



Prevalence of Intestinal Parasites and the Therapeutic Potential of *Andrographis Paniculata* Leaf Extract in Managing Coccidiosis and Enhancing Growth Performance in Broiler Chickens

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Abstract

Intestinal parasitic infections remain a persistent challenge in poultry production, with coccidiosis standing out as one of the most economically damaging diseases due to its effects on growth, feed utilization, and flock health. The growing concerns surrounding drug resistance, residue accumulation, and rising costs of conventional anticoccidial agents have intensified the search for safer and more sustainable alternatives. This study investigated the prevalence of intestinal parasites in broiler chickens sourced from selected markets in Uyo metropolis and evaluated the therapeutic efficacy and growth-promoting potential of *Andrographis paniculata* leaf extract in broilers experimentally infected with coccidiosis. A total of 420 broilers were sampled from Itam and Akpan Andem markets, and fecal samples were analyzed using standard parasitological techniques. Additionally, an experimental trial involving *Eimeria tenella*-infected broilers treated with graded doses (100, 200, and 400 mg/kg body weight) of the plant extract was conducted, alongside a conventional drug control (diclazuril). Results revealed an overall parasite prevalence of 43.1%, with *Eimeria* spp. being the most dominant. The extract significantly reduced oocyst shedding and improved growth indices in a dose-dependent manner. These findings highlight the potential of *A. paniculata* as a viable natural alternative in poultry disease management.

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Keywords: Coccidiosis, Intestinal parasites, *Andrographis paniculata*, *Eimeria tenella*, Broiler chickens

Article history

Received: January 24, 2026

Received in revised form: March 27, 2026

Accepted for publication: April 3, 2026

Available online: April 8, 2026

1.0 Introduction

Poultry production has become an essential pillar in the effort to meet the growing demand for affordable animal protein, particularly across developing nations where population growth continues to outpace food supply (Attia et al. 2022). In many households, broiler chicken represents not just a source of nutrition, but also a means of livelihood and economic stability.

Despite its importance, the sector is constantly navigating a range of challenges that threaten its efficiency and long-term sustainability (Caisin, & Scripnic, 2026). Among these challenges, disease outbreaks stand out as one of the most persistent and costly constraints.

A closer look at these disease pressures reveals that intestinal parasitic infections remain deeply

embedded within poultry production systems. These infections often operate quietly, without immediate dramatic signs, yet their impact accumulates over time. Birds that appear healthy may still experience reduced nutrient absorption, weakened immunity, and slower growth (Riber, et al., 2024; Chapman, 2009; Choi et al., 2021). The result is a gradual but significant decline in overall productivity. Farmers may notice increased feed consumption without corresponding weight gain, inconsistent flock performance, and in severe cases, higher mortality rates (Abbas et al., 2011).

Within this group of parasites, coccidiosis has maintained its reputation as one of the most economically damaging diseases in poultry (Lee et al., 2022). Caused by protozoan parasites of the genus *Eimeria*, it thrives in environments where birds are raised in close confinement. The disease spreads easily through contaminated litter, feed, and water, making it difficult to control once established (Islam et al., 2025). Its effects are far-reaching, ranging from mild subclinical infections that reduce performance to severe outbreaks characterized by diarrhea, dehydration, and death. What makes coccidiosis particularly challenging is its ability to persist within production systems, re-emerging even after control measures have been applied.

For decades, the poultry industry has relied heavily on synthetic anticoccidial drugs to manage this disease (Abraham et al., 2025). These drugs have played a crucial role in reducing losses and maintaining productivity. However, their effectiveness is no longer as consistent as it once was. Continuous and sometimes indiscriminate use has contributed to the development of drug-resistant strains of *Eimeria* (Ratcliffe et al., 2024). At the same time, the cost of these medications continues to rise, placing additional financial pressure on farmers, especially small-scale producers. There is also a growing awareness among consumers regarding the presence of chemical residues in animal products, which has influenced

preferences toward safer and more natural production systems (Chaachouay & Zidane, 2024).

In response to these evolving concerns, attention is gradually shifting toward alternative approaches that can complement or even replace conventional drugs (Cantin-Rosas et al., 2025). Among these, plant-based therapeutics have emerged as a promising direction (Asiru & Abifarin, 2016; Badparva & Kheirandish, 2017; Baeshen et al., 2023). Plants have been used for centuries in traditional medicine, offering a wide range of bioactive compounds capable of combating infections and supporting overall health. Unlike many synthetic drugs, plant-derived treatments often provide multiple benefits at once, including antimicrobial, antioxidant, and immune-boosting effects (Efriem et al., 2023; Oliveira et al., 2025). Their accessibility and relatively low cost further enhance their appeal, particularly in regions where resources are limited.

One plant that has gained increasing attention in this context is *Andrographis paniculata* (Xu et al., 2025). Commonly known as the “king of bitters,” it has a long history of use in herbal medicine across Asia and parts of Africa. Its therapeutic properties are well documented, with studies highlighting its antimicrobial, anti-inflammatory, and antiparasitic activities. Despite this, its potential application in poultry production, especially in the control of coccidiosis, has not been fully explored. This presents an opportunity to investigate whether a plant with established medicinal value in humans can be effectively translated into animal health management.

This study is therefore designed to take a more integrated approach. It does not only seek to understand the prevalence of intestinal parasites within a real-world poultry supply system, but also to evaluate a practical solution under controlled conditions. By combining field-based epidemiological assessment with experimental validation, the research moves beyond

observation into application. It aims to provide insights that are both scientifically grounded and directly relevant to improving poultry health and productivity in a sustainable manner.

2. Materials and Methods

2.1 Study Area and Sample Collection

Uyo has a typically humid tropical climate characteristic of the Niger Delta, with two distinct seasons: a long rainy season (March/April–October) and a shorter dry season (November–March) (Georgen et al., 2014). Temperatures remain consistently warm, averaging about 26.4 °C, while annual rainfall exceeds 2,000 mm, sustaining lush vegetation and supporting year-round farming (Georgen et al., 2014).

Located within Nigeria's tropical rainforest belt, Uyo was originally covered by dense evergreen rainforest and swamp vegetation. However, urban expansion and agriculture have transformed much of this into secondary vegetation and cultivated land (George et al., 2015). The persistently warm and humid conditions in this environment also favour the survival and transmission of intestinal parasites.

The study was carried out in Uyo metropolis (Fig.1), a vibrant urban center in Akwa Ibom State, Nigeria, where poultry trading forms an important part of the local economy. Two major markets, Itam and Akpan Aдем, were selected because of their high turnover of broiler chickens and their role as key supply points to farmers and households within and beyond the city.

A total of 420 broiler chickens were randomly sampled from these markets. Care was taken to ensure that the selection reflected a mix of ages and sources, as typically encountered in commercial circulation. Fresh fecal samples were collected directly from the birds in clean, sterile containers to avoid contamination. Each sample was properly labeled and stored in cooled carriers to preserve integrity during transport to the laboratory. The handling process

was done promptly and carefully to ensure that the samples remained suitable for accurate parasitological examination.

2.2 Parasitological Examination

In the laboratory, each fecal sample was processed using established flotation and sedimentation techniques, methods widely recognized for their reliability in detecting both helminth eggs and protozoan oocysts. The flotation technique helped to recover lighter parasitic stages, while sedimentation allowed for the detection of heavier eggs that may not float easily.

Microscopic examination was carried out using a light microscope, where identification relied on observable morphological features such as size, shape, shell thickness, and internal structures. To improve accuracy, multiple fields of view were examined for each sample. Where necessary, repeated observations were conducted to confirm findings and reduce the chances of misidentification.

2.3 Sample Size and Experimental Design

The sample size was estimated using the formula provided in Equation 1, as described by Thrusfield et al. (2018) for survey studies. A predicted prevalence of 50% was assumed, since the actual prevalence was not known.

$$n = \frac{z^2 \times p_{\text{exp}} (1 - p_{\text{exp}})}{d^2} \quad (1)$$

where n = required number, z = standard normal variate (z -score) for the desired confidence interval (1.96 for a 95% confidence interval), P = expected prevalence or proportion of the characteristics in the population, often estimated from previous studies or pilot study (if unknown 50% is commonly used to maximize the sample size and d = absolute desired precision (margin of error) usually set at 0.05 (5%)

The sample size (n) was determined using Equation 1, yielding a value of 384.16, which was rounded up to 400 for practical implementation. To enhance the robustness and

reliability of the study, the sample size was further increased to 420, consistent with standard practice that allows for a modest upward adjustment beyond the minimum required estimate.

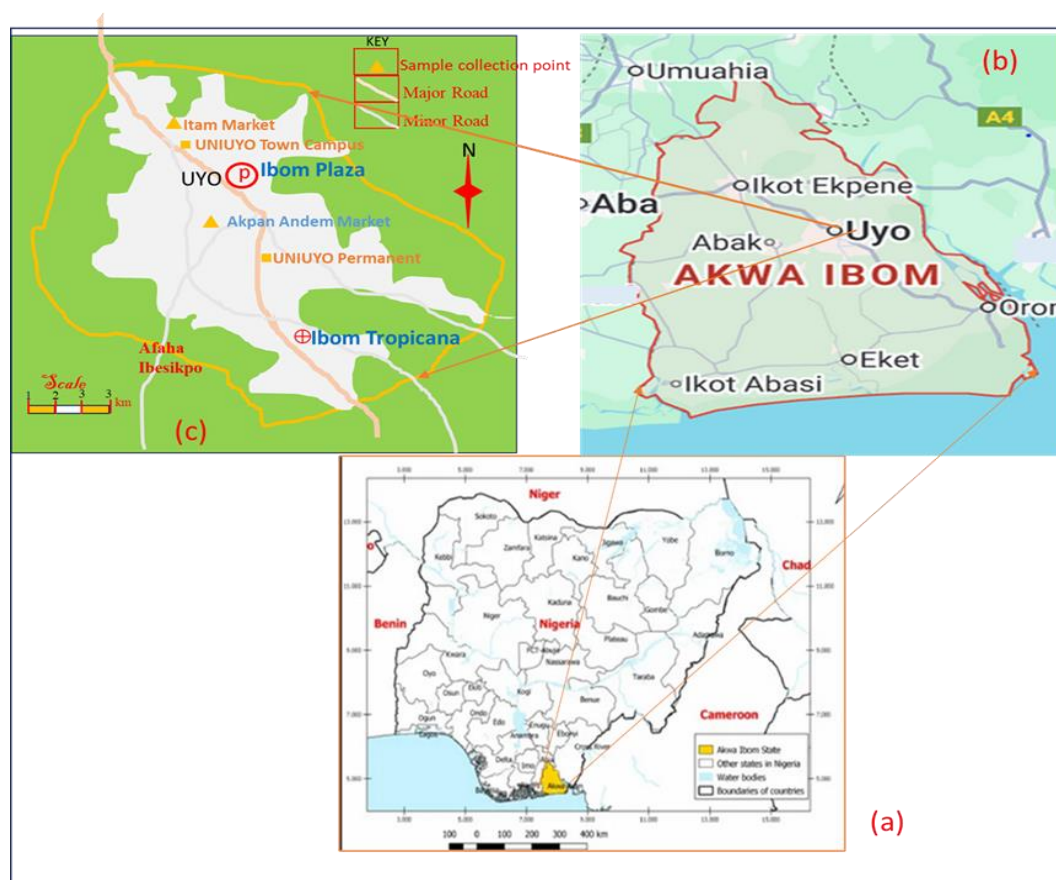


Fig. 1: Location maps illustrating (a) Nigeria with Akwa Ibom State highlighted, (b) Akwa Ibom State showing Uyo, and (c) Uyo Metropolis indicating the major market sampling points (adapted from George et al., 2014).

To evaluate the therapeutic potential of *Andrographis paniculata*, a controlled experimental trial was designed using healthy broiler chicks. The birds were acclimatized under standard management conditions before the commencement of the study to ensure that stress-related factors did not influence the outcome.

The birds were then experimentally infected with a known dose of *Eimeria tenella*, a highly pathogenic species responsible for caecal coccidiosis. Following infection, the birds were randomly assigned into five groups, each receiving a different treatment regime.

The first group served as the negative control and remained uninfected and untreated, providing a baseline for normal physiological performance. The second group, the positive

control, was infected but received no treatment, allowing for observation of disease progression in the absence of intervention. The third group was treated with diclazuril, a standard anticoccidial drug, to serve as a reference for conventional therapy.

The remaining three groups received *A. paniculata* leaf extract at doses of 100, 200, and 400 mg per kilogram of body weight, respectively. Treatments were administered orally, and the birds were monitored closely throughout the study period.

2.4 Preparation of Plant Extract

Fresh leaves of *Andrographis paniculata* were collected from a reliable local source and authenticated to ensure botanical accuracy. The leaves were thoroughly washed to remove dirt and debris, then air dried under shade to preserve

their active constituents. Once fully dried, the leaves were ground into a fine powder using a mechanical grinder.

The powdered material was subjected to solvent extraction using an appropriate solvent system, ensuring maximum recovery of bioactive compounds. The extract obtained was filtered and concentrated using controlled evaporation techniques to remove the solvent without degrading the active ingredients. The final extract was stored under suitable conditions until use and was reconstituted to the required concentrations before administration to the experimental birds.

2.5 Growth and Health Assessment

Throughout the experimental period, the birds were observed daily, and key performance indicators were carefully recorded (Kabeta et al., 2024). Feed intake was measured by tracking the quantity of feed consumed over time, while body weight gain was monitored at regular intervals using a sensitive weighing scale.

Feed conversion ratio was calculated to assess how efficiently the birds converted feed into body mass, providing insight into overall productivity. Clinical observations were also made, focusing on visible signs such as diarrhea, reduced activity, ruffled feathers, and general weakness, which are commonly associated with coccidiosis (Marzouk & Alajaji, 2025; Martins et al., 2022; McDougald & Fitz-Coy, 2013).

Oocyst shedding was evaluated through periodic fecal examinations. This served as a direct measure of infection intensity and treatment effectiveness. By combining growth performance data with clinical and parasitological observations, a comprehensive picture of the birds' health status was obtained (Murshed et al., 2023).

2.6 Phytochemical Analysis

To better understand the therapeutic effects observed, the plant extract was analyzed using Gas Chromatography Mass Spectrometry. This technique allowed for the identification and characterization of the chemical constituents

present in the extract (Akinmoladun et al., 2007).

The analysis provided detailed information on the composition of the extract, including the presence of compounds known for their biological activity. These findings helped to link the observed anticoccidial and growth-promoting effects with specific phytochemical components.

2.7 Statistical Analysis

All data generated from the study were analyzed using appropriate statistical software. Descriptive statistics were first employed to organize and summarize the data in a clear and meaningful way. This was followed by the application of relevant inferential tests to evaluate differences between groups. Statistical significance was determined at a probability level of $p < 0.05$, ensuring that any observed variations were unlikely to have occurred by chance.

Where significant differences were detected, post hoc analyses were conducted to further identify the specific groups responsible for these variations. In addition, the chi-square test was used to examine relationships between categorical variables, including comparisons between infected and treated birds as well as variations across different sampling locations. The use of SPSS enhanced the accuracy and consistency of the computations, thereby increasing confidence in the findings.

The statistical approach provided a reliable framework for interpreting the data and drawing well-informed conclusions regarding the patterns of parasitic infection and the observed responses to treatment within the study.

3. Results and discussion

3.1 Prevalence of Intestinal Parasites

The parasitological survey revealed that intestinal parasites remain a common occurrence among broiler chickens within the study area, with an overall prevalence of 43.1 percent. This finding reflects the level of exposure that birds

face within typical market environments, where biosecurity measures may be limited (Andersen, et al., 2021; Kovács et al., 2025). The parasites *Heterakis gallinarum*, *Ascaridia galli*, *Raillietina* species, and *Eimeria* species are shown in Figs. 2–5, with the microscopic images highlighting their distinctive features.



Fig. 2: Microscopic image showing an *Heterakis gallinarum* egg recovered from the intestine of a broiler chicken during the prevalence survey ($\times 40$)



Fig.3: Microscopic image showing an *Ascaridia galli* egg recovered from the intestine of a broiler chicken during the prevalence survey ($\times 40$)

Among the parasites detected, *Eimeria* species were the most prevalent, accounting for 26.0 percent of infections. This was followed by *Heterakis gallinarum* at 10.5 percent, while both *Ascaridia galli* and *Raillietina* species were recorded at 8.3 percent each (Table 2 and Fig. 6). Although coinfections were observed in some birds, they did not dominate the infection pattern. Interestingly, statistical analysis showed that parasite occurrence did not vary significantly with respect to market location,

age, sex, or sampling period (Figs. 6-9). This suggests that the risk of infection is relatively uniform across the population and not confined to specific groups or conditions.



Fig. 4: Microscopic image showing coinfection of *Raillietina* species and *Eimeria* species eggs recovered from the intestine of a broiler chicken during the prevalence survey ($\times 40$)



Fig. 5: Microscopic image showing an *Eimeria* species oocyst recovered from the intestine of a broiler chicken during the prevalence survey ($\times 40$)

3. 2 Intestinal Parasite Coinfection Patterns in Broiler Chickens

A total of 420 broiler chickens were examined to determine the prevalence and patterns of intestinal parasite coinfections. Across all categories, the most commonly observed coinfections were *Heterakis gallinarum* + *Eimeria* spp. and *Raillietina* spp. + *Eimeria* spp., each accounting for 3.1 percent of the sampled population. Analysis by market location revealed notable differences (Fig. 10). *Heterakis gallinarum* + *Eimeria* spp. was significantly more prevalent in Akpan Andem, affecting 4.8 percent of birds, compared to 1.4 percent in Itam ($p = 0.049$).

Table 1: Prevalence of parasite infection among broiler chickens across markets, months, sex, and age categories

Categories	Number Examined	Infected (%)	Uninfected (%)
Markets			
Itam	210	85 (40.5)	125 (59.5)
Akpan Andem	210	96 (45.7)	114 (54.3)
Total	420	181 (43.1)	239 (56.9)
Chi-square		1.175	
df		1	
p Value		0.276 ns	
Months			
June	70	32 (45.7)	38 (54.3)
July	70	29 (41.4)	41 (58.6)
August	70	31 (44.3)	39 (55.7)
September	70	37 (52.9)	33 (47.1)
October	70	28 (40.0)	42 (60.0)
November	70	24 (34.3)	46 (65.7)
Total	420	181 (43.1)	239 (56.9)
Chi-square		5.524	
df		5	
p Value		0.355 ns	
Sex			
Male	195	77 (39.5)	118 (60.5)
Female	225	104 (46.2)	121 (53.8)
Total	420	181 (43.1)	239 (56.9)
Chi-square		1.932	
df		1	
p Value		0.165 ns	
Age Groups (Weeks)			
4 to 5	129	58 (45.0)	71 (55.0)
6 to 7	272	114 (41.9)	158 (58.1)
8 to 9	19	9 (47.4)	10 (52.6)
Total	420	181 (43.1)	239 (56.9)
Chi-square		0.48	
df		2	
p Value		0.787 ns	

* Source: Field Data, 2025

Table 2: Prevalence of Broiler Chicken Infection by Parasite Species Across Markets

Categories	<i>Heterakis gallinarum</i> (%)	<i>Ascaridia galli</i> (%)	<i>Rellitinia</i> spp. (%)	<i>Eimeria</i> spp. (%)
Markets				
Itam	14 (6.7%)	23 (11.0)	17 (8.1)	48 (22.9)
Akpan Andem	30 (14.3)	12 (5.7)	18 (8.6)	61 (29.0)
Total	44 (10.5)	35 (8.3)	35 (8.3)	109 (26.0)
Chi-square	6.499	3.771	0.031	2.094
df	1	1	1	1
p Value	0.011*	0.052*	0.860ns	0.148ns

Analysis by market location revealed notable differences (Fig. 10). *Heterakis gallinarum* + *Eimeria* spp. was significantly more prevalent in Akpan Andem, affecting 4.8 percent of birds, compared to 1.4 percent in Itam ($p = 0.049$). Similarly, *Rellitina* spp. + *Eimeria* spp. appeared more frequently in Akpan Andem (4.3%) than in Itam (1.9%). Other combinations, such as *Ascaridia galli* + *Eimeria* spp., did not show significant differences across markets ($p = 0.522$). These results indicate that market-specific factors, including management practices or environmental conditions, may influence the likelihood of certain coinfections (Andersen et al., 2021).

Temporal trends over the six-month survey period revealed seasonal variations (Figure 11). August recorded the highest prevalence of *Rellitina* spp. + *Eimeria* spp., reaching 10 percent, with a statistically significant monthly variation ($p = 0.005$). Other coinfections, such as *Heterakis gallinarum* + *Eimeria* spp., declined in October and November, with several dropping to zero, suggesting a clear seasonal effect on parasite transmission.

Sex did not appear to have a significant impact on coinfection prevalence (Fig. 12). While females had a slightly higher occurrence of *Ascaridia galli* + *Eimeria* spp. (3.6%) compared to males (1.0%), this difference was not statistically significant ($p = 0.090$). The prevalence of *Rellitina* spp. + *Eimeria* spp. was identical in both sexes at 3.1 percent ($p = 0.984$), indicating similar susceptibility between male and female broilers.

When analyzed by age group (Fig. 13), chickens aged 6 – 7 weeks showed the greatest diversity of coinfections, including the only recorded instance of *Heterakis gallinarum* + *Ascaridia galli* (0.4%). No age group demonstrated a statistically significant predisposition to specific coinfections, with all p -values above 0.05. Among older birds aged 8–9 weeks, which represented a smaller portion of the population

($n = 19$), a prevalence of 5.3 percent was observed across three coinfection types, highlighting that older birds, despite being fewer, can still harbor multiple parasites.

Generally, these findings demonstrate that intestinal parasite coinfections in broiler chickens are influenced by market location and seasonal factors, while sex and age show less pronounced effects. The patterns underscore the need for targeted parasite management strategies, particularly in high-prevalence markets and peak transmission periods.

3.3 Phytochemical Analysis of *Andrographis paniculata*

To evaluate the bioactive potential of *Andrographis paniculata*, its leaf extract was subjected to chromatographic analysis, as shown in Fig. 14. Examination of the chromatogram allowed for the identification of several compounds, with their respective retention times (RT in minutes), molecular weights (M.W. in g/mol), peak areas (%), and deviation times (Dev. in minutes) quantified and summarized in Table 3. The distribution and relative abundance of these compounds were further explored through statistical representations in Figs. 15 to 18. These figures provide insights into the variability and consistency of the phytochemical constituents, highlighting compounds that are predominant and potentially responsible for the observed bioactivity. For instance, compounds with higher peak areas indicate a stronger presence, which may correlate with enhanced therapeutic properties.

Analytically, the data suggest that the leaf extract of *Andrographis paniculata* contains a complex mixture of bioactive molecules, some of which are consistently detected across replicates (Liu et al., 2024), while others exhibit minor deviations in retention times and peak intensities. This pattern underscores both the richness and reproducibility of the extract's phytochemical profile. The integration of chromatographic findings with statistical distributions thus provides a robust framework for understanding the extract's potential efficacy and lays the foundation for targeted pharmacological investigations.

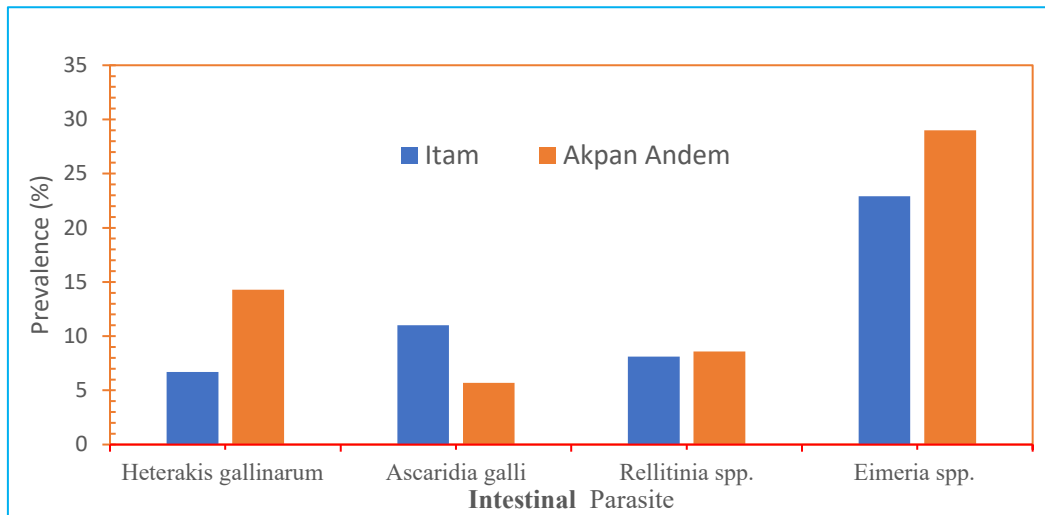


Fig. 6: Bar chart showing Statistical distribution of intestinal parasite in broiler chickens across markets

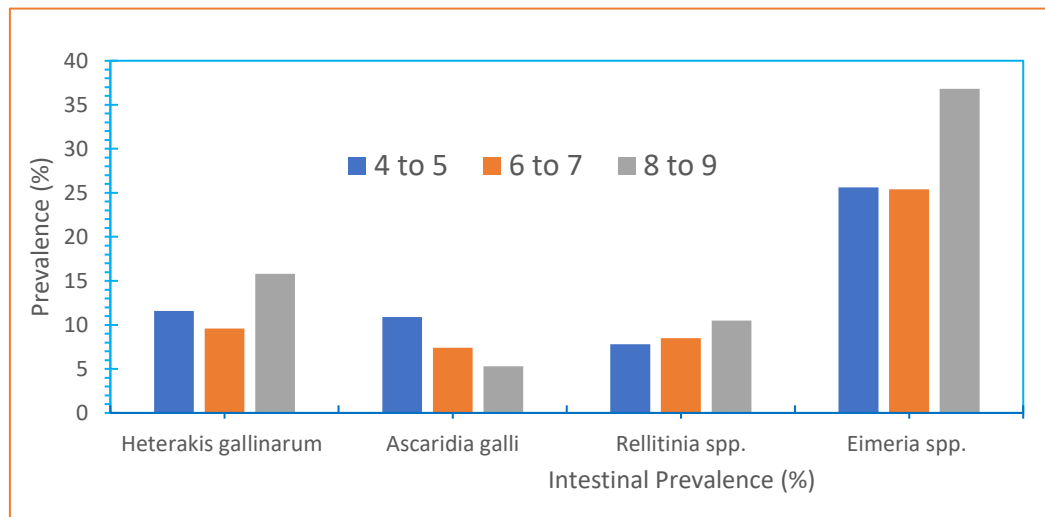


Fig. 7: Bar chart showing Statistical distribution of intestinal parasite in broiler chickens by age

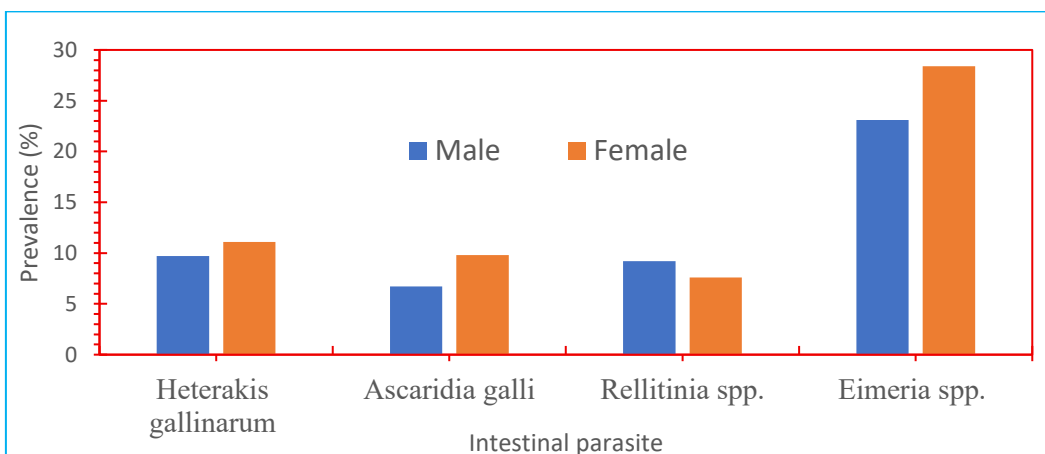


Fig. 8: Bar chart showing Statistical distribution of intestinal parasite in broiler chickens by sex

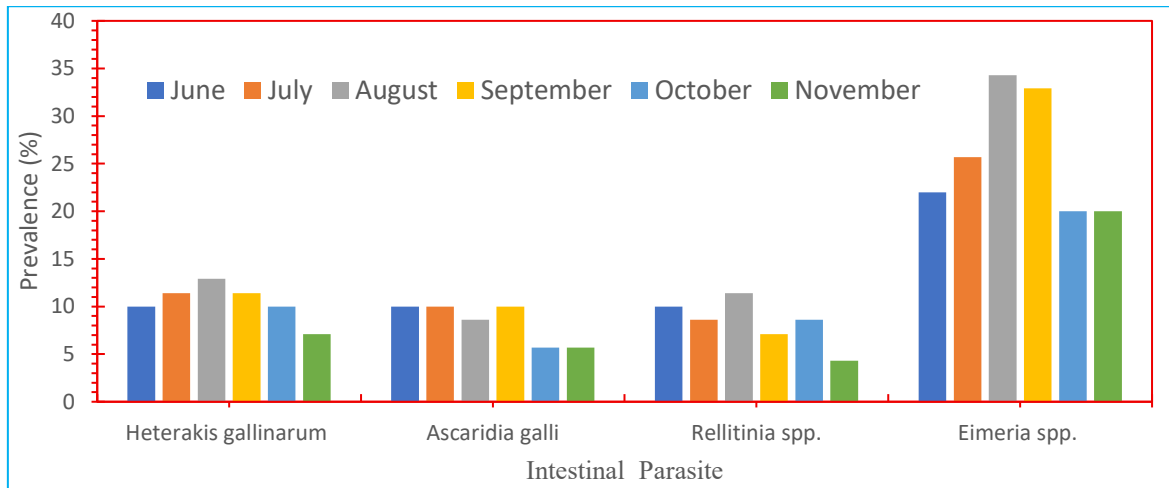


Fig. 9: Bar chart showing Statistical distribution of intestinal parasite in broiler chickens across months

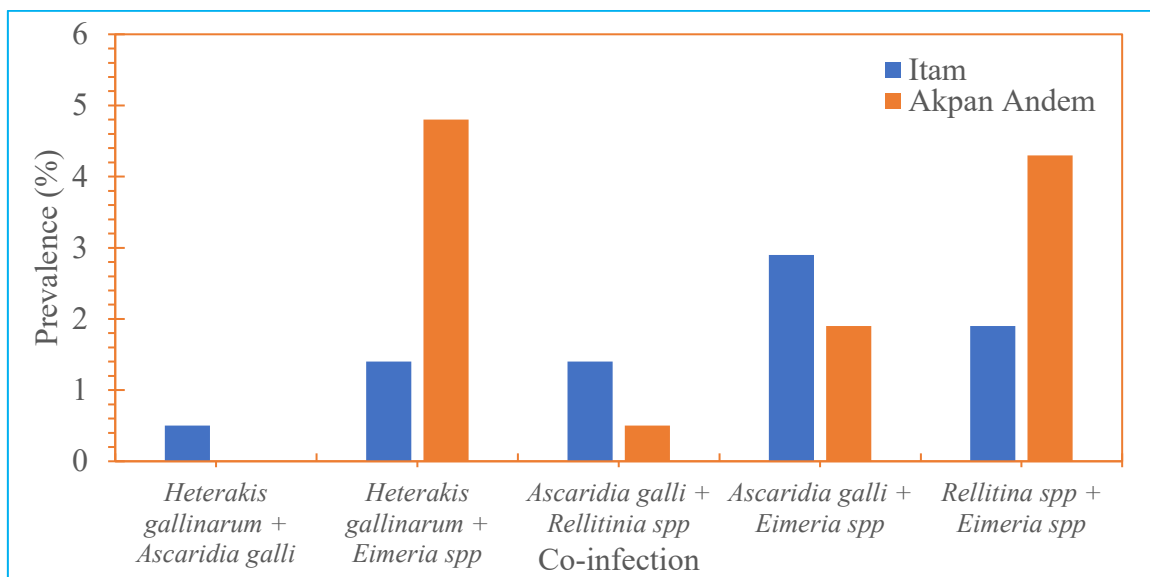


Figure 10: Bar chart showing Statistical distribution of intestinal parasite coinfections in broiler chickens across markets (Itam and Akpan Andem)

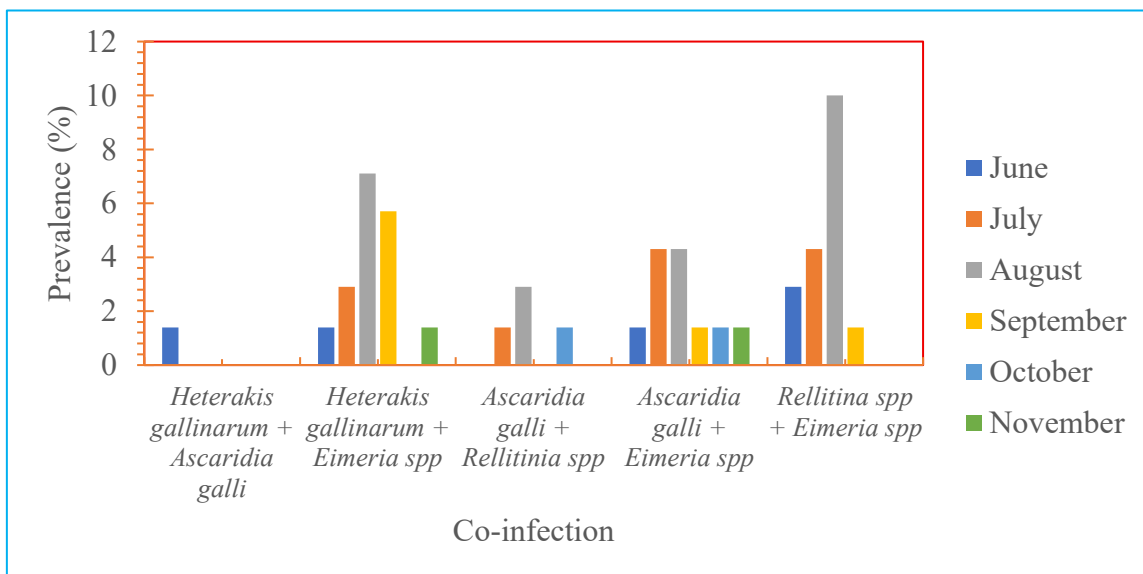


Fig. 11: Bar chart showing Statistical distribution of intestinal parasite coinfections in broiler chickens across months

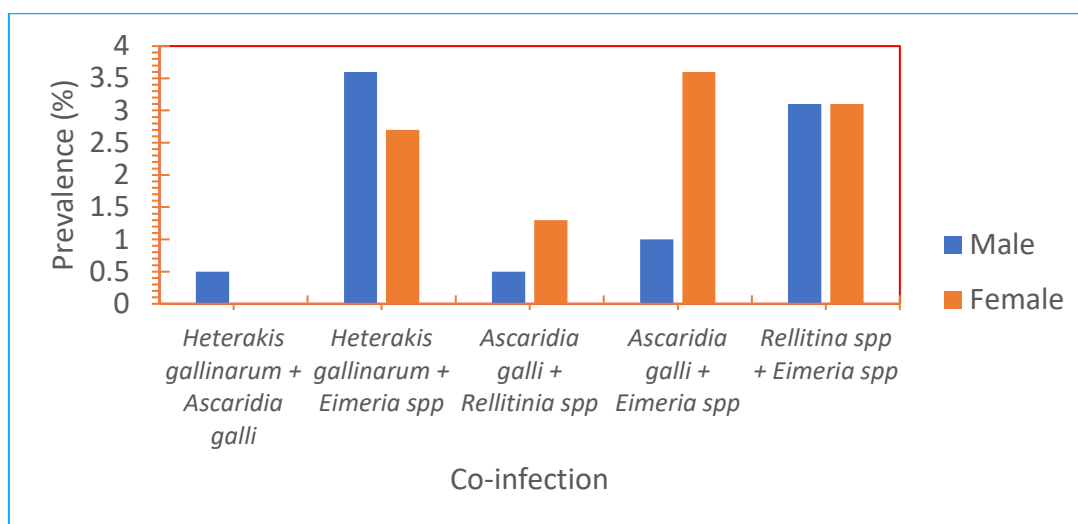


Fig. 12: Bar chart showing Statistical distribution of intestinal parasite coinfections in broiler chickens by sex

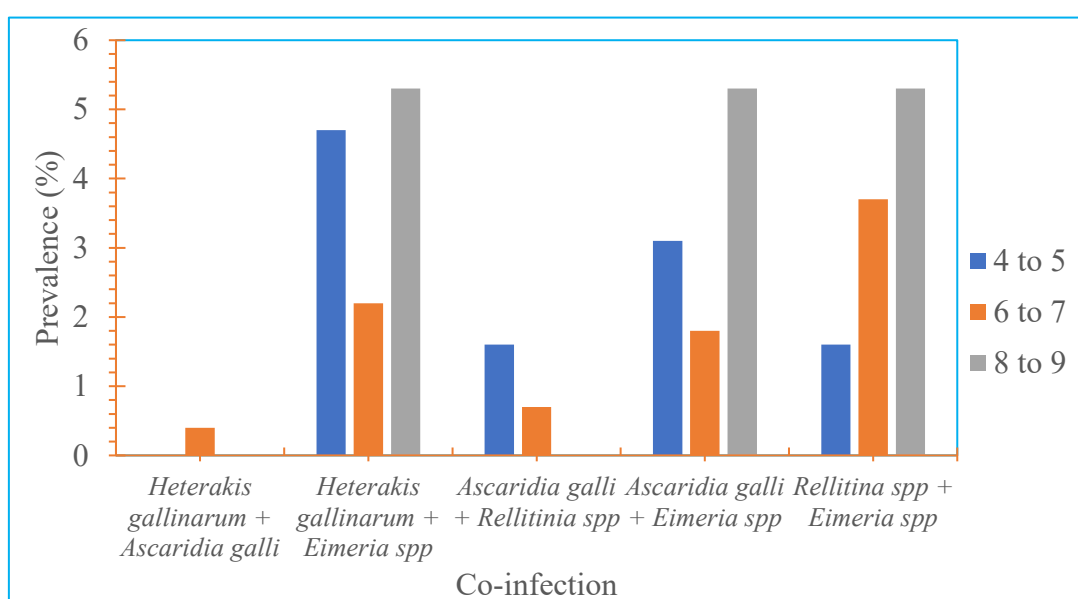


Fig. 13: Bar chart showing Statistical distribution of intestinal parasite coinfections in broiler chickens by age groups

3.3.1 Phytochemical Profile of the Extract

GC–MS analysis of *Andrographis paniculata* leaf extract (Table 3) identified approximately thirty phytochemical constituents spanning terpenes, flavonoids, fatty acids, and other secondary metabolites, indicating broad therapeutic potential (López-Abán et al., 2025). The most abundant compounds were Sylvestrene (9.44%), IsoCaryophyllene (9.24%), and Epicatechin (9.19%), highlighting a strong presence of terpenoids and catechin-type flavonoids. Terpenoids contribute antimicrobial, anti-inflammatory, and antiparasitic activities, while Epicatechin provides antioxidant protection, potentially mitigating oxidative stress in broilers during coccidial infection. Other

notable constituents, including D-Limonene (8.35%), Kauranes (7.32%), and Phorbol esters (8.42%), further suggest the extract's capacity to reduce intestinal inflammation and modulate parasite-host interactions.

The compounds exhibited a wide range of molecular weights, from small, rapidly absorbed molecules like Methacrolein (70.09 g/mol) to larger, complex structures such as Phorbol esters (616.83 g/mol). This duality implies both systemic and localized mechanisms, with smaller molecules exerting antioxidant and antimicrobial effects in the bloodstream, while larger molecules remain in the intestinal lumen to interact directly with parasites.

Figures 15 to 18 illustrate the chemical diversity, abundance, and analytical precision of the extract. Retention times (Fig. 15) confirm variation in volatility and polarity, while molecular weight distribution (Fig. 16) highlights structural complexity. Relative abundance (Fig. 17) emphasizes the dominant role of Sylvestrene, IsoCaryophyllene, and Epicatechin in biological activity, supported by minor compounds through potential synergistic effects. Deviation times (Fig. 18) demonstrate

reliable identification of all major compounds despite the complex plant matrix.

In all, the phytochemical composition of *Andrographis paniculata* combines antioxidant, antimicrobial, and anti-inflammatory compounds in a chemically diverse matrix, providing a multi-faceted mechanism likely to support the control of coccidiosis in broiler chickens (Mahizan et al., 2019).

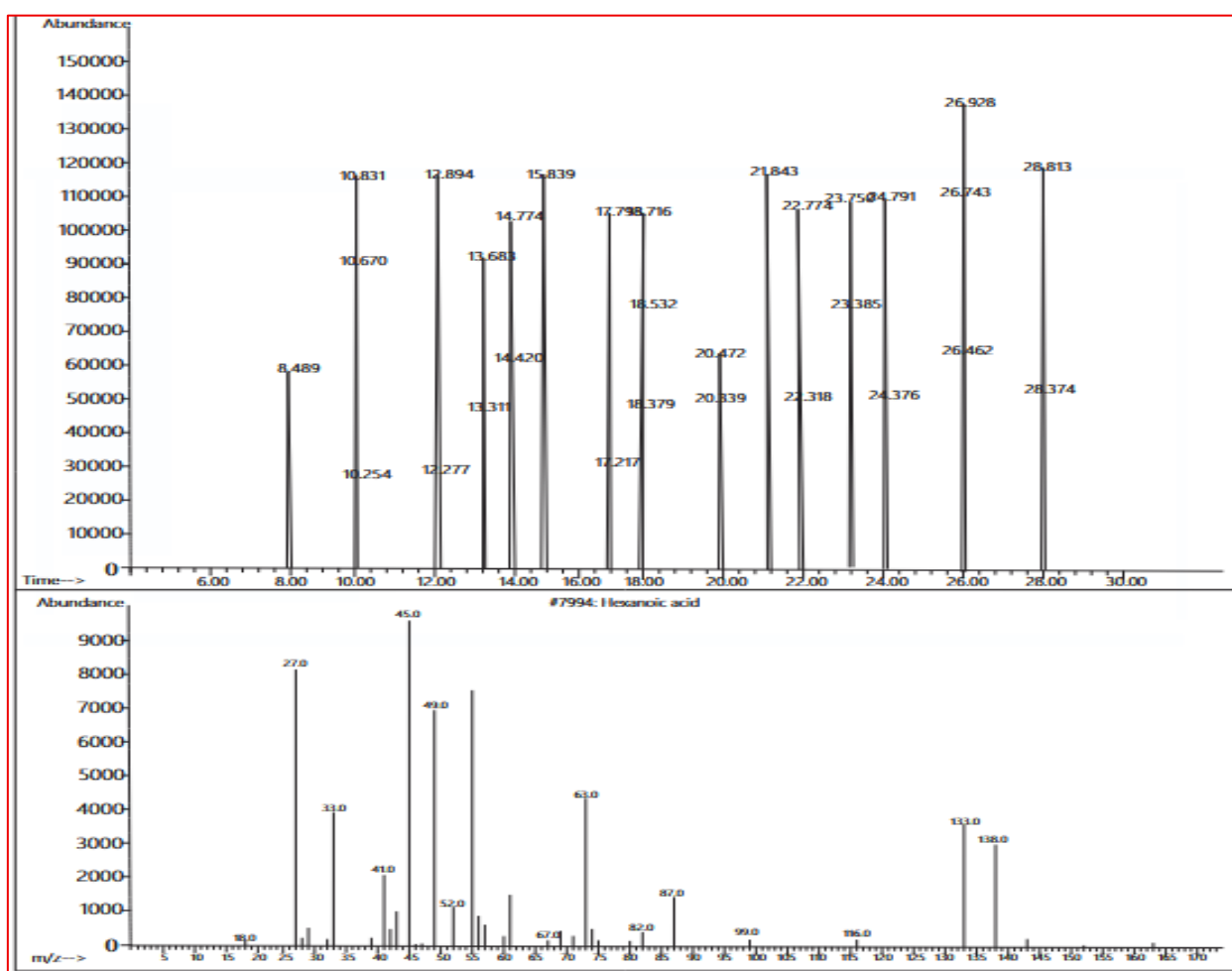


Fig. 14: GC–MS Chromatogram of *Andrographis paniculata* leaf extract species.

Table 3: GC–MS-identified phytochemical constituents of *Andrographis paniculata* leaf extract, showing the retention time (RT), molecular weight (MW), peak area, and deviation time (Dev.) of each compound

S/N	compound	RT (min)	M.W(g/m ol)	Peak Area (%)	Dev.(min)
1	Phorbol esters	8.489	616.83	8.42	33
2	Clerodanes	10.254	376.53	2.78	41
3	Cembranoid	10.670	272.43	1.15	57
4	Halimanes	10.831	275.34	4.36	61
5	Sigmasterol	12.277	412.69	1.87	44
6	1,2,4-Trthiolane,3,5-diethyl	12.894	180.4	3.08	38

7	Carpaine	13.311	478.7	4.14	42
8	Cedrene	13.683	204.35	2.49	19
9	Kauranes	14.420	272.43	7.32	52
10	Labdanes	14.774	278.52	1.08	82
11	Trachylobanes	15.839	272.32	2.54	16
12	Sarcopetalanes	17.217	288.51	3.28	33
13	Benzenepropanoic acid	17.793	150.17	4.17	29
14	IsoCaryopyllene	18.379	204.35	9.24	52
15	Hexadecenoic acid ethyl ester	18.532	200.31	6.15	48
16	Tran-sesquisabinene hydrate	18.716	222.37	2.39	31
17	Methacrolein	20.339	70.09	1.51	30
18	Sylvestrene	20.472	136.23	9.44	53
19	Alpha-Farnesene	21.843	204.36	2.19	69
20	D-Limonene	22.318	136.23	8.35	18
21	2-Furancarboaldehyde	22.774	96.08	1.03	37
22	2-propanone, 1-hydroxy	23.385	74.07	6.18	42
23	Methyl,8-Methyl-nonanoate	23.750	186.29	3.74	61
24	8-Octadecenoic acid, methyl ester	24.376	298.51	1.26	74
25	Neoisolongifolene, oxo	24.791	218.33	5.14	36
26	n-Hexadecanoic acid	26.462	256.42	3.20	19
27	Epicatechin	26.743	290.26	9.19	42
28	Callo catechin	26.928	306.267	4.57	70
29	p-Coumaric acid ethyl ester	28.374	192.21	1.89	64
30	2-hydroxy-gamma-butyrolactone	28.813	102.09	2.21	83

* Source: Field Data, 2025

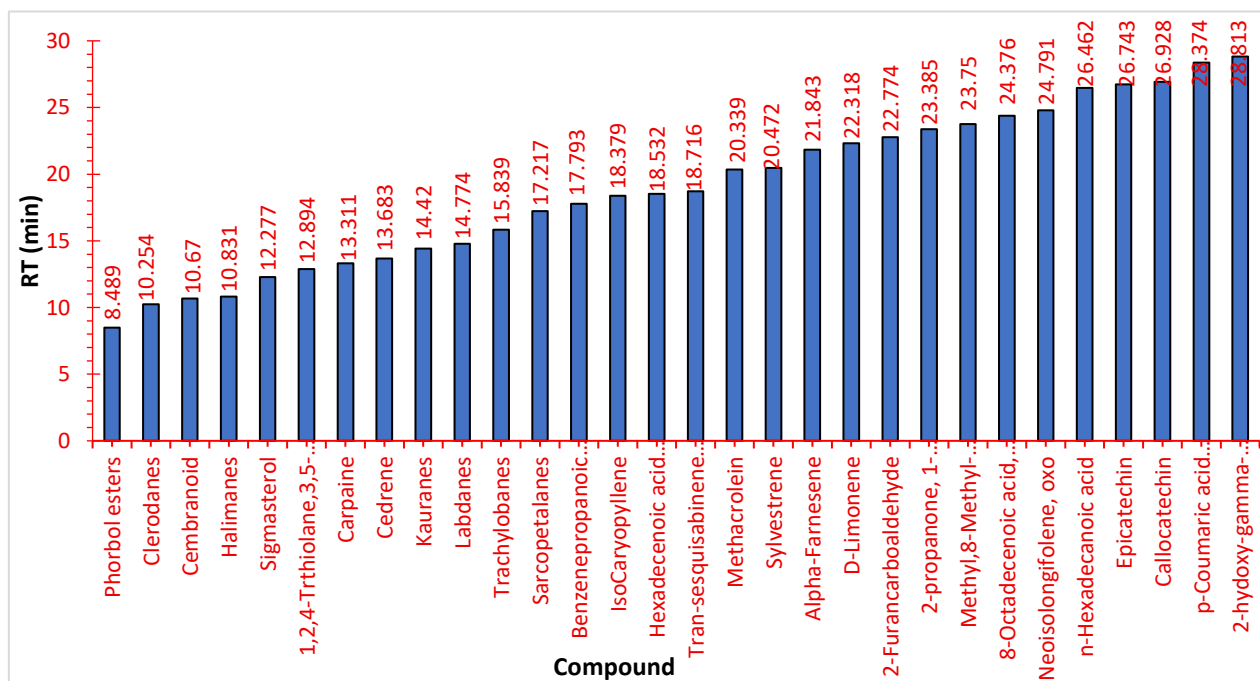


Fig. 15: bar chart showing retention time of bioactive compounds in *Andrographis paniculata* leaf extract

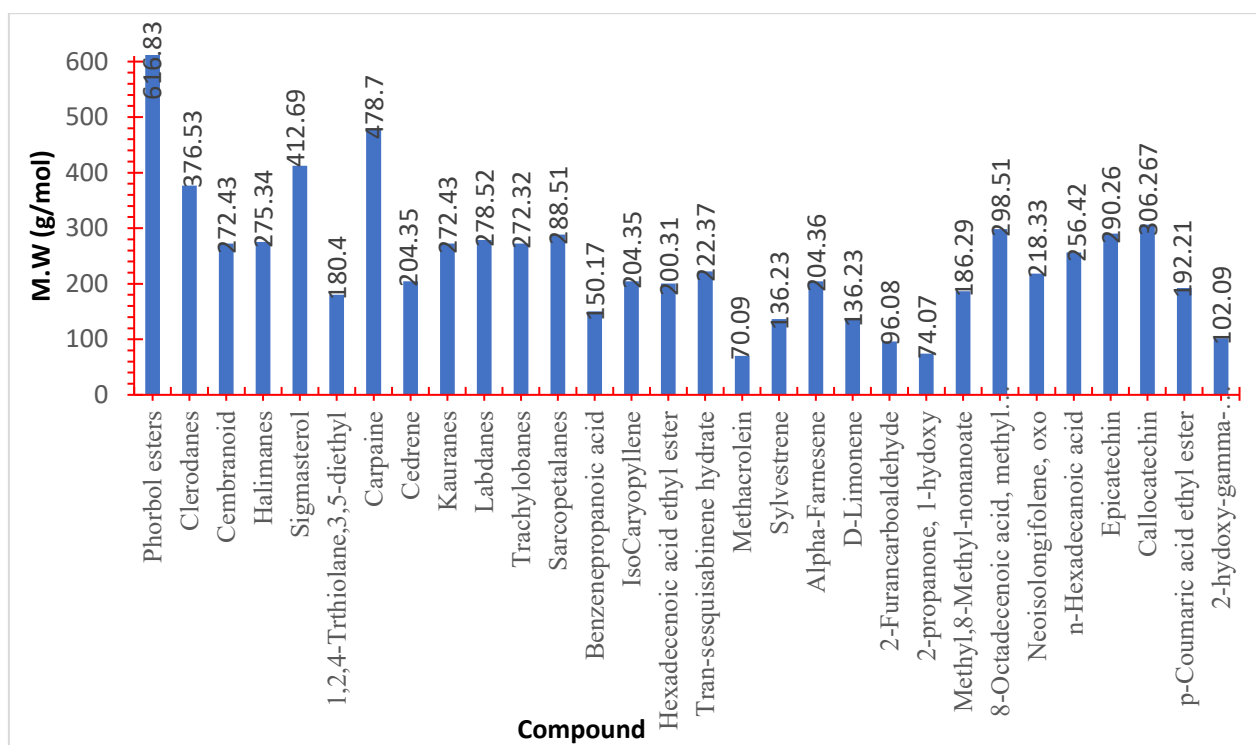


Fig. 16: bar chart showing molecular weight of bioactive compounds in *Andrographis paniculata* leaf extract.

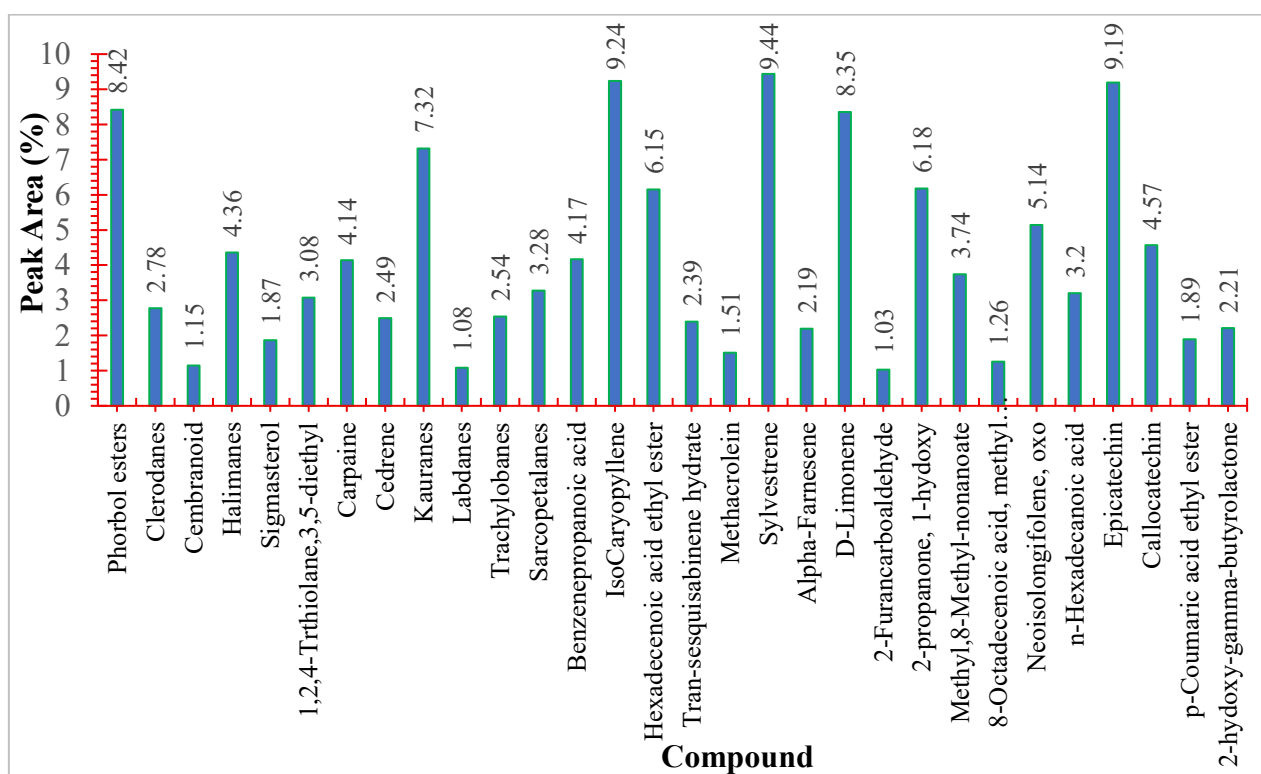


Fig.17: bar chart showing peak area of bioactive compounds in *Andrographis paniculata* leaf extract.

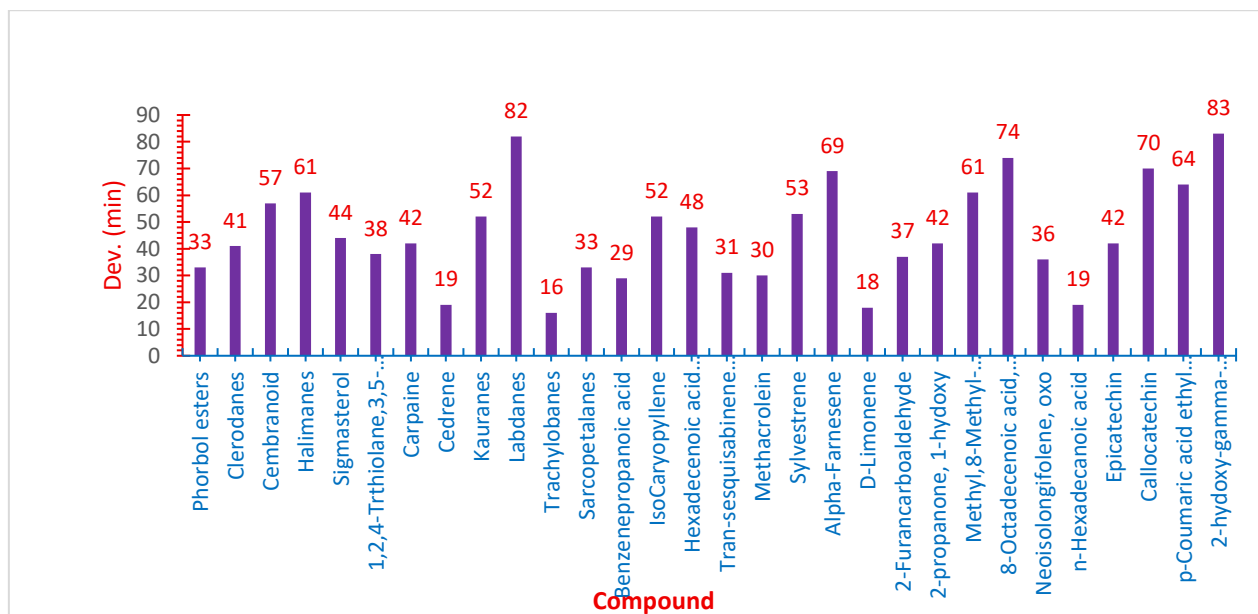


Fig. 18: bar chart showing deviation time of bioactive compounds in *Andrographis paniculata* leaf extract.

Figure 14 shows the GC–MS chromatogram of the *Andrographis paniculata* leaf extract, illustrating the diverse range of chemical constituents identified. The therapeutic effects of *Andrographis paniculata* leaf extract against coccidiosis in experimentally infected broiler chickens are summarized in Table 4. In addition to its antiparasitic activity, the extract's impact on growth performance and feed conversion ratio (FCR) was assessed, with results presented in Tables 5 and 6. Table 5 specifically compares the effectiveness of the plant extract to that of

conventional synthetic drugs, highlighting its potential as a natural alternative.

3.3.2 Effects on Clinical Signs in Experimentally Infected Broilers

Broiler chickens treated with *Andrographis paniculata* leaf extract after experimental infection with *Eimeria tenella* exhibited a range of behavioral and clinical responses during the treatment period. These observations are detailed in columns 2 to 4 of Table 7, reflecting the extract's influence on disease symptoms and overall health status (Martins et al., 2025).

Table 4: Statistical indices demonstrating the therapeutic effects of *Andrographis paniculata* leaf extract in the treatment of coccidiosis in experimentally infected broiler chickens

Groups	Oocyst B/4 extract amin	Oocyst 24 hrs after extract admin	Oocyst 48 hrs after extract admin	Oocyst 72 hrs after extract admin
400 mg/KgBW	580.00±37.42a	280.00±37.42c	140.00±24.49bc	-
200 mg/KgBW	560.00±40.00a	320.00±37.42bc	120.00±20.00c	-
100 mg/KgBW	620.00±37.42a	380.00±58.31b	220.00±20.00b	140.00±24.49
Infected Untreated	500.00±31.62a	600.00±44.72a	880.00±48.99a	1320.00±58.31
Standard Drug	540.00±40.00a	220.00±37.42bc	120.00±20.00c	-
Total	560.00±17.32	360.00±32.15	296.00±61.23	730.00±198.91
p Value	0.261ns	<0.001*	<0.001*	<0.001*

* Source: Field Data, 2025

Table 5: Statistical indices illustrating the effects of *Andrographis paniculata* leaf extract on the growth performance of experimentally infected broiler chickens

	Final weight (g/b)	Initial weight (g/b)	Weight Gain (g)
400 mg/KgBW	2258.45±28.54a	1836.18±15.04a	422.27±19.60a
200 mg/KgBW	2026.41±35.87b	1626.16±9.27d	400.24±31.63a
100 mg/KgBW	2076.42±27.13b	1816.18±10.77a	260.23±24.50b
Infected Untreated	1336.27±72.86c	1756.18±6.78b	-419.91±66.35c
Standard Drug	2128.43±35.14b	1724.17±5.10c	404.25±31.88a
Average	1965.19±68.42	1751.78±15.77	213.42±67.57
p Value	<0.001*	<0.001*	<0.001*

* Source: Field Data, 2025

Table 6: Statistical indices illustrating the influence of *Andrographis paniculata* leaf extract on the feed intake of experimentally infected broiler chickens

Groups	FI 24 Hrs b/4 infection (g/b)	FI for 1st day of extract admin(g/b)	FI for 2nd day of extract admin(g/b)	FI for 3rd day of extract admin(g/b)	FCR
400 mg/KgBW	87.00±0.00c	85.20±0.00b	84.00±0.00c	85.20±0.00b	0.61±0.03b
200 mg/KgBW	90.00±0.00a	86.40±0.00a	87.00±0.00a	87.00±0.00a	0.67±0.06b
100 mg/KgBW	88.80±0.00b	80.40±0.00d	83.40±0.00d	85.20±0.00b	0.99±0.10a
Infected Untreated	87.00±0.00c	78.00±0.00e	75.00±0.00e	60.00±0.00c	-0.58±0.13c
Standard Drug	85.44±0.36d	84.00±0.00c	85.20±0.00b	85.20±0.00b	0.65±0.06b
Average	87.65±0.33	82.80±0.64	82.92±0.85	80.52±2.10	0.47±0.12
p Value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

* Source: Field Data, 2025. FI= Feed intake, FCR= Feed Conversion Ratio

The administration of *Andrographis paniculata* leaf extract demonstrated clear therapeutic benefits against coccidiosis in experimentally infected broiler chickens. Table 4 shows a dose-dependent reduction in oocyst shedding, with the 400 mg/KgBW group dropping from 580 ± 37.42 before treatment to 280 ± 37.42 at 24 hours and 140 ± 24.49 at 48 hours, closely matching the standard drug (220 ± 37.42 and 120±20). Lower doses (200 and 100 mg/KgBW) also reduced parasite load, though less dramatically, while the infected untreated group's oocyst counts increased to 1320 ± 58.31 by 72 hours, underscoring the severity of untreated infection. According to Naidoo et al. (2008), this antiparasitic effect translated into improved growth performance (Table 5). Birds receiving 400 mg/KgBW gained 422.27 ± 19.60

g, reaching a final weight of 2258.45 ± 28.54 g, whereas infected untreated birds lost weight (-419.91 ± 66.35 g). The 200 mg/KgBW and standard drug groups also exhibited strong weight gains (400.24 ± 31.63 g and 404.25 ± 31.88 g, respectively), reflecting enhanced nutrient utilization following parasite reduction. Feed intake and efficiency (Table 6) further supported these findings. Higher doses of the extract maintained consistent feed intake (400 mg/KgBW: 85.20–84.00 g/b) with a low FCR of 0.61±0.03, while lower doses showed moderate improvement (200 mg/KgBW FCR: 0.67±0.06; 100 mg/KgBW FCR: 0.99±0.10). Infected untreated birds exhibited declining feed intake (60–78 g/b) and poor conversion efficiency, highlighting the negative impact of coccidiosis on appetite and growth.

Table 7: Effect of *Andrographis paniculata* leaf extract on clinical signs of experimentally infected broiler chickens

Treatment Group	Clinical signs at 24 hrs. before extract administration	Clinical signs at 24 hrs. after extract administration	Clinical signs at 48 hrs. after extract administration	Clinical signs at 72 hrs. after extract administration
400mg/kg BW	Slight reduction in feed intake	Slight reduction in feed intake	Feed intake improved	Feed intake improved
	Slight weight loss	Slight weight loss	Weight improved	Weight improved
	Slightly loose droppings	Slightly loose droppings	Faecal sample improved	Faecal sample normalized
	Reduced activity	Reduced activity	Activity improved	Normal activity
200mg/kg BW	Slight reduction in feed intake	Slight reduction in feed intake	Feed intake improved	Feed intake improved
	Slight weight loss	Slight weight loss	Weight improved	Weight improved
	Slightly loose droppings	Slightly loose droppings	Faecal sample improved	Faecal sample normalized
	Reduced activity	Reduced activity	Activity improved	Normal activity
100mg/kg BW	Slight reduction in feed intake	Slight reduction in feed intake	Feed intake improved	Feed intake improved
	Slight weight loss	Slight weight loss	Weight improved	Weight improved
	Slightly loose droppings	Slightly loose droppings	Faecal sample improved	Faecal sample slightly loose
	Reduced activity	Reduced activity	Activity improved	Normal activity
Uninfected untreated	Normal faeces Normal feed intake Normal activity	Normal faeces Normal feed intake Normal activity	Normal faeces Normal feed intake Normal activity	Normal faeces Normal feed intake Normal activity
Infected untreated	Slight reduction in feed intake	Reduced feed intake	Reduced feed intake Ruffled feathers	Reduced feed intake Ruffled feathers
	Slight weight loss	weight loss	Weight loss	Weight loss
	Slightly loose droppings	loose droppings	watery brownish diarrhea	Bloody diarrhea
	Reduced activity	Reduced activity	weakness	General body weakness
Standard drug (Diclazuril)	Slight reduction in feed intake	Slight reduction in feed intake	Feed intake improved	Feed intake improved
	Slight weight loss	Slight weight loss	Weight improved	Weight improved
	Slightly loose droppings	Slightly loose droppings	Faecal sample improved	Faecal sample normalized
	Reduced activity	Reduced activity	Activity improved	Normal activity

* Source: Field data, 2025

Clinical observations (Table 7) mirrored these quantitative trends. Treated birds, especially at 400 and 200 mg/KgBW, displayed rapid improvements in feed intake, weight, droppings consistency, and activity within 48–72 hours,

approaching normalcy. Birds on the lowest dose improved more slowly, while untreated birds showed progressive worsening, including bloody diarrhea, weight loss, reduced activity, and ruffled feathers (Rizwan et al., 2023).

Put together, these results indicate that *Andrographis paniculata* leaf extract exerts multi-faceted, dose-dependent therapeutic effects: it effectively reduces parasite burden, restores feed intake, improves growth, and alleviates clinical signs of coccidiosis. At 400 mg/KgBW, its efficacy is comparable to the standard drug (diclazuril), suggesting its potential as a natural, growth-supportive alternative for managing coccidiosis in broiler chickens.

4.0 Conclusion

The findings from this study point to a clear and persistent challenge: broiler chickens sourced from market environments remain highly exposed to intestinal parasites, largely due to inadequate sanitary conditions. The predominance of *Eimeria* spp. further confirms that coccidiosis is not only widespread but deeply established within these production systems (Rosłóń et al., 2025). Against this backdrop, the performance of *Andrographis paniculata* leaf extract is particularly noteworthy. Beyond reducing parasite burden, the extract also supported improved growth performance, indicating that its benefits extend beyond therapy to include a positive influence on productivity. This dual role is especially relevant in today's drive toward more sustainable and health-conscious poultry production practices.

The observed dose-dependent response highlights the importance of proper dosage in achieving optimal outcomes. Higher concentrations were associated with a faster reduction in oocyst shedding, suggesting a strong anticoccidial effect that may be attributed to the combined activity of its bioactive phytochemical constituents.

Generally, this study establishes *Andrographis paniculata* as a viable, plant-based alternative to conventional anticoccidial drugs. Its effectiveness in controlling coccidiosis while enhancing growth performance positions it as a practical option for improving poultry health and productivity in a more sustainable manner. In addition to

providing valuable epidemiological insights, this work offers experimental evidence that supports the broader adoption of phyto-genic interventions in animal health management.

To strengthen and extend these findings, future research should focus on evaluating the long-term safety of the extract under field conditions and across different production systems. There is also a need to standardize extraction procedures and dosage levels to ensure consistency and reproducibility. Furthermore, exploring its incorporation into commercial feed formulations could facilitate large-scale application and enhance its accessibility to poultry producers.

Acknowledgements

The first author sincerely appreciates her supervisors for their guidance and support throughout the study, which led to its successful completion. The authors are also grateful to their colleagues and the laboratory staff in the Department of Animal and Environmental Biology, University of Uyo, Nigeria, for their valuable assistance during data collection, curation, and processing.

Funding: This research was funded by the authors.

Declaration of Interest: The authors declare no known financial or personal relationships that could have influenced the work reported in this paper.

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