

Potential of *Andrographis paniculata* and *Morinda citrifolia* as Natural Anticoccidial Agents in Broiler Chickens: An In Vitro Study

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ABSTRACT

Coccidiosis is a parasitic disease that commonly affects broiler chickens, caused by protozoa of the genus *Eimeria*. This disease significantly reduces productivity, impairs feed efficiency, and increases mortality rates. The continuous use of synthetic anticoccidial drugs has raised concerns over resistance and drug residues accumulation in poultry products. This study aimed to evaluate and compare the in vitro anticoccidial efficacy of herbal powders from *Andrographis paniculata* (sambiloto) and *Morinda citrifolia* (noni leaves) as potential natural alternatives. A total of 150 fecal samples were collected from five poultry farms using a simple random sampling method. Eight treatment groups were established: a negative control (without coccidiostat), a positive control (trimethoprim-sulfamethoxazole), and six herbal treatments (0.25%, 0.5% and 0.75% of both *A. paniculata* and *M. citrifolia* powders). Each treatment group comprised five biological replicates. Oocyst per gram (OPG) counts were determined using McMaster technique. Both herbal powders significantly reduced OPG compared to the negative control ($p < 0.05$). The most effective treatment was *M. citrifolia* at 0.75%, showing the lowest OPG value and comparable efficacy to the synthetic anticoccidial. *M. citrifolia* 0.75% reduced mean OPG to $4,098 \pm 250$ ($p < 0.001$) compared to the negative control. This study provides the direct comparative evidence of *A. paniculata* and *M. citrifolia* anticoccidial activity using standardized OPG quantification, suggesting their promise as sustainable herbal alternatives for coccidiosis control.

Keywords: anticoccidial, *Andrographis paniculata*, *Morinda citrifolia*, *Eimeria*

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INTRODUCTION

Coccidiosis is a parasitic disease caused by protozoan endoparasites of the genus *Eimeria* (Olabode *et al.*, 2020). This disease negatively affects growth performance, body weight, carcass quality, and may cause significant mortality in broiler chickens. In Indonesia, the prevalence of coccidiosis varies across regions and tends to be higher in humid environments (Arsyitahlia *et al.*, 2019; Dewi *et al.*, 2025; Fitri *et al.*, 2021; Yuwono *et al.*, 2023). Coccidiosis is commonly controlled with synthetic anticoccidial

drugs such as sulfonamides, amprolium, and diclazuril (Ekawasti and Martindah, 2019). However, improper dosing can lead to drug resistance, rendering treatments ineffective and posing a threat to the poultry industry. Continuous administration of sulfonamide-based anticoccidials without proper regulation, may also result in drug residues in meat, liver, and eggs (Martins *et al.*, 2022). Therefore, there is an urgent need for safer and more sustainable alternatives.

Herbal therapeutics, such as *Andrographis paniculata* (commonly known as sambiloto) and *Morinda citrifolia* (noni leaves), have been identified as promising natural anticoccidial agents. *A. paniculata* contains bioactive compounds such as flavonoids and tannins, which exhibit antiparasitic and immunomodulatory effects (Ramonah *et al.*, 2023). Similarly, *M. citrifolia* leaves are rich in flavonoids, alkaloids, and saponins. Saponins are known to disrupt membrane integrity in protozoa by interacting with membrane sterols, a plausible mechanism for the observed *in vitro* effect (Pahlani *et al.*, 2022; Masneno *et al.*, 2023). Previous studies have reported that extracts from *A. paniculata* and *M. citrifolia* reduce the number of *Eimeria* oocysts in broiler feces (Irianti *et al.*, 2022; Karimy *et al.*, 2013).

Therefore, this study aimed to compare the *in vitro* anticoccidial efficacy of *A. paniculata* and *M. citrifolia* herbal powders at multiple concentrations using standardized oocyst-per-gram (OPG) quantification. We hypothesized that increasing concentrations of both herbal powders would significantly reduce OPG compared to the negative control and that *M. citrifolia* would demonstrate stronger efficacy than *A. paniculata*. This investigation provides new insights into the comparative potential of these two herbal agents as sustainable and safe alternatives to synthetic coccidiostats.

METHODS

Study design

This study employed an *in vitro* quantitative experimental design. A total of 150 fecal samples were collected using a simple random sampling method from five broiler farms in Malang, with 30 samples per farm. Samples were divided

into eight treatment groups: (1) Negative Control (K1): 0.5 mL of 0.9% NaCl solution; (2) Positive Control (K2): 0.5 mL of trimethoprim-sulfamethoxazole (Interbat®, Indonesia); (3–5) *A. paniculata* treatments at 0.25%, 0.5%, and 0.75%; (6–8) *M. citrifolia* treatments at 0.25%, 0.5%, and 0.75%.

Treatment concentrations were determined based on preliminary range-finding tests and literature on non-toxic phytochemical levels for poultry (Xu *et al.*, 2025; Choi & Kim, 2020). Each treatment group consisted of five biological replicates: each derived from distinct pooled fecal samples to account for biological variation. Sample allocation and treatment assignments were randomized using a computer-generated sequence to minimize bias.

Fecal sample examination

Both qualitative and quantitative examinations were performed. The qualitative test was conducted using direct smear and flotation methods to confirm the presence of *Eimeria* oocysts. The quantitative analysis employed the McMaster technique (Ngwese *et al.*, 2020) to calculate oocyst per gram (OPG). Each measurement was performed in triplicate (technical replicates) to ensure precision.

Preparation of herbal concentration solutions

Herbal powders of *A. paniculata* and *M. citrifolia* were obtained from a certified herbal supplier (CV. Herbalindo, Malang, Indonesia) and standardized by dry weight. Concentration solutions of 0.25%, 0.5%, and 0.75% were prepared by dissolving 0.025 g, 0.050 g, and 0.075 g of each powder, respectively, in 10 mL of 0.9% NaCl. All solutions were freshly prepared before each trial and homogenized for 10 minutes using a vortex mixer to ensure uniform dispersion.

In vitro incubation procedure

In vitro incubation was carried out using fecal samples with pre-determined OPG values. The weight of feces per microtube was calculated using the formula: $\text{Weight} = 5000 / \text{total OPG}$. Each sample was placed in a microtube and treated accordingly (0.5 ml of either control or herbal solution). Incubation was conducted at 26°C for 72 hours in the Laboratory of Veterinary Histology, Faculty of Veterinary Medicine, Universitas Brawijaya. The number of replications was determined using the Federer formula. With 8 groups ($t = 8$), a minimum of three replications was required. Following precedent (Cahyaningsih *et al.*, 2012), each treatment group was replicated five times.

Oocyst counting

After incubation, 0.5 g of fecal material from each treatment was mixed with 7.5 mL of saturated sugar solution, homogenized, and filtered through a strainer. A 0.5 mL aliquot was placed in a McMaster chamber (Jorgensen Laboratories, USA) and examined under an Olympus CX-23® microscope at 100× magnification. OPG values were calculated according to Correia *et al.* (2021). Each count was performed independently by two trained observers, and mean values were used for analysis to reduce observer bias.

Data analysis

Data were analyzed using RStudio version 4.4.2. Data normality was assessed using the Shapiro–Wilk test, confirming nonparametric distribution. The Kruskal–Wallis H test ($p < 0.05$) was used to determine statistical differences among groups, followed by pairwise comparisons using the Mann–Whitney U post hoc test to assess significant differences between treatments (Okoye and Hosseini, 2024).

RESULT AND DISCUSSION

Microscopic examination and oocyst per gram (OPG) count of *Eimeria* spp.

Microscopic analysis at 400× magnification (Figure 1) revealed the presence of *Eimeria* oocysts. They were characterized by unsporulated and an oval shape. Visible structures included the outer wall, inner membrane, and sporocysts. *Eimeria* species infecting chickens typically exhibit oval, smooth and transparent oocysts lacking micropyles or residual bodies but containing polar granules. Each oocyst typically contains four sporocysts, each containing two sporozoites (Mares *et al.*, 2023). The morphological features observed in this study were consistent with descriptions of *Eimeria tenella* as reported by Ristanti *et al.* (2024), identified by its oval shape, smooth surface, and absence of micropyles.

The mean OPG in the negative control group was 24,054 oocysts/g ($n = 5$ biological replicates; median 23,900; IQR 21,600–26,300). This level is indicative of a high oocyst burden and is comparable to values reported in severe infection scenarios in other field surveys (e.g., Arsyitahlia *et al.*, 2019). According to this study, the average OPG across five poultry farms in Malang is indicative of a severe infection classification ($>15,000$ OPG).

Anticoccidial effectiveness of *Andrographis paniculata* Powder

Treatment with *A. paniculata* significantly reduced *Eimeria* oocyst counts across all tested concentrations (Table 1, Figure 2). The Kruskal–Walli's test indicated a significant difference among treatment groups ($H = 25.37$, $p < 0.001$). Post hoc Mann–Whitney tests showed that *A. paniculata* at 0.25% reduced OPG by 49.5% compared to the negative control ($U = 2.0$, $p = 0.023$). Meanwhile, 0.5% and 0.75%

concentrations showed greater reductions (66.4% and 74.7%, respectively) and did not differ significantly from the positive control ($p > 0.05$).

These findings are consistent with Irianti *et al.* (2024), who reported that *A. paniculata* significantly reduced oocyst counts at a 20% concentration, resulting in an inhibition value of 24.67 ± 5.03 . The reduction is attributed to bioactive compounds such as flavonoids and tannins. Indrati and Titisari (2020) also reported significant OPG reduction at a 1125 mg dose (75.15% decrease).

Flavonoids present in *A. paniculata* can induce oxidative stress in host cells as part of the immune defense mechanism, thereby disrupting the redox balance within *Eimeria* oocysts and increasing reactive oxygen species (ROS), which ultimately damage oocyst membranes (Irianti *et al.*, 2022; Scicutela *et al.*, 2021; El-Ghareeb *et al.*, 2023). The observed reduction in OPG may also be associated with phytochemicals such as flavonoids and tannins, which have been reported to cause oxidative damage and inhibit key enzymatic activities in protozoa (Irianti *et al.*, 2022; Masneno *et al.*, 2023). However, mechanistic confirmation, such as assessments of membrane

integrity or ROS quantification, was beyond the scope of this *in vitro* study.

Tannins also reduce intestinal inflammation by suppressing pro-inflammatory cytokines (TNF- α , IL-6, IL-1 β), thus accelerating tissue regeneration (Yuan *et al.*, 2023). However, excessive doses can be toxic; Choi and Kim (2020) reported that tannins at 25 g/kg adversely affect growth and liver function, while Xu *et al.* (2025) recommended a safe dose of $\leq 0.375\%$. Overdosage may reduce nutrient digestibility and productivity, induce immunosuppression, and delay growth (Ramah *et al.*, 2022). Xu *et al.* (2023) recommended 500–750 mg/kg for broiler diets. Flavonoids also have a tolerable dose limit of 300 mg/kg, beyond which the onset of hepatic toxicity may occur, as indicated by elevated transaminase enzyme levels (Ortiz-Andrade *et al.*, 2020). In summary, *A. paniculata* powder is effective as an anticoccidial agent at various concentrations, particularly at 0.5% and 0.75%, demonstrating efficacy comparable to synthetic anticoccidials such as trimethoprim. Therefore, while *A. paniculata* demonstrates significant *in vitro* anticoccidial potential, optimization of dosage is essential to balance efficacy and safety in potential *in vivo* applications.

Table 1. Kruskal–Wallis test results for *A. paniculata* treatments

Treatment Group	Mean OPG	Confidence Interval (95%)	Significance (p-value)
Negative Control	24,054	21,625 – 26,300	
Positive Control	6,838	5,675 – 7,300	
0.25% <i>A. paniculata</i>	12,166	8,800 – 14,925	< 0.000
0.5% <i>A. paniculata</i>	8,082	6,175 – 9,800	
0.75% <i>A. paniculata</i>	6,076	4,275 – 7,250	

Table 2. Kruskal–Wallis test results for *M. citrifolia* treatments

Treatment Group	Mean OPG	Confidence Interval (95%)	Significance (p-value)
Negative Control	24,054	21,625 – 26,300	< 0.000
Positive Control	6,838	5,675 – 7,300	
0.25% <i>M. citrifolia</i>	5,132	3,650 – 6,450	
0.5% <i>M. citrifolia</i>	5,118	3,600 – 6,250	
0.75% <i>M. citrifolia</i>	4,098	2,825 – 5,000	

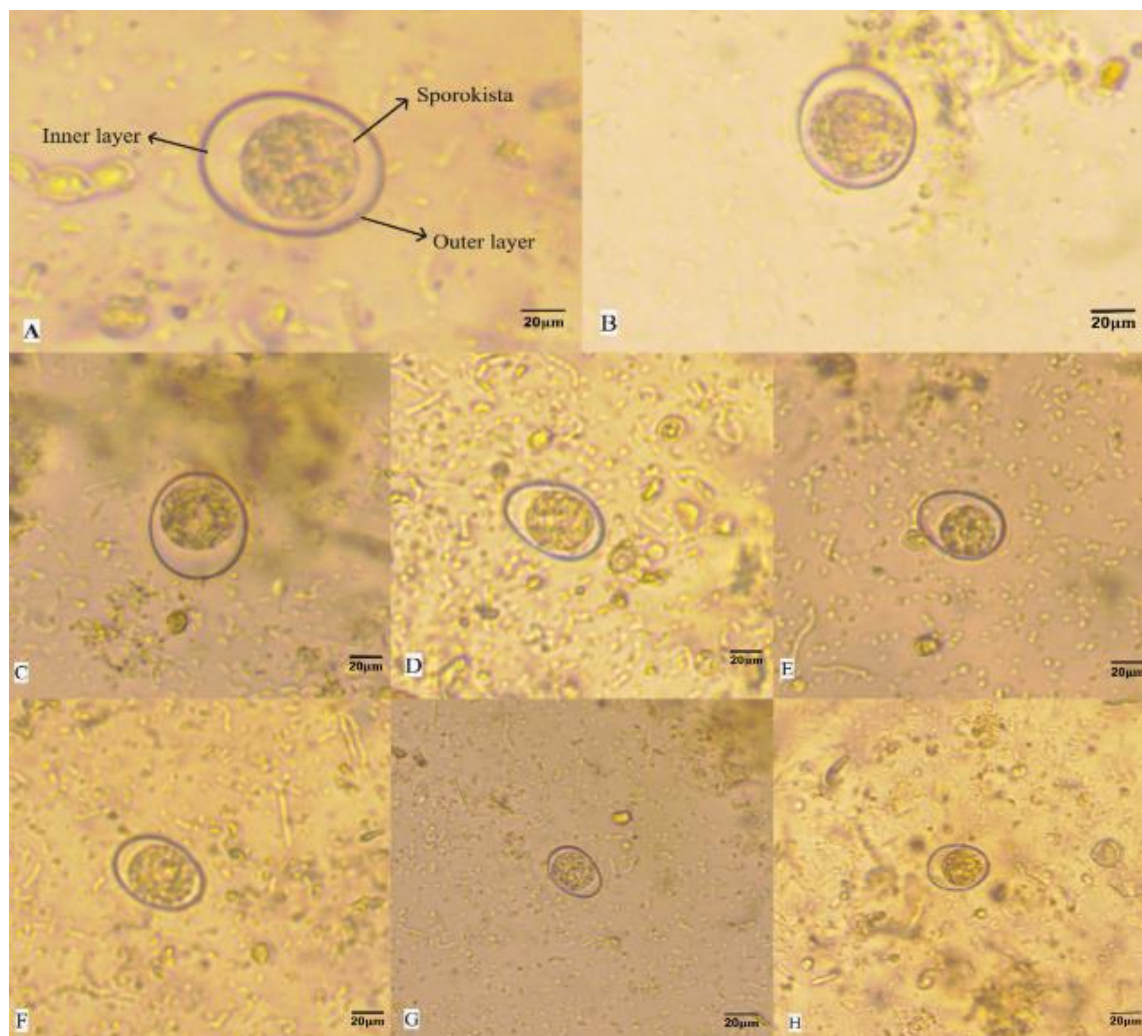


Figure 1. Unsporulated *Eimeria* oocysts at 400× magnification. Description: (A) Negative Control, (B) Positive Control, (C) 0.25% *Andrographis paniculata*, (D) 0.5% *A. paniculata*, (E) 0.75% *A. paniculata*, (F) 0.25% *Morinda citrifolia*, (G) 0.5% *M. citrifolia*, (H) 0.75% *M. citrifolia*.

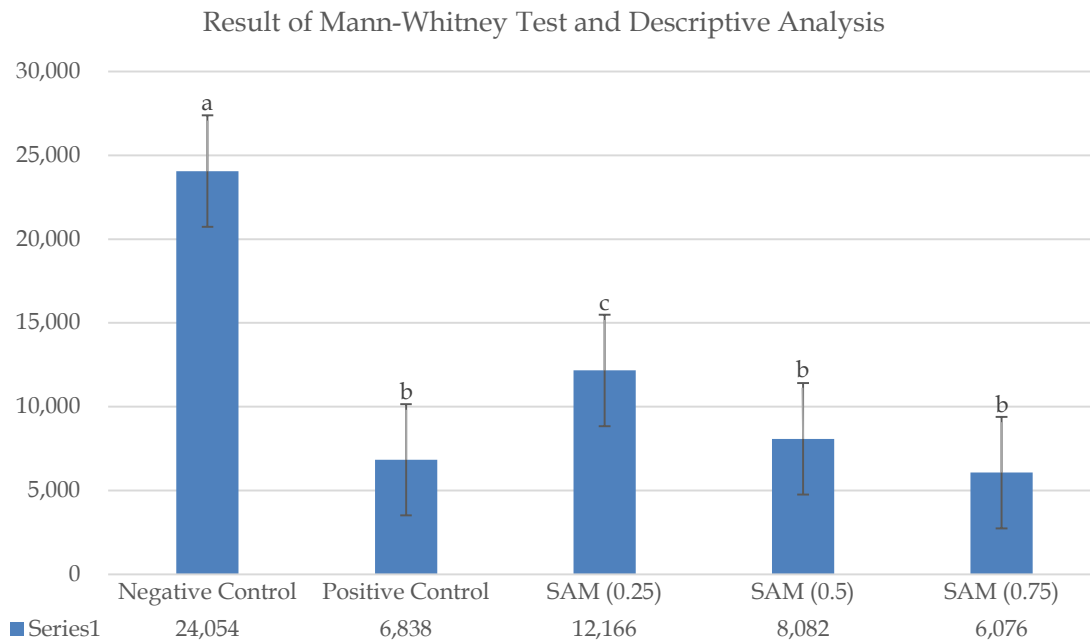


Figure 2. Average OPG of *A. paniculata* Treatment Groups. Different letters indicate significant differences ($p < 0.05$). Description: SAM (0.25, 0.5, 0.75): Mann–Whitney test results for *Andrographis paniculata* (Sambiloto) leaf powder at concentrations of 0.25%, 0.50%, and 0.75%.

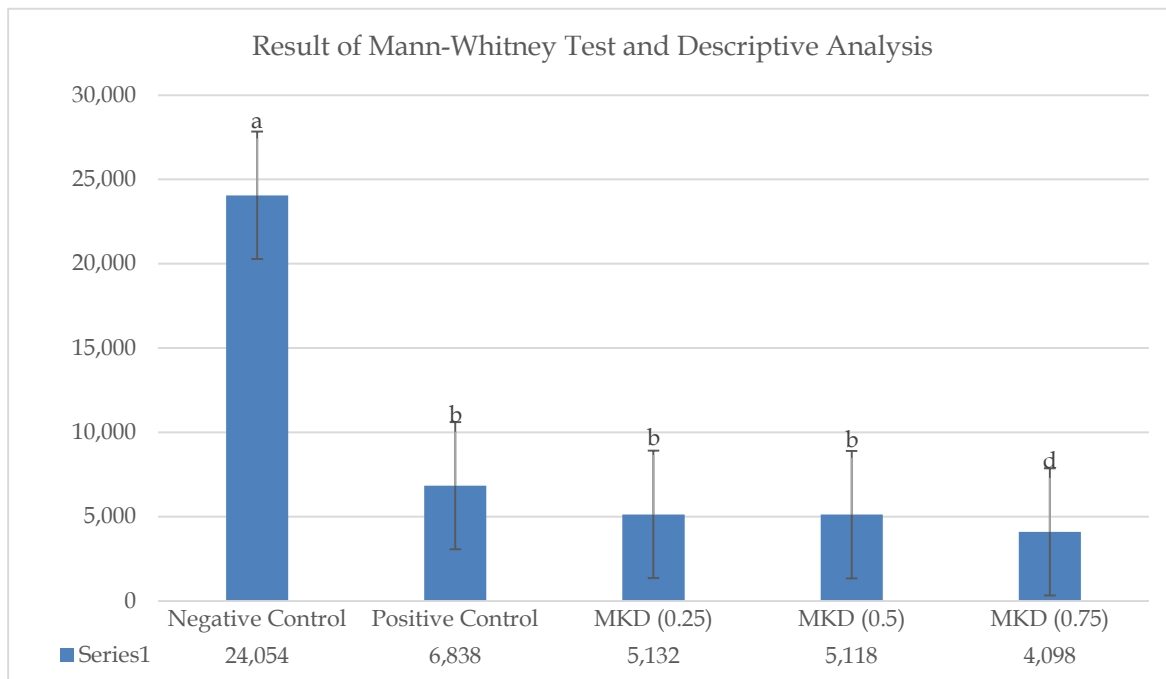


Figure 3. Average OPG of *M. citrifolia* Treatment Groups. Different letters indicate significant differences ($p < 0.05$). Description: MKD (0.25, 0.5, 0.75): Mann–Whitney test results for *Morinda citrifolia* (Noni) leaf powder at concentrations of 0.25%, 0.50%, and 0.75%.

Anticoccidial effectiveness of *Morinda citrifolia* leaf powder

M. citrifolia treatment also resulted in significant reductions in OPG at all tested concentrations (Table 2, Figure 3).

The Kruskal–Walli’s analysis showed a significant overall effect ($H = 27.91$, $p < 0.001$). The 0.25% and 0.5% treatments showed OPG reductions of 78.7% and 78.8%, respectively, compared to the negative control ($p < 0.05$), with efficacy comparable to the positive control ($p > 0.05$). The 0.75% concentration yielded the lowest mean OPG ($4,098 \pm 250$), significantly lower than both controls ($p < 0.01$), indicating the strongest inhibitory activity. These results align with Karimy *et al.* (2013), who demonstrated that *M. citrifolia* extract effectively prevented oocyst development in *Eimeria tenella* infections at a 200 mg/kg BW dose, with an average elimination of 2.34 log₁₀ OPG. This effect is attributed to active compounds such as alkaloids, flavonoids, and tannins.

Alkaloids inhibit oocyst sporulation by interfering with maturation enzymes and inducing oxidative stress through ROS generation (Li *et al.*, 2022). Flavonoids disrupt oocyst morphology by penetrating the wall and causing cytoplasmic damage (Amrane-Abider *et al.*, 2023). Tannins interfere with sporulation and sporocyst development by binding endogenous enzymes (Ristanti *et al.*, 2024). Although these compounds are beneficial, overdosing may be toxic. Zhao *et al.* (2020) reported a lethal dose of 5.48 g/kg for alkaloids, may cause respiratory distress, tremors, and mortality.

Comparative insights and mechanistic interpretation

The comparative analysis revealed that both *A. paniculata* and *M. citrifolia*

powders effectively reduced oocyst counts, with *M. citrifolia* demonstrating greater efficacy at equivalent concentrations. The concentration-dependent decline in OPG for *M. citrifolia*, illustrated in Figure 3, supports the hypothesis that increasing herbal dosage enhances anticoccidial effects. The phytochemical interactions within these herbs may differ in their target mechanisms: flavonoid-dominant *A. paniculata* likely acts through oxidative stress and enzyme inhibition, whereas saponin-rich *M. citrifolia* primarily disrupts membrane integrity.

These biochemical differences underscore the potential for combined or sequential herbal formulations that leverage complementary mechanisms to optimize anticoccidial efficacy. The exploration of herbal medicine often employs integrative therapeutic approaches, which can enhance treatment efficacy through the synergistic effects of various compounds. While prior research indicates that the combination of different herbal constituents can lead to improved therapeutic outcomes, specific studies have highlighted the need for thorough mechanistic investigations, such as enzyme activity assays and cell membrane integrity analyses (Asare *et al.*, 2021; Vranish *et al.*, 2018). Future mechanistic studies would help elucidate these interactions in more detail, revealing the underlying pathways that contribute to enhanced efficacy.

This study was confined to in vitro analysis, which, despite offering controlled and reproducible conditions, may not adequately reflect the complex physiological environment present in living organisms (Chaves de Jesus *et al.*, 2025). The herbal powders were assessed in their crude forms, without quantitative determination of individual active constituents, and no dose–

response modeling or phytochemical quantification, such as total phenolic or flavonoid content, was conducted (Singh *et.al.*, 2022). Furthermore, the absence of detailed phytochemical profiling limits the interpretability and translational potential of the findings (Abdel-Tawab, 2021).

Future research should prioritize *in vivo* investigations to substantiate both efficacy and safety, as well as employ advanced analytical techniques such as liquid chromatography–mass spectrometry (LC–MS) for comprehensive identification and quantification of key bioactive compounds (Singh *et.al.*, 2022). Additionally, exploring the synergistic interactions of combined herbal formulations may enhance therapeutic efficacy and potentially reduce the required dosages (Abdel-Tawab, 2021).

CONCLUSION

The present study demonstrates that both *Andrographis paniculata* and *Morinda citrifolia* powders possess promising *in vitro* anticoccidial activity. Increasing concentrations of both herbs significantly reduced oocyst output, with *M. citrifolia* at 0.75% exhibiting the strongest inhibition and comparable efficacy to synthetic coccidiostats. These findings suggest that *M. citrifolia* powder, particularly at 0.75%, could serve as a viable natural substitute for chemical anticoccidials in poultry production, pending further *in vivo* validation and feed safety assessments. Further research on dose optimization and compound characterization is warranted to support its practical application in sustainable poultry health management.

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Author Contribution

Reza Yesica: Conceptualization; Methodology; Validation; Formal analysis; Writing - Original Draft; Writing - Review & Editing; Project Administration; and Funding Acquisition. Angelica Maharani Putri: Methodology; Software; Formal analysis; Investigation; Resources; Data Curation; Writing - Original Draft; Visualization. Shelly Kusumarini R: Methodology; Data Curation; Supervision. Heri Irawan: Methodology; Data Curation; Supervision. Fajar Shodiq Permata: Conceptualization; Methodology; Funding Acquisition.

Competing Interest

None.

Ethical Approval

All animal-related procedures in this study were reviewed and approved by the Animal Ethics Committee, Universitas Brawijaya (Approval No. 137-KEP-UB-2024).

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