

Anticoccidial activities of combination of aqueous extracts of different medicinal plants on eimeria strain in Abor Acre + strain of broilers

Atividades anticocídias da combinação de extratos aquosos de diferentes plantas medicinais na estirpe eimeria em Abor Acre + estirpe de frangos de corte

Actividades anticoccidiales de la combinación de extractos acuosos de diferentes plantas medicinales sobre la cepa eimeria en Abor Acre + cepa de pollos de engorde

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ABSTRACT

This study investigated the anticoccidial activities of combinations of aqueous extracts of different medicinal plants on *Eimeria* strain in Abor Acre + strain of broilers. *Zingiber officinale* (ginger) rhizome and *Carica papaya* (pawpaw) leaves were used for the study. Aqueous extracts of the plant materials were subjected to GC-MS analysis. Forty-five broilers divided into nine groups of five birds each, were used for in vivo study. Group A was normal control (uninfected and no extract), B - negative control (infected and untreated), C - positive control (infected + 25 mg/ml diclazuril), and D, E, F, G, H and I were infected and treated with 50 mg/ml ginger, 100 mg/ml ginger, 50 mg/ml pawpaw, 100 mg/ml pawpaw, 50 mg/ml of 1:1 combination of both and 100 mg/ml combination in 1 L of water, respectively, for 5 days. Weights were measured at weeks 3, 4 and 5 and feed conversion rates (FCR) determined. After treatment, blood samples were collected to assess haematological parameters and liver function. The results showed that both extracts contained

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bioactive compounds in varying quantities. Weight gain was observed across all groups while FCR values increased at week 5 after a drop at week 4. Also, the extracts induced significant ($p<0.05$) increase in Hb, PCV, MCHC and RBC, and significant ($p<0.05$) decrease in TWBC, lymphocyte, AST, ALT and ALP compared to the disease control. In addition, extracts induced significant ($p<0.05$) increase in platelet count except high dose ginger and low dose pawpaw. Thus, the extracts contained bioactive compounds, and improved health parameters showing potential in ameliorating coccidiosis and coccidiosis-related complications in birds.

Keywords: Abor Acre + broilers, anticoccidial activities, aqueous extract, *Eimeria* strain, medicinal plants

RESUMO

Este estudo investigou as atividades anticoccidiais de combinações de extratos aquosos de diferentes plantas medicinais na cepa *Eimeria* em frangos da raça Abor Acre +. Foram utilizados para o estudo o rizoma de *Zingiber officinale* (gingibre) e as folhas de *Carica papaya* (mamão). Os extratos aquosos dos materiais vegetais foram submetidos à análise GC-MS. Quarenta e cinco frangos de corte, divididos em nove grupos de cinco aves cada, foram utilizados para o estudo in vivo. O grupo A foi o controle normal (não infectado e sem extrato), B - controle negativo (infectado e não tratado), C - controle positivo (infectado + 25 mg/ml de diclazuril) e D, E, F, G, H e I foram infectados e tratados com 50 mg/ml de gengibre, 100 mg/ml de gengibre, 50 mg/ml de mamão, 100 mg/ml de mamão, 50 mg/ml de uma combinação 1:1 de ambos e 100 mg/ml da combinação em 1 L de água, respectivamente, durante 5 dias. Os pesos foram medidos nas semanas 3, 4 e 5 e as taxas de conversão alimentar (FCR) foram determinadas. Após o tratamento, amostras de sangue foram coletadas para avaliar os parâmetros hematológicos e a função hepática. Os resultados mostraram que ambos os extratos continham compostos bioativos em quantidades variáveis. O ganho de peso foi observado em todos os grupos, enquanto os valores de FCR aumentaram na semana 5, após uma queda na semana 4. Além disso, os extratos induziram um aumento significativo ($p<0,05$) na Hb, PCV, MCHC e RBC, e uma diminuição significativa ($p<0,05$) no TWBC, linfócitos, AST, ALT e ALP em comparação com o controle da doença. Além disso, os extratos induziram um aumento significativo ($p<0,05$) na contagem de plaquetas, exceto no caso do gengibre em alta dose e da papaia em baixa dose. Assim, os extratos continham compostos bioativos e melhoraram os parâmetros de saúde, mostrando potencial para amenizar a coccidiose e as complicações relacionadas à coccidiose em aves.

Palavras-chave: Abor Acre + frangos de corte, atividades anticoccidiais, extrato aquoso, cepa *Eimeria*, plantas medicinais

RESUMEN

Este estudio investigó las actividades anticoccidiales de combinaciones de extractos acuosos de diferentes plantas medicinales en la cepa *Eimeria* en pollos de engorde de la cepa Abor Acre +. Para el estudio se utilizaron rizomas de *Zingiber officinale* (jengibre) y hojas de *Carica papaya* (papaya). Los extractos acuosos de los materiales vegetales se sometieron a análisis GC-MS. Se utilizaron cuarenta y cinco pollos de engorde divididos en nueve grupos de cinco aves cada uno para el estudio in vivo. El grupo A era el control normal (sin infección y sin extracto), el B era el control negativo (infectado y sin tratar), el C era el control positivo (infectado + 25 mg/ml de diclazuril) y los grupos D, E, F, G, H e I estaban infectados y fueron tratados con 50 mg/ml de jengibre, 100 mg/ml de jengibre, 50 mg/ml de papaya, 100 mg/ml de papaya, 50 mg/ml de una combinación 1:1 de ambos y 100 mg/ml de la combinación en 1 l de agua, respectivamente, durante 5 días. Se midieron los pesos en las semanas 3, 4 y 5 y se determinaron los índices de conversión alimenticia (ICA). Después del tratamiento, se tomaron muestras de sangre para evaluar los parámetros hematológicos y la función hepática. Los resultados mostraron que ambos extractos contenían compuestos bioactivos

en cantidades variables. Se observó un aumento de peso en todos los grupos, mientras que los valores del ICA aumentaron en la semana 5 después de una caída en la semana 4. Además, los extractos indujeron un aumento significativo ($p < 0,05$) en Hb, PCV, MCHC y RBC, y una disminución significativa ($p < 0,05$) en TWBC, linfocitos, AST, ALT y ALP en comparación con el control de la enfermedad. Además, los extractos indujeron un aumento significativo ($p < 0,05$) en el recuento de plaquetas, excepto en el caso de la dosis alta de jengibre y la dosis baja de papaya. Por lo tanto, los extractos contenían compuestos bioactivos y mejoraban los parámetros de salud, lo que demuestra su potencial para mejorar la coccidiosis y las complicaciones relacionadas con la coccidiosis en las aves.

Palabras clave: Abor Acre + pollos de engorde, actividades anticoccidiales, extracto acuoso, cepa de *Eimeria*, plantas medicinales.

1 INTRODUCTION

Coccidiosis is an intestinal tract infection that is caused by single-celled, eukaryotic, heterospecific organisms (protozoa) called coccidians. About 1800 *Eimeria* species infect the intestinal tract of different animals and birds (Haug et al., 2008) and infection with this parasite normally occurs through ingestion of feed or water contaminated with sporulated oocysts (Chen et al., 2025) and it is one of the most alarming problems for chicken, porcine and calf rearing industries (Pandit, 2007). Parasitic diseases have remained a major problem limiting the expansion and profitability of developing agricultural countries, where skilled husbandry inputs have not matched the rate of expansion and intensification of livestock holdings (Mohammed and Sunday, 2015).

Coccidiosis caused by *Eimeria* is a serious parasitic disease threatening all poultry species. *Eimeria* invades the digestive tract and causes severe morbidity and mortality in broilers (Jebessa et al., 2025). Diarrhoea, which may become bloody in severe cases, is the primary symptom of coccidiosis. Other symptoms might include; dysentery, enteritis, emaciation, lower feed conversion rate, delayed sexual maturity, drooping wings, poor growth and low production with attendant high mortality and morbidity rates (Awais, Akhtar, Iqbal, Muhammad, & Anwar, 2012).

Most animals suffering from coccidiosis show no symptoms, but immunocompromised and young animals may be severely affected and even die (Andrews, 2022). Coccidian parasites can infect a wide variety of animals including humans, birds, and livestock, they are usually species-specific and re-infection by these species is possible (Javaregowda, 2016). Coccidiosis is endemic in most of the tropical and subtropical regions where ecological and poor management conditions favour an all year-round development and propagation of casual agents (Obasi, Ifut, & Offiong, 2006).

Medicinal plants are species of plants whose parts; flowers, leaves, roots, stems, fruits, or seeds, are directly used or used in some preparation as medicine to treat a condition or disease.

Knowledge of the beneficial properties of medicinal plants to treat diseases represents a valuable resource to preserve the biological and cultural diversity of different ethnicities (Mekonnen, Mohammed & Tefera, 2022). There are a number of medicinal plants which represent an alternative treatment to various diseases, and their use is increasingly prevalent throughout the world.

Zingiber officinale (Ginger) contains active compounds that have been found to be effective in alleviating inflammation (Luettig, Rosenthal, Lee, Krug & Schulzke, 2016) and exhibit antibacterial, antifungal, and antiviral activities (Moon, Lee & Lee, 2018). Ginger extract improved markers of liver function (Abdel-Moneim, Morsy, Mahmoud, Abo-Seif & Zanaty, 2013), possessed anticarcinogenic properties (Sakulnarmrat, Srzednicki & Konczak, 2015), and might be useful in the management and prevention of neurodegenerative diseases (Huh et al., 2018). The plant was reported to possess cardioprotective effects (Khosravani et al., 2016), effective in the management and prevention of obesity (Rjabi et al., 2025) as well as, antiallergic effects, bronchodilating activity and could help with nausea and emesis (Mao et al., 2019).

Carica papaya plant, commonly papaya, belongs to the family Caricaceae. It is also known as paw-paw (Babalola, 2019). Ripe papaya fruits are rich in vitamins A and C. The plant has been reported to be used in the treatment of diseases (Singh et al., 2022). Papaya leaves is one of the most useful parts with a wide array of health-promoting compounds and activities. Research has shown that papaya leaves contain glycosides, flavonoids, alkaloids, saponins, phenolic compounds, amino acids, lipids, carbohydrates, enzymes, vitamins, and minerals (Alara, Abdurahman & Alara, 2022).

Avian coccidiosis poses a serious challenge to most farmers. This parasitic disease cause gut damage (i.e., inflammation, hemorrhage, diarrhoea, etc.), morbidity, and mortality in poultry. This disease annually causes a global loss of over 2.4 billion US dollars in the poultry industry, including poor growth performance, replacement of chicks and medication (Quiroz-Castañeda & Dantán-González, 2015). Anticoccidial chemicals, coccidiocides, coccidiostats, and ionophores, have long been used as a mainstream strategy to control avian coccidiosis in modern poultry production (Chapman et al., 2010). Although this strategy is effective and successful, the presence of drug resistance and public demands for residue-free meat has encouraged development of alternative control strategies. Natural products are emerging as an attractive way to combat coccidiosis. Besides, investigation of the compounds and/or their derivatives present in anticoccidial plants may inspire the research and development of anticoccidial chemicals.

Zingiber officinale and *Carica papaya* are plants with well-known medicinal value and applications. Scientific investigations of these plants have reported that they possess a wide range of chemical components that have been proven to produce a good number of effects that are beneficial to human and animal health, especially individually. These benefits offered by these

plants have been beneficial in their use as natural products/source for combating avian coccidiosis. There is a possibility to achieve synergistic effects when these medicinal plants are combined in the treatment of avian coccidiosis.

This study aimed at determining the anticoccidial activities of combination of aqueous extracts of different medicinal plants on *Eimeria* strain in Abor Acre + strain of broilers. The objectives were to identify bioactive components in *Zingiber officinale* and *Carica papaya* extracts, and determine their effects on haematological parameters and liver function enzymes.

2 METHODOLOGY

Collection of Plant Materials

Fresh *Zingiber officinale* rhizome was purchased at Relief Market in Owerri, Imo State, Nigeria, while *Carica papaya* was sourced around Federal Polytechnic Nekede, Owerri. They were authenticated by a taxonomist.

Preparation of Plant Material

Each plant sample was handled separately. They were washed thoroughly under running tap water, cut into small pieces, allowed to air dry and pulverized to coarse powder. The powdered samples were allowed to soak in potable water at a ratio of 1:10 (sample:solvent) for 72 h with intermittent shaking (Arawande et al., 2021). Afterwards, they were filtered and the filtrates collected and concentrated in a water bath at 65 °C to obtain the crude extracts.

Experimental Animals

A total of 45 healthy day old chicks of both sexes weighing between 75 to 85g were sourced from reputable hatchery vendors for the study. The chicks were brooded for the first week and allowed to acclimatize. The animals were maintained under good bio-secured environment to ensure that no prior coccidian pathogen were introduced before this study. All animals had free access to food and water.

The birds were divided into different groups at third week in a randomized manner to allow even weight distribution across all group and sexes. Each treatment occupied an area of 2.25 m² where feed and water were provided. Incandescent bulbs provided light and heat day and night during the brooding period.

Experimental design

The experimental design is a complete randomized design (CRD).

Identification of biologically active compounds in the plant extracts

Biologically active compounds in the plant samples were detected using gas chromatography-mass spectrometry (GC-MS) analysis.

Extract Administration		
Group	No. of birds	Treatment dosage
A (Normal control)	5	Feed and water only
B (Disease control)	5	Infected and untreated
C (Standard control)	5	Infected + Standard drug (25 mg/ml diclazuril in 1 L of water)
D	5	Infected + 50 mg/ml of <i>Z. officinale</i> extract in 1 L of water
E	5	Infected + 100 mg/ml of <i>Z. officinale</i> extract in 1 L of water
F	5	Infected + 50 mg/ml of <i>C. papaya</i> extract in 1 L of water
G	5	Infected + 100 mg/ml of <i>C. papaya</i> extract in 1 L of water
H	5	Infected + 50 mg/ml of <i>Z. officinale</i> and <i>C. papaya</i> extract in 1 L of water
I	5	Infected + 50 mg/ml of <i>Z. officinale</i> and <i>C. papaya</i> extract in 1 L of water

The treatment lasted for five days. At the end of the treatment, the chicks were fasted overnight and blood collected via under wing veni-puncture into plain and heparinized tubes. The blood in heparinized tubes were used immediately for assessment of haematological indices. The ones in plain tubes were allowed to clot, sera were separated by centrifugation within 1 h of clotting and collected using Pasteur pipettes. The sera were stored in a refrigerator for use.

Determination of weight gain

The body weights of the birds were recorded weekly throughout the period of experiment. Starting from week 3, the weekly weight gain recorded per group. The average daily feed administration was also measured in Kg.

Feed conversion rate (FCR)

This was calculated as follows:

Total feed consumed (kg)/Total weight gain

Where, total weight gain = final weight – initial weight

Haematological analysis

The parameters were determined using Sysmex K-21n automatic multi-parameter blood cell counter. Counting of blood cells is based on the volumetric impedance method, directly measuring white blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), platelets and automatically calculating mean corpuscular haemoglobin concentration (MCHC).

Principle: The machine employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block and the RBC and Platelets count

are taken by the RBC detector block using the DC detection method. The HB detector block measures the Hb concentration using a non-cyanide haemoglobin method. Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into each transducer. The transducer chamber has a minute hole, the aperture, which has electrodes on both sides between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse sizes.

Procedure: The power was turned on and the machine was allowed to undergo “Machine self-check” and “background check”. When the machine displayed ‘ready’, the sample number was set and sample introduced to the sample probe. Then, the start switch was pressed to execute analysis. At the end of analysis, results were displayed on liquid crystal display (LCD) screen and printed onto a ticket format. The ‘ready’ was displayed again for subsequent samples.

Liver function assay

Assay of serum aspartate aminotransferase (AST) activity

Determination of AST activity was based on the method of Reitman & Frankel (1957). The AST catalyzes the transfer of the α -amino group from L-aspartate to α -ketoglutarate resulting in oxaloacetate and L-glutamate formation. The AST is measured by the reaction of oxaloacetate with 2, 4-dinitrophenylhydrazine (DNPH) to form 2, 4 – dinitrophenylhydrazone in an alkaline medium.

The reagents were brought to room temperature, test tubes labelled Reagent blank (RB) and Sample were set up. Then, 0.1 ml of distilled water and serum were placed in the tubes, respectively. Thereafter, 0.5 ml of reagent 1 (100 mmol/l phosphate buffer pH 7.4, 100 mmol/l L-aspartate and 2 mmol/l α -oxoglutarate) were added, mixed and incubated for exactly 30 mins at 37°C. Then, 0.5 ml of reagent 2 (2mmol/l DNPH) was added, mixed and incubated for exactly 20 mins at 25 °C. Thereafter, 5.0 ml of 0.4mol/L sodium hydroxide (NaOH) was added and the content of the test tubes mixed thoroughly and left at room temperature for 5 mins. Absorbance was read in a turner® 390 spectrophotometer against the reagent blank at 510nm. The activity of AST in the serum was read from the AST calibration curve in U/L.

Assay of serum alanine aminotransferase (ALT) activity

This was based on the method of Reitman & Frankel (1957). The principle of the test is that ALT catalyzes the transfer of the amino group from L-alanine to α -ketoglutarate resulting in the

formation of pyruvate and L-glutamate. The ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

The reagents were brought to room temperature, test tubes labelled Reagent blank (RB) and Sample were set up. Then, 0.1 ml of distilled water and serum were placed in the tubes, respectively. Thereafter, 0.5 ml of solution R1 (100mmol/l phosphate buffer pH 7.4, 200mmol/l L-alanine and 2.0mmol/l α -oxoglutarate) were added, mixed and incubated for exactly 30 mins at 37°C. Then, 0.5 ml of solution R2 (2mmol/l DNPH) was added, mixed and incubated for exactly 20 mins at 25°C. Thereafter, 5.0 ml of NaOH (0.4mol/L) was added and the content of the tubes mixed thoroughly and left at room temperature for 5 mins. Absorbance was read against the reagent blank at 510 nm. The activity of ALT in the serum was read from the ALT calibration curve in U/L.

Assay of serum alkaline phosphatase (ALP) activity

The ALP activity was determined according to the method described by Englehardt (1970). The ALP catalyses the hydrolysis of p-nitrophenylphosphate to p-nitrophenol - with the simultaneous release of phosphate group. The rate of reduction of p-nitrophenylphosphate, measured by determining the rate of increase in absorbance at 405nm, is directly proportional to the ALP activity.

The reagents were brought to room temperature, test tubes labelled Reagent blank (RB) and Sample were set up. Then, 50 μ l of distilled water and serum were placed in the tubes, respectively, and 3.0 ml of reagent solution was added. These were mixed, initial absorbance read against the blank and timer started simultaneously. And read again after 1, 2 and 3 minutes.

Calculations: ALP activity (U/L) = $(\Delta A_{405nm}/min) \times 3300$

Where; ΔA = Change in absorbance, min = minute.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) with repeated measures using Statistical Product and Service Solutions (SPSS) version 23. Results are expressed as mean $\pm$ standard deviation (SD). Differences between means were considered to be significant at $P < 0.05$ using Duncan Multiple Range Test (DMRT).

3 RESULTS

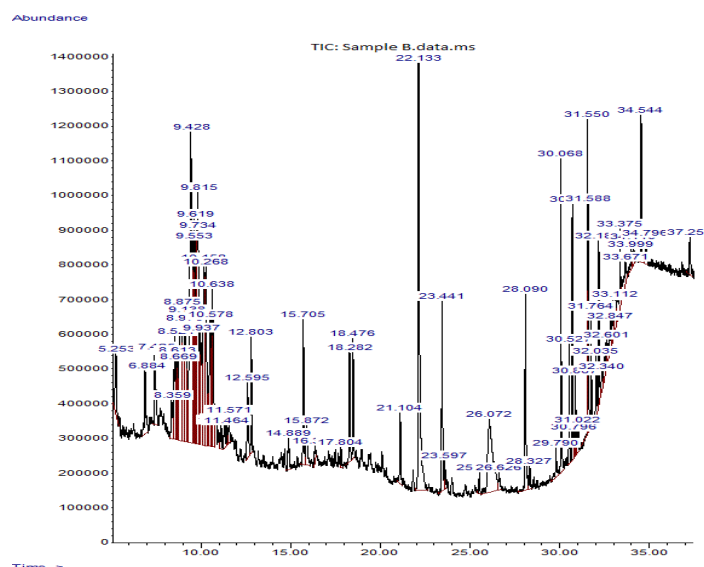
The results from this study are presented in tables 3.1 to 3.7 and figures 3.1 and 3.2.

Table 3.1 below shows the bioactive compounds detected in the *Zingiber officinale* extract.

Table 3.1: Bioactive compounds identified in *Zingiber officinale* extract

S/N	Retention time (min)	Identified compound	Peak area (%)	Reported biological activities
1	22.133	2,4-Di-tert-butylphenol	7.74	Antioxidant, antibacterial, antifungal
2	29.790–33.671	Oleic acid	0.48–1.25	Anti-inflammatory, cardioprotective, antimicrobial
3	30.068	Hexadecanoic acid, methyl ester (Methyl palmitate)	2.35	Antioxidant, anti-inflammatory, antimicrobial
4	31.550	9,12-Octadecadienoic acid, methyl ester (Methyl linoleate)	1.57	Anti-inflammatory, hypocholesterolemic
5	31.764	Stearic acid, methyl ester	0.72	Antimicrobial, lipid metabolism regulation
6	32.035	Palmitoleic acid	0.53	Anti-inflammatory, antimicrobial
7	12.595	Azulene	1.21	Anti-inflammatory, antioxidant
8	15.872–16.352	Methyl naphthalene derivatives	0.15–0.77	Antimicrobial, insecticidal
9	23.441	Z-8-Hexadecene	2.07	Antimicrobial, insect pheromone activity
10	30.527	Dibutyl phthalate	0.88	Antimicrobial, antifouling activity

Figure 3.1: Chromatogram of GC-MS analysis of the *Zingiber officinale* extract.



6	33.375	Erucic acid	1.08	Antimicrobial, anti-inflammatory
7	19.354	Citronellol	0.23	Antimicrobial, antioxidant, anti-inflammatory
8	19.743	1-Hexacosanol	0.15	Antioxidant, anti-inflammatory
9	34.116	L-Ascorbic acid, 6-octadecanoate	0.36	Antioxidant, immune-supportive

Figure 3.2: Chromatogram of GC-MS analysis of the *Carica papaya* leaf extract.

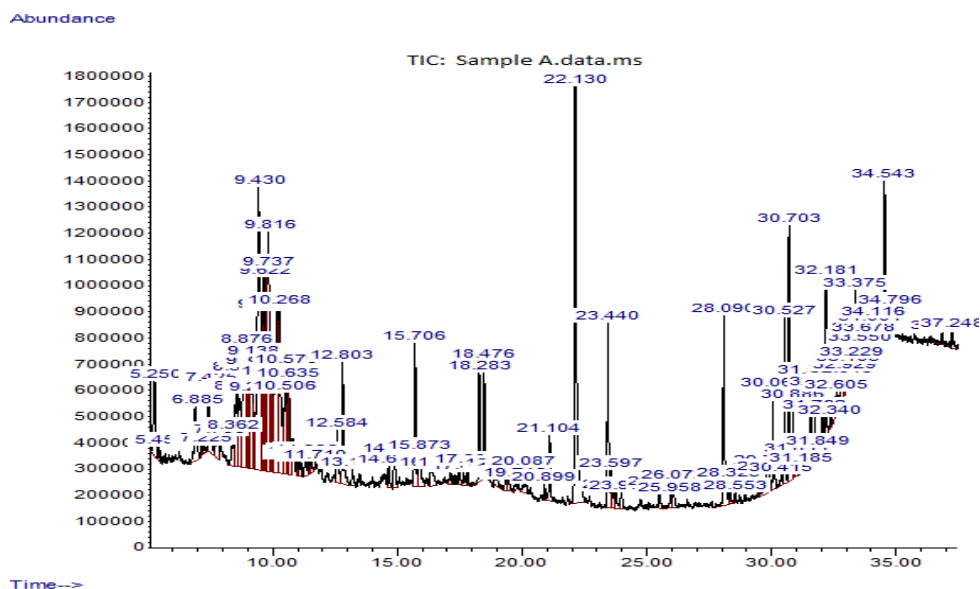


Table 3.3 below presents the results of effects of aqueous extracts of *Zingiber officinale* rhizome and *Carica papaya* leaf on weight gain of Abor Acre + strain of broilers. The results showed steady increase in weight gain in all groups. At week 3, there was no significant difference ($p > 0.05$) in the weight gain. At week 4, the weight gain in all infected groups were significantly lower than the normal control, while at week 5, only the weight gain of birds treated with high dose ginger extract and low dose combined extract were statistically similar to the normal control.

Table 3.3: Effects of aqueous extracts of *Zingiber officinale* rhizome and *Carica papaya* leaf on weight gain of Abor Acre + strain of broilers

Values are presented as mean $\pm$ standard deviation of triplicate determinations. Values with different superscript letters are significantly different ($p < 0.05$).

Table 3.4 below presents the results of effects of aqueous extracts of *Zingiber officinale* rhizome and *Carica papaya* leaf on feed conversion rate (FCR) of Abor Acre + strain of broilers. The results showed no significant difference ($p > 0.05$) in the FCR among the groups at week 3. At week 4, a general drop in FCR was observed and the values from the infected groups were significantly higher than the normal. At week 5, FCR of all treatment groups compared well with

the normal control, except for groups treated with low dose ginger extract and high dose pawpaw extract that were significantly higher.

Table 3.4: Effects of aqueous extracts of *Zingiber officinale* rhizome and *Carica papaya* leaf on FCR of Abor Acre + strain of broilers

Group	Week 3 (kg)	Week 4 (kg)	Week 5 (kg)
Normal control	0.58 ± 0.07 ^a	0.40 ± 0.01 ^a	0.45 ± 0.02 ^a
Disease control	0.63 ± 0.05 ^a	0.47 ± 0.02 ^b	0.45 ± 0.02 ^a
Standard control	0.59 ± 0.06 ^a	0.49 ± 0.00 ^b	0.51 ± 0.04 ^{ab}
50 mg/ml of <i>Z. officinale</i>	0.67 ± 0.07 ^a	0.48 ± 0.05 ^b	0.54 ± 0.06 ^b
100 mg/ml of <i>Z. officinale</i>	0.60 ± 0.06 ^a	0.46 ± 0.04 ^b	0.50 ± 0.04 ^{ab}
50 mg/ml of <i>C. papaya</i>	0.63 ± 0.03 ^a	0.47 ± 0.03 ^b	0.53 ± 0.04 ^{ab}
100 mg/ml of <i>C. papaya</i>	0.62 ± 0.05 ^a	0.46 ± 0.03 ^b	0.54 ± 0.06 ^b
50 mg/ml of <i>Z. officinale</i> and <i>C. papaya</i>	0.65 ± 0.07 ^a	0.46 ± 0.03 ^b	0.51 ± 0.04 ^{ab}
100 mg/ml of <i>Z. officinale</i> and <i>C. papaya</i>	0.67 ± 0.09 ^a	0.46 ± 0.02 ^b	0.53 ± 0.03 ^{ab}

Values are presented as mean ± standard deviation of triplicate determinations. Values with different superscript letters are significantly different ($p < 0.05$).

Table 3.5 below presents the results of effects of aqueous extracts of *Zingiber officinale* rhizome and *Carica papaya* leaf on Hb, PCV and MCHC of Abor Acre + strain of broilers. The results showed that the extracts induced significant ($p < 0.05$) increase in the values of the three parameters compared to the disease control.

Table 3.5: Effects of aqueous extracts of *Zingiber officinale* rhizome and *Carica papaya* leaf on Hb, PCV and MCHC of Abor Acre + strain of broilers

Group	Hb concentration (g/dl)	PCV (%)	MCHC (g/dl)
Normal control	12.8 ± 0.7 ^c	38.3 ± 2.1 ^c	33.3 ± 0.8 ^{bcd}
Disease control	7.2 ± 0.8 ^a	21.6 ± 2.5 ^a	29.2 ± 0.8 ^a
Standard control	10.1 ± 0.8 ^b	30.2 ± 2.3 ^b	34.3 ± 0.4 ^d
50 mg/ml of <i>Z. officinale</i>	10.9 ± 0.9 ^b	32.6 ± 2.6 ^b	32.7 ± 0.4 ^b
100 mg/ml of <i>Z. officinale</i>	10.2 ± 0.4 ^b	30.5 ± 1.1 ^b	33.6 ± 0.9 ^{bcd}
50 mg/ml of <i>C. papaya</i>	11.3 ± 1.2 ^b	33.8 ± 3.6 ^b	33.6 ± 0.4 ^{bcd}
100 mg/ml of <i>C. papaya</i>	11.0 ± 0.5 ^b	33.0 ± 1.4 ^b	33.1 ± 0.4 ^{bc}
50 mg/ml of <i>Z. officinale</i> and <i>C. papaya</i>	12.6 ± 0.5 ^c	37.8 ± 1.4 ^c	33.3 ± 0.3 ^{bcd}
100 mg/ml of <i>Z. officinale</i> and <i>C. papaya</i>	11.2 ± 0.3 ^b	33.5 ± 0.9 ^b	33.9 ± 0.8 ^{cd}

Values are presented as mean ± standard deviation of triplicate determinations. Values with different superscript letters are significantly different ($p < 0.05$).

Table 3.6 below presents the results of effects of aqueous extracts of *Zingiber officinale* rhizome and *Carica papaya* leaf on RBC, TWBC, platelet and lymphocyte counts of Abor Acre + strain of broilers. The results showed that the extracts induced significant ($p < 0.05$) increases in the RBC and platelet counts while inducing significant ($p < 0.05$) reductions in TWBC and lymphocyte counts compared to the disease control.

Table 3.6: Effects of aqueous extracts of *Zingiber officinale* rhizome and *Carica papaya* leaf on RBC, TWBC, platelet and lymphocyte counts of Abor Acre + strain of broilers

Group	RBC count (x 10 ⁶ /μl)	TWBC count (x 10 ³ /μl)	PLT count (x 10 ⁶ /μl)	Lymphocyte count (%)
Normal control	4.83 ± 0.55 ^b	18.14 ± 0.24 ^a	2.0 ± 0.2 ^{bc}	31.86 ± 1.24 ^a
Disease control	3.28 ± 0.53 ^a	35.63 ± 2.25 ^b	1.2 ± 0.1 ^a	51.62 ± 4.53 ^b
Standard control	4.69 ± 0.64 ^b	17.38 ± 1.35 ^a	2.4 ± 0.4 ^d	29.84 ± 1.47 ^a
50 mg/ml of <i>Z. officinale</i>	5.11 ± 0.3 ^{bc}	18.06 ± 0.21 ^a	1.7 ± 0.1 ^{bc}	31.07 ± 1.92 ^a
100 mg/ml of <i>Z. officinale</i>	4.73 ± 0.39 ^b	17.73 ± 0.36 ^a	1.6 ± 0.3 ^{ab}	31.61 ± 1.53 ^a
50 mg/ml of <i>C. papaya</i>	5.14 ± 0.29 ^{bc}	18.13 ± 0.22 ^a	1.5 ± 0.3 ^{ab}	28.60 ± 2.60 ^a
100 mg/ml of <i>C. papaya</i>	4.93 ± 0.19 ^b	17.98 ± 0.21 ^a	1.8 ± 0.3 ^{bc}	31.36 ± 1.88 ^a
50 mg/ml of <i>Z. officinale</i> and <i>C. papaya</i>	5.84 ± 0.24 ^c	18.94 ± 0.28 ^a	1.7 ± 0.2 ^{bc}	30.08 ± 1.47 ^a
100 mg/ml of <i>Z. officinale</i> and <i>C. papaya</i>	4.99 ± 0.39 ^b	17.85 ± 0.95 ^a	2.1 ± 0.3 ^{cd}	31.71 ± 1.60 ^a

Values are presented as mean ± standard deviation of triplicate determinations. Values with different superscript letters are significantly different ($p < 0.05$).

Table 3.7 below presents the results of effects of aqueous extracts of *Zingiber officinale* rhizome and *Carica papaya* leaf on serum activities of AST, ALT and ALP of Abor Acre + strain of broilers. The results showed that the extracts induced significant ($p < 0.05$) reductions in the activities of the enzymes compared to the disease control.

Table 3.7: Effects of aqueous extracts of *Zingiber officinale* rhizome and *Carica papaya* leaf on serum activities of AST, ALT and ALP of Abor Acre + strain of broilers

Group	AST (U/l)	ALT (U/l)	ALP (U/l)
Normal control	91.67 ± 3.06 ^a	4.73 ± 0.40 ^{ab}	609.7 ± 11.5 ^{ab}
Disease control	162.67 ± 9.61 ^d	14.50 ± 0.82 ^c	711.7 ± 21.5 ^c
Standard control	121.33 ± 4.04 ^b	6.17 ± 0.55 ^{cd}	615.7 ± 13.0 ^{ab}
50 mg/ml of <i>Z. officinale</i>	114.33 ± 11.59 ^b	6.13 ± 0.15 ^{cd}	613.0 ± 15.7 ^{ab}
100 mg/ml of <i>Z. officinale</i>	91.00 ± 6.25 ^a	5.17 ± 1.07 ^{abc}	611.7 ± 36.0 ^{ab}
50 mg/ml of <i>C. papaya</i>	87.67 ± 2.08 ^a	4.20 ± 0.63 ^a	609.0 ± 25.1 ^{ab}
100 mg/ml of <i>C. papaya</i>	99.00 ± 7.55 ^a	5.80 ± 0.50 ^{bcd}	589.3 ± 17.8 ^a
50 mg/ml of <i>Z. officinale</i> and <i>C. papaya</i>	137.00 ± 7.55 ^c	6.77 ± 0.35 ^d	646.7 ± 14.6 ^b
100 mg/ml of <i>Z. officinale</i> and <i>C. papaya</i>	92.67 ± 5.69 ^a	5.00 ± 0.72 ^{abc}	603.7 ± 15.8 ^a

Values are presented as mean ± standard deviation of triplicate determinations. Values with different superscript letters are significantly different ($p < 0.05$).

4 DISCUSSION

This study investigated the anticoccidial activities of combinations of aqueous extracts of different medicinal plants (*Zingiber officinale* rhizome and *Carica papaya* leaves) on *Eimeria* strain in Abor Acre + strain of broilers. The results obtained are presented in tables 3.1 to 3.7.

GC-MS analysis

The GC-MS results (figures 3.1 and 3.2) showed that 67 compounds were detected in the ginger extract, while 94 compounds were detected in the pawpaw extract. The results (tables 3.1) revealed that fatty acids, fatty acid esters, phenolic compounds, and sesquiterpene-related molecules were the predominant compounds detected in the ginger extract. Compounds like 2, 4-di-tert-butylphenol, azulene, and unsaturated fatty acids have been attributed with antioxidant potential

(Liu et al., 2023; Ren et al., 2023; Slon, & Kowalczyk, 2024) were detected. Phenols, fatty acid esters, and long-chain hydrocarbons were also detected and have been associated with antimicrobial activity (Walsh et al., 2020; Lamba, Kopel, Westenberg, & Kapila, 2023). Additional compounds detected were palmitoleic acid which have been suggested to have anti-inflammatory effects (de Souza et al., 2018), and oleic acid and linoleic acid derivatives which have been reported to possess anti-inflammatory and cardioprotective effects in addition to metabolic benefits (Shramko, Polonskaya, Kashtanova, Stakhneva & Ragino, 2020; Bertoni et al., 2023). Minor aromatic compounds such as methyl naphthalenes may contribute insecticidal or preservative effects, commonly reported in plant essential oils. These agree with the reports of antimicrobial, anti-inflammatory, antioxidant, anticancer and anti-allergic activities of ginger (Semwal, Combrinck, & Viljoen, 2015).

The bioactive compounds detected in the pawpaw extract (table 3.2) were majorly phenolic compounds, fatty acid esters, terpenoids, fatty acids and long chain alcohols. It contained phenolic compounds such as 2, 4-di-tert-butylphenol which is known for its antimicrobial and anti-inflammatory properties, in addition to its antioxidant potential (Yang et al., 2024). Unsaturated fatty acids such as the oleic and cis-vaccenic acids were detected. Terpenoids and alcohols like citronellol and hexacosanol which possess antibacterial, antifungal, insect-repellent and antioxidant properties (Guimarães et al., 2019; Câmara et al., 2024) were present. Fatty acid esters such as palmitic acid and linoleic acid methyl esters were detected. These compounds possess antioxidant and antimicrobial properties.

Overall, both plants extracts has bioactive compounds which support their pharmacological relevance, especially as antioxidants, antimicrobials, and anti-inflammatory agents.

Weight gain and feed conversion ratio

The results of weight gain showed that a general increase in weight gain throughout the weeks. At week 3, the weight gain observed across all groups were statistically similar, at week 4 the weight gain observed in all the other groups were significantly ($p < 0.05$) lower than the normal control while at week 5 the values obtained for the high dose ginger extract group and the low dose combined extract group were not significantly ($p > 0.05$) different from the normal control.

Weight gain remained steady across most groups, but the impact of infection was visible in feed efficiency. The disease control group showed a persistent decrease in FCR into the fifth week. The normal control shows a remarkable performance at the third and fourth week recording an average of 0.58 ± 0.07^a and 0.40 ± 0.01^a as against 100 mg/ml of ginger extract with an average of 0.60 ± 0.06^a and 0.46 ± 0.04^b . In contrast, at week five, birds treated with 100 mg/ml of ginger

extracts showed a recovery in FCR values, suggesting that the extract helped maintain intestinal integrity and nutrient absorption.

Haematological profile

Haematological parameters are used to measure the effects of environmental, nutritional and/or pathological factors on animals (Afolabi, Akinsoyinu, Olajide & Akinleye, 2010). *Eimeria* infection typically causes intestinal hemorrhaging, leading to anaemia (Andrews, 2022). Values for haematological markers from this study suggested that the disease control exhibited significantly lower Hb (7.2 ± 0.8 g/dl). The lower Hb level and the lower PCV ($21.6 \pm 2.5\%$) compared to the normal control are pointers to the anaemia induced by the infection. The results showed that 50 mg/ml combination treatment effectively boosted these levels to 12.6 ± 0.5 g/dl (Hb) and $37.8 \pm 1.4\%$ (PCV), outperforming the standard drug diclazuril in some metrics. The TWBC and lymphocyte counts were significantly higher in the disease control ($35.63 \pm 2.25 \times 10^3/\mu\text{l}$) indicating infection. Extract treatment returned these values to the normal range, indicating a reduction in systemic inflammation.

It was also observed that the values obtained for all the groups except the disease control were within normal range for all parameters assessed. Among all the treated groups, only the combined extract at low dose induced effects similar to the normal control for all the haematological indices except for RBC count where it induced the highest value obtained. In addition, the test extracts induced reductions in TWBC count in a dose-dependent manner. These results indicated that the test extracts are capable of improving haematological indices of birds. These results are similar to the findings of Nghonjuyi et al. (2015) who stated that *Carica papaya* leaf extract reduced oocytes count and improved RBC and WBC counts in chickens. Okolo, Otu, Edicha and Abdulkareem (2023) also reported that ginger extract did not cause negative effects on haematological indices suggesting that it could boost immunity in broiler birds.

Liver Function

The AST, ALT and ALP are liver enzymes often measured to monitor liver health. Abnormally high activities of these enzymes in the serum suggest liver damage (Diri, Eu & Johnson, 2025). This present study showed that the plant extracts induced significant ($p < 0.05$) reductions in the activities of these enzymes. The values were observed to be within normal ranges. These results indicated that the test extract improved liver function. These results agree with the works of Diri et al. (2025) who reported that garlic-and ginger-based diets fed to broilers improved liver health and integrity. Mbakwe, Sabo, Jibia and Sulaiman (2025) also reported that pawpaw extract improved

haematological and liver function parameters in broilers. They further stated that the liver cells were likely protected by the antioxidant properties of the extract.

5 CONCLUSION

From the findings of this study, aqueous extracts of *Zingiber officinale* rhizome and *Carica papaya* leaf possessed various compounds with beneficial biological activities in varying concentrations. The in vivo studies revealed that the extracts and their combinations improved weight gain and feed conversion rates. The extracts improved the haematological profile and demonstrated significant hepatoprotective properties in the birds by reducing elevated liver enzymes (AST, ALT, and ALP) induced by the infection, specifically, the 100 mg/ml combination.

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