. ciliata* as a potential source of naturally derived anticoccidial compounds.

The greater inhibition observed a later time point may reflect cumulative interference with processes required for oocyst maturation and sporulation, although the underlying mechanism remains to be established (Murshed *et al.*, 2023). *Eimeria* oocysts are known for their considerable environmental resistance, largely due to the protective architecture of the oocyst wall. Therefore, the progressive suppression of sporulation observed over time suggests that *M. ciliata* contains constituents capable of interfering with oocyst development despite these protective barriers. Further bioassay-guided fractionation is warranted to determine whether the activity is primarily associated with polar phenolic constituents, less-polar terpenoid or sterol-related compounds, or the combined effects of multiple phytochemicals (El-Shall *et al.*, 2022).

Taken together, the present findings indicate that *M. ciliata* leaf extract possesses measurable in vitro biological activity, particularly against *E. tenella* oocyst sporulation, while exerting limited cytotoxic effects on MCF-7 cells. However, these observations should be considered preliminary because they are based exclusively on vitro assays. Further studies should therefore focus on bioassay-guided fractionation, chemical identification and standardization of the active constituents, evaluation of selectivity toward malignant versus non-malignant cells, mechanistic characterization and *in vivo* assessment of anticoccidial efficacy and safety in chickens. Such investigations will be necessary to determine whether *M. ciliata* can serve as a viable source of phytotherapeutic anticoccidial compounds and to clarify the significance of its cytotoxic activity in cancer-related applications.

CONCLUSION

M. ciliata leaf extract demonstrated significant *in vitro* anticoccidial activity against *E. tenella* oocyst sporulation and a concentration-dependent but limited cytotoxic effect on MCF-7 cells. These findings support further investigation of *M. ciliata* as a potential source of bioactive constituents for anticoccidial applications and warrant additional studies to clarify the relevance of its cytotoxic activity in cancer-related research.

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Declaration of AI involvement in writing

The authors acknowledge the use of AI to assist in language refinement.

Disclaimers

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal Care and handling techniques were approved by the University of Animal Care Committee approval number (KSU-SE-25-10).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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