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An experimental investigation on the anthelmintic efficacy of crude aqueous extracts of selected medicinal herbs against gastrointestinal helminths in native chickens

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ABSTRACT

This study assessed the anthelmintic efficacy of selected crude aqueous herbal extracts against gastrointestinal helminths in native chickens raised under semi-scavenging conditions. Forty-five naturally infected birds were randomly allocated to five treatment groups: T1 (distilled water), T2 (commercial levamisole), T3 (*Basella alba*), T4 (*Carica papaya*), and T5 (*Allium sativum*). Treatments were administered orally at 3 mL/kg body weight, and fecal egg counts (EPG) were measured at baseline (day 0) and at days 10 and 14 post-treatment. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, saponins, steroids, and tannins in all tested species. By Day 10, substantial reductions in EPG occurred, with *C. papaya* (966.7 ± 76.4) and *A. sativum* (1100.0 ± 217.9) achieving significantly lower counts compared to distilled water (5283.3 ± 236.3) and the commercial anthelmintic levamisole (8650.0 ± 1468.3) ($P < 0.05$). *B. alba* exhibited intermediate efficacy (1233.3 ± 361.7), comparable statistically to the two most effective botanicals. However, EPG values rebounded significantly across all groups by day 14, though *C. papaya* (5166.7 ± 464.6), *A. sativum* (5266.7 ± 189.3), and *B. alba* (5250.0 ± 482.2) maintained significantly lower burdens compared to the control (10300.0 ± 3404.4) and levamisole (6716.7 ± 2878.5) groups ($P < 0.05$). These results show that botanical extracts could be good short-term replacements for traditional anthelmintics. They also suggest that parasite control may require repeated or optimized dosing.

Keywords: *Anthelmintic, Allium sativum, Basella alba, Carica papaya, Native chicken*.

INTRODUCTION

Native chicken farmers in tropical areas continue to face persistent helminth challenges due to climatic conditions that support parasite development and survival (Shifaw *et al.,* 2021). This vulnerability is evident in a study by Ybañez *et al.* (2018), who found that native chickens raised under free-range and semi-scavenging systems in the Philippines are highly susceptible to gastrointestinal helminths, with *Ascaridia spp.* and *Heterakis spp.* reported at prevalences of 41.2% and 59.3%, respectively, and an overall detection rate of 92.2% in small-scale layer farms. These nematode infections impair feed efficiency, reduce egg and meat production, and elevate mortality risks, thereby threatening food security and rural livelihoods (Zalizar *et al.,* 2021; Wuthijaree *et al.,* 2024).

Nowadays, the primary approach to helminth control in poultry has long relied on synthetic anthelmintics such as levamisole and albendazole. While initially effective, the repeated and often indiscriminate use of these drugs has led to the emergence of resistant helminth populations, a phenomenon documented across various geographic settings (Beech *et al.,* 2011; Kaplan and Vidyashankar, 2012; Martin *et al.,* 2012). In addition, concerns over chemical residues in eggs and meat products, environmental contamination, and consumer safety have prompted the search for safer, sustainable alternatives (Sattar *et al.,* 2014; Mund *et al.,* 2016; Owusu-Doubreh *et al.,* 2023; Mesfin *et al.,* 2024).

Herbal extracts derived from ethnomedicinal plants have gained renewed interest for their anthelmintic potential (Nghonjuyi *et al.,* 2020; Kuralkar *et al.,* 2021; Adak and Kumar, 2022; Jamil *et al.,* 2022). Phytochemicals are known to exert anthelmintic activity through diverse mechanisms targeting parasite viability and reproduction. For instance, *C. papaya* possesses cysteine proteinases such as papain and chymopapain, which are believed to degrade the structural proteins of the helminth cuticle, leading to parasite death (Behnke *et al.,* 2008; Sen *et al.,* 2020; Adak and Kumar, 2022; Zirintunda *et al.,* 2022; Ugbogu *et al.,* 2023). Moreover, George and Kousalya (2018) reported that *B. alba* exhibited potent anthelmintic effects, inducing paralysis and death of *Eisenia fetida* within 10 and 32 minutes, respectively, at a concentration of 300 mg/mL. This effect is likely due to its rich phytochemical content, particularly saponins, flavonoids, and tannins, which are known to disrupt helminth cuticle integrity and neuromuscular function (Deshmukh and Gaikwad, 2014). On the other hand, *A. sativum* contains various sulfur compounds, including allyl disulfide (Ayaz *et al.,* 2008), ajoenes (Kothari *et al.,* 2019; Ahmad *et al.,* 2023), and allicin (Velkers *et al.,* 2011; Kothari *et al.,* 2019), which can impair parasite metabolism and development by potentially disrupting cell membranes and inhibiting essential enzymatic systems (Kothari *et al.,* 2019; Ranasinghe *et al.,* 2023). These findings are consistent with the study by Ahmad *et al.* (2023), which showed that flavonoids, tannins, and alkaloids may impede nutrient absorption in parasites and disrupt essential metabolic pathways vital for the survival of helminths.

Ethnoveterinary practices in the province of Agusan del Norte, Philippines, indicate the widespread use of these plants, yet their efficacy remains scientifically unverified. This study evaluates the anthelmintic potential of these plant extracts in naturally infected native chickens to validate their use and support their role in sustainable parasite control. Moreover, this study hypothesized that selected crude aqueous herbal extracts exhibit significant short-term anthelmintic efficacy comparable to commercial levamisole in reducing gastrointestinal helminth burden in native chickens.

MATERIALS AND METHODS

Experimental Animals, Housing, and Treatment Design

All experimental procedures strictly adhered to the Philippine Animal Welfare Act (Republic Act No. 8485, as amended by Republic Act No. 10631). Animal handling, care, and use were performed following established Good Animal Husbandry Practices (GAHP PNS/BAFS 184:2016), ensuring birds' well-being and minimal distress. A total of forty-five (45) native chickens, aged 4 to 6 months, were naturally exposed to gastrointestinal helminths under free-range farm conditions and their infection was confirmed through pre-treatment fecal egg count screening (≥150 eggs per gram [EPG]) using the Modified McMaster Technique (MAFF, 1986). The birds, of mixed sex and varying body weights, were sourced from the Department of Agriculture-Tagbina Research Station, Surigao del Sur. They were acclimatized for seven days in individual bamboo cages (0.5 m^2 per bird) under natural ventilation and ambient temperature (27–32°C), with a 12-hour light-dark cycle. Commercial poultry mash and clean water were provided *ad libitum* throughout the study.

The experiment employed a Completely Randomized Design (CRD) with five treatments, each replicated three times with three birds per replicate. Treatments were administered *via* oral gavage on day 0 using a sterile 5 mL syringe. The five treatment groups were as follows: T1, negative control, which received distilled water; T2, positive control, administered a commercial synthetic anthelmintic (Levamisole HCl, 8 mg/kg body weight); T3, 3 mL/kg BW of *Basella alba* (Alugbati) aqueous extract; T4, 3 mL/kg BW of *Carica papaya* (Papaya leaf) aqueous extract; and T5, 3 mL/kg BW of *Allium sativum* (Garlic) aqueous extract. These dose levels were adapted for safe oral administration in poultry, as similarly employed in herbal efficacy studies using aqueous extracts (Raza *et al.,* 2015).

Preparation of Crude Aqueous Extracts

Fresh leaves of *B. alba*, *C. papaya* and bulbs of *A. sativum* were identified and collected from multiple locations where ethnoveterinary use was reported. Voucher specimens were taxonomically verified and authenticated using standard morphological descriptors. Each plant sample was washed, shade-dried for 10 days, ground up, and then cold macerated in distilled water 300 g in 1.5 L) for 72 hours, with occasional agitation. Filtration was performed using Whatman No. 1 filter paper. The extracts were stored in amber bottles at 4°C until use, following established extract preparation methods (Yamson *et al.,* 2019).

Fecal Egg Count and Anthelmintic Efficacy Assessment

Fresh fecal samples were collected per bird on day 0 (baseline), day 10, and day 14 post-treatment. Fecal analyses were carried out at the Regional Animal Disease Diagnostic Laboratory (RADDL) in Taguibo, Butuan City. The Modified McMaster Technique was employed to quantify EPG, using saturated NaCl solution as the flotation medium. Briefly, 5 g of feces was homogenized with 28 mL of flotation fluid, filtered, and loaded into McMaster chambers (0.15 mL each). Ova were counted under low-power microscopy, and EPG was computed as

Fecal Egg Count (FEC) = $(\boldsymbol{C}_1 + \boldsymbol{C}_2) \times 50$

Where: $\boldsymbol{C}_1$ and $\boldsymbol{C}_2$ represent the egg counts in McMaster chambers 1 and 2, respectively.

Percent Fecal Egg Count Reduction (% FECR) = $100 \times (1 - FEC_{post} / FEC_{pre})$

Where FEC_{pre} is the mean fecal egg count before treatment and FEC_{post} is the mean fecal egg count after treatment.

Interpretation of infection intensity followed RADDL (2022): Light (100–500 EPG), Moderate (501–2,000 EPG), and Heavy (>2,000 EPG).

Phytochemical Screening

Qualitative phytochemical screening of aqueous extracts was conducted to detect saponins, alkaloids, flavonoids, tannins, steroids, anthraquinones, and cyanogenic glycosides. Standard phytochemical tests were used, including Culvenor–Fitzgerald for alkaloids, Froth (foam) for saponins, Ferric chloride for tannins, Bate-Smith & Metcalf for flavonoids, Keller–Kiliani for steroids, Modified Borntrager's for anthraquinones, and Guignard's (picrate paper) for cyanogenic glycosides, as described by Harborne (1998) and Trease and Evans (2002). The assays were performed at the Department of Chemistry, Mindanao State University – Iligan Institute of Technology.

Statistical Analysis

EPG data were analyzed using a Linear Mixed-Effects Model (LMM) with Treatment, Day, and Treatment × Day interaction as fixed effects, and replicate nested within Treatment as a random effect. Pairwise comparisons between treatments at each time point were performed using independent t-tests with Bonferroni correction. Significance was set at $P < 0.05$.

RESULTS

Phytochemical Composition of Aqueous Extracts

Figure 1 shows the qualitative distribution of seven key phytochemical groups in the aqueous extracts of *B. alba*, *C. papaya*, and *A. sativum*, categorized by their biofunctional roles. All three species exhibited the presence of at least four phytochemical groups, with notable variation in composition and intensity. Saponins, classified as surface disruptors, were most intensely detected (+++) in *B. alba*, moderately present (++) in *C. papaya*, and minimally present (+) in *A. sativum*. In a similar manner, tannins, another surface-active compound, were found in moderate (++) levels in *A. sativum*, weakly (+) in *C. papaya* and absent (−) in *B. alba*. Moreover, among neuromuscular inhibitors, alkaloids were consistently detected in all three species, with the highest intensity (+++) in *C. papaya*, moderate levels (++) in *A. sativum*, and trace presence (+) in *B. alba*. However, cyanogenic glycosides were not detected (−) in any of the tested extracts.

Furthermore, flavonoids, recognized for their metabolic and enzymatic interference, were most abundant (+++) in *C. papaya*, followed by

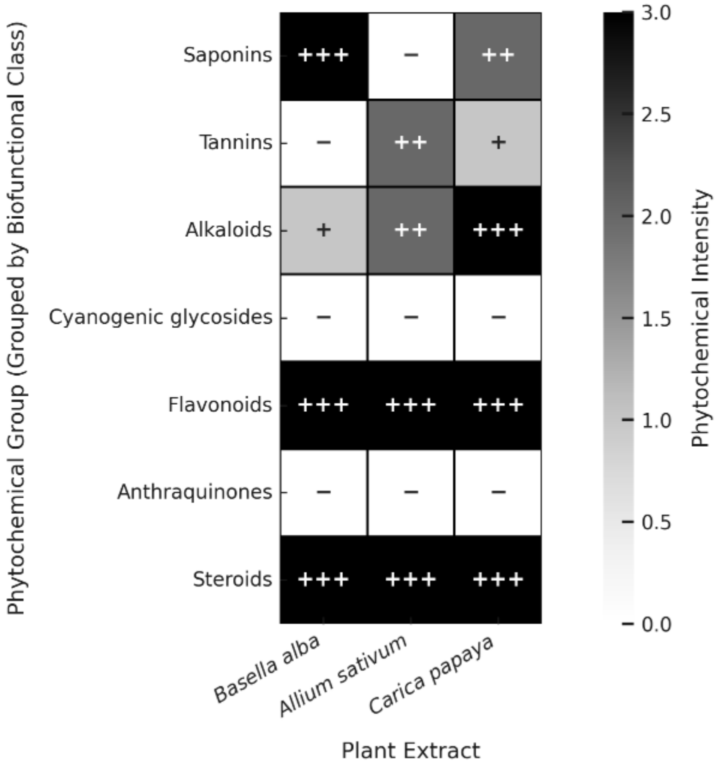

Figure 1. Qualitative intensity levels of seven phytochemical groups identified in aqueous extracts of the tested medicinal plants.

Table 1. Fecal Egg Counts (EPG; mean ± SD) in Native Chickens Measured Before and After a Single Oral Dose

Treatment	EPG Day 0	EPG Day 10	EPG Day 14
T1 - Distilled water	3066.67 ± 550.76[b]	5283.33 ± 236.29[d]	10300.00 ± 3404.40[d]
T2 - Commercial Anthelmintic	4250.00 ± 650.00[c]	8650.00 ± 1468.27[e]	6716.67 ± 2878.51[e]
T3 - *B. alba*	1616.67 ± 275.37[a]	1233.33 ± 361.71[c]	5250.00 ± 482.18[c]
T4 - *C. papaya*	7300.00 ± 264.57[e]	966.67 ± 76.38[a]	5166.67 ± 464.57[a]
T5 - *A. sativum*	6133.33 ± 3005.97[d]	1100.00 ± 217.94[b]	5266.67 ± 189.29[b]

Means within a column that bear different superscripts differ significantly at P < 0.05.

B. alba (++) and *A. sativum* (+). Anthraquinones, also categorized under metabolic modulators, were absent (−) in all extracts. Lastly, steroids, associated with structural and immunomodulatory functions, were weakly detected (+) in both *B. alba* and *C. papaya*, and absent (−) in *A. sativum*.

Fecal Egg counts (EPG) at Days 0, 10, and 14 Post-treatment

Table 1 summarizes mean ± SD EPG for each treatment over time. Baseline burdens differed (P < 0.05), with the highest counts in *C. papaya* (7300.00 ± 264.57) and *A. sativum* (6133.33 ± 3005.97), while *B. alba* (T3) exhibited the lowest EPG (1616.67 ± 275.37). By day 10, significant differences persisted among treatments (P < 0.05), with marked reductions noted particularly for *C. papaya* (966.67 ± 76.38) and *A. sativum* (1100.00 ± 217.94). These values differed significantly from the higher EPGs seen in T1 (Distilled Water: 5283.33 ± 236.29) and T2 (Commercial Anthelmintic: 8650.00 ± 1468.27).

B. alba achieved an intermediate reduction of 1233.33 ± 361.71, statistically comparable to the two highly effective botanicals. At day 14, despite a rebound in egg counts across all treatments, significant treatment differences remained ($P < 0.05$). The EPG values for *B. alba* (5250.00 ± 482.18), *C. papaya* (5166.67 ± 464.57), and *A. sativum* (5266.67 ± 189.29) continued to be lower than those of the control (10300.00 ± 3404.40) and commercial anthelmintic (6716.67 ± 2878.51). Although the rebound suggested repeated or optimized dosing regimens to sustain long-term parasite control, these observations indicate that tested extracts have significant short-term efficacy.

Distribution of EPG Values by Treatment and Time

Figure 2 illustrates the distribution of fecal egg counts (EPG) per treatment group across day 0, day 10, and day 14 using boxplots. At day 0, all groups exhibited relatively consistent worm burdens. By day 10, treated groups (T2, T4, T5) showed narrowed distributions, indicating reduced and more uniform parasitic loads. In contrast, day 14 displayed wider interquartile ranges and notable outliers, particularly in T2 (Commercial Drug) and T3 (*B. alba*), which could reflect variability in treatment response and possible parasite resurgence among some birds.

Mean EPG per Treatment Over Time

As illustrated in Figure 3, EPG values varied substantially across treatments and timepoints. At day 10 post-treatment, all groups exhibited a reduction in parasite egg counts compared to their pre-treatment EPG's. The most pronounced decreases were observed in chickens treated with *C. papaya* (T4), *A. sativum* (T5), and the commercial anthelmintic (T2), reflecting short-term efficacy. In contrast, the distilled water group (T1) showed a continued rise in EPG from day 0 through day 14, validating its role as a negative control. However, by day 14, all treatment groups, including those administered with herbal extracts, experienced a resurgence in EPG. The EPG levels in *B. alba* (T3), *C. papaya* (T4), and *A. sativum* (T5) groups exceeded 5,000 EPG, suggesting a decline in anthelmintic effectiveness over time. The commercial anthelmintic group (T2) also failed to sustain parasite suppression, with EPG values rebounding above baseline in some replicates.

Percentage Fecal Egg Count Reduction (% FECR).

Figure 4 illustrates the %FECR of native chickens following a single administration of aqueous herbal extracts and a commercial anthelmintic at days 10 and 14 post-treatment. The commercial drug (T2) showed a moderate % FECR at day 10 (32.16%) but exhibited a negative %FECR by day 14 (–58.04%), indicating an increase in worm burden compared to the baseline. In contrast, *C. papaya* (T4) and *A. sativum* (T5) extracts demonstrated strong anthelmintic activity at day 10, with %FECR values of 86.76% and 82.07%, respectively. However, their effectiveness reduced by day 14, falling to 29.22% and 14.13%, respectively. Moreover, *B. alba* (T3) showed a mild reduction at day 10 (23.71%) but a significant increase in EPG at day 14 (–224.74%). The negative control (T1, distilled water) consistently displayed increased worm load at both time points, confirming the absence of anthelmintic effect.

DISCUSSION

Phytochemical Basis for Anthelmintic Activity

The detection of alkaloids, saponins, tannins, flavonoids, and steroids in the tested plants supports their ethnomedicinal use through diverse, often synergistic, anthelmintic actions. For instance, saponins, which were found in high amounts in *B. alba* and moderate amounts in *C. papaya*, can disrupt mitochondrial function and damage helminth cell membranes due to their steroidal or triterpenoid structures (Zirintunda *et al.,* 2025). This membrane-active property will lead to vacuolization and mitochondrial disruption in nematodes (Tiwari *et al.,* 2020; Ahmad *et al.,* 2023). Likewise, tannins, which are detected strongly in *A. sativum*, bind to surface proteins on helminth cuticles, reducing motility and inducing starvation through impaired nutrient absorption.

Moreover, tannins have been shown to inhibit larval development (Mubarokah *et al.,* 2019; Stephen *et al.,* 2021; Ahmad *et al.,* 2023) and disrupt gastrointestinal function by interfering with energy metabolism and binding to para-

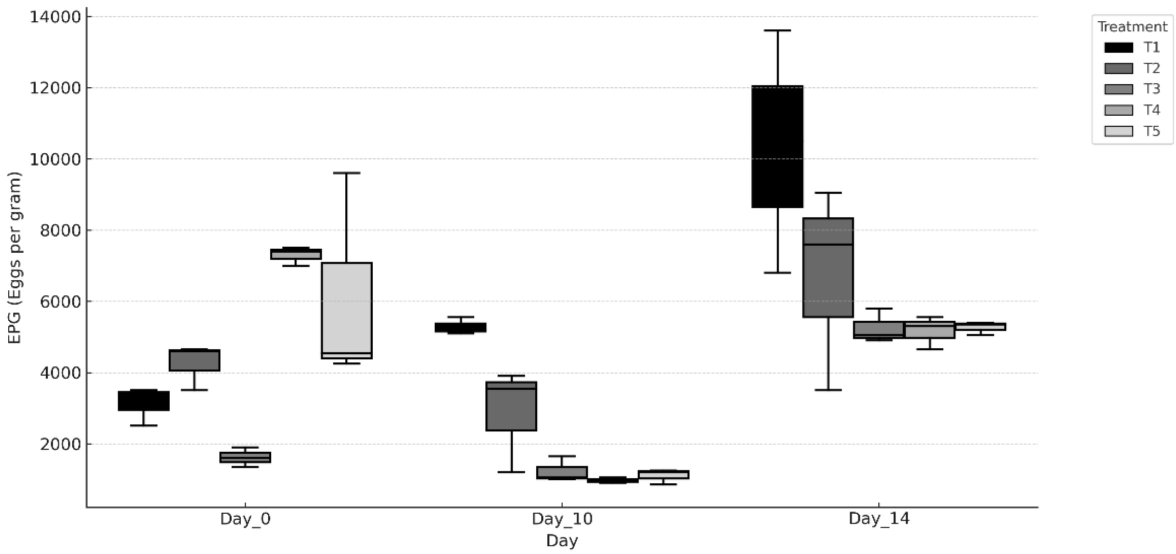

Figure 2. Boxplot howing fecal egg counts (EPG) distributions across treatment groups on Days 0, 10, and 14 post-treatments.

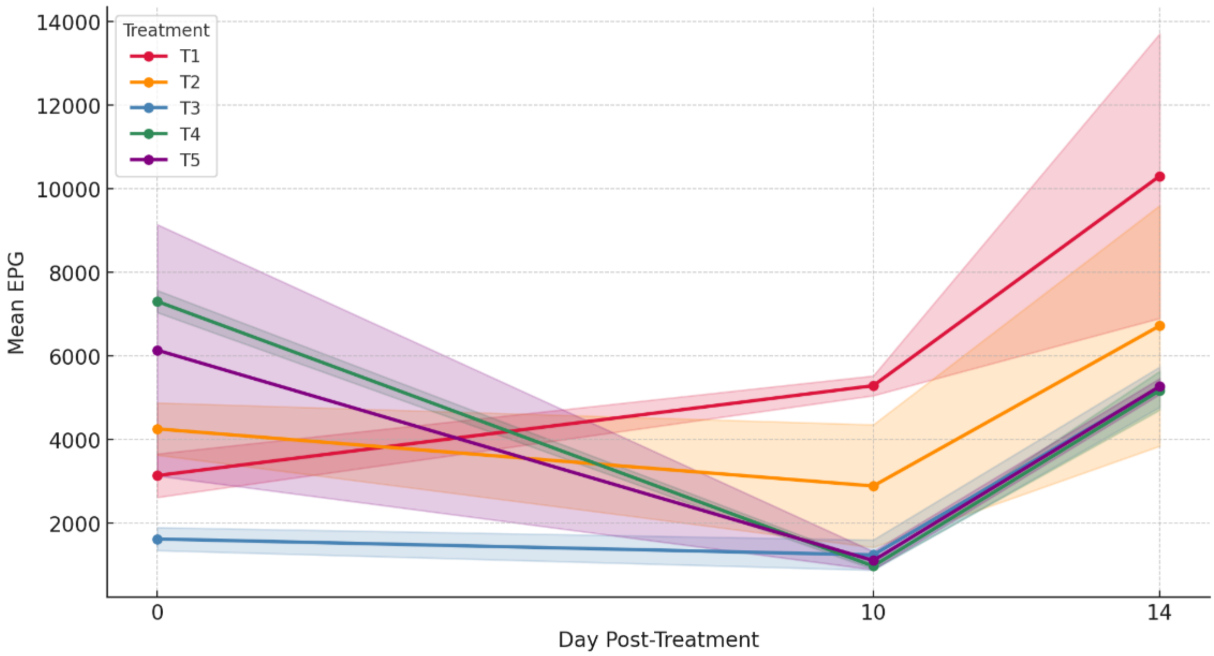

Figure 3. Mean EPG per treatment over time (Day 0, 10, 14).

site surfaces, thereby exerting anti-nutritional effects that impair parasite viability (Athanasiadou *et al.,* 2001; Wadekar *et al.,* 2011; Gasaliyu *et al.,* 2022; Roy *et al.,* 2024). In a similar manner, alkaloids, detected in all three extracts, act primarily through neurotoxic effects by interfering with neural receptors, including acetylcholine receptors, leading to spastic or flaccid paralysis in helminths. This paralysis can result in the expulsion of the parasitic worms from the host (Zirintunda *et al.,* 2022; Ranasinghe *et al.,* 2023). In addition, alkaloids exert anthelmintic effects by targeting acetylcholine receptors and inhibiting glucose uptake, leading to energy depletion and eventual death of the parasite through starvation (Badarina *et al.,* 2017).

Furthermore, flavonoids, which are particularly abundant in *C. papaya*, may contribute to its anthelmintic activity (Ugbogu *et al.,* 2023).

Flavonoids have been shown to disrupt parasite enzymes such as phosphodiesterase and Ca^{2+}-ATPase, potentially hindering essential physiological processes (Zirintunda *et al.,* 2022). Moreover, steroids that detected at lower intensities in *B. alba* and *C. papaya*, may indirectly benefits by modulating host immune function and suppressing helminth reproduction. Plant steroids can inhibit embryogenesis and fecundity, possibly through hormonal mimicry or mitochondrial interference (Patel and Savjani, 2015; Stephen *et al.,* 2021).

EPG Outcomes Across Anthelmintic Treatments

The significant reduction in EPG observed by day 10 in chickens treated with *C. papaya* and *A. sativum* supports their rapid anthelmintic action. This result may be attributed to the activity of papain, a cysteine protease in *C. papaya*, known to degrade cuticular proteins and compromise the structural integrity of helminths (Behnke *et al.,* 2008; Zirintunda *et al.,* 2025). Similarly, allicin from *A. sativum* inactivates thiol-dependent enzymes essential for parasite metabolism and oxidative defense, leading to its antiparasitic effect after short-term exposure (Ayaz *et al.,* 2008; Velkers *et al.,* 2011). Moreover, Kothari *et al.* (2019) reported garlic's role in metabolic enzyme suppression, in contrast to papain's structural degradation pathway (Sen *et al.,* 2020).

However, by day 14, the resurgence in EPG across all treatments, including synthetic levamisole, suggests a limitation in the residual efficacy of single-dose aqueous extracts. This could be attributed to the poor systemic bioavailability and rapid degradation of the active compounds, particularly in the case of allicin, which is known to be unstable in solution without encapsulation or sustained-release carriers (Ayaz *et al.,* 2008; Velkers *et al.,* 2011).

Moreover, the T2, while initially effective, exhibited reduced efficacy and greater variability by day 14, a trend that supports emerging reports of anthelmintic resistance in poultry helminths. This phenomenon is consistent with reports linking anthelmintic resistance to mutations in nicotinic acetylcholine receptor (nAChR) sub units, which alter receptor conformation and reduce drug binding affinity (Kaplan and Vidyashankar, 2012; Martin *et al.,* 2012; Choudhary *et al.,* 2022; Ahmad *et al.,* 2023). Such resistance has been increasingly reported under field conditions

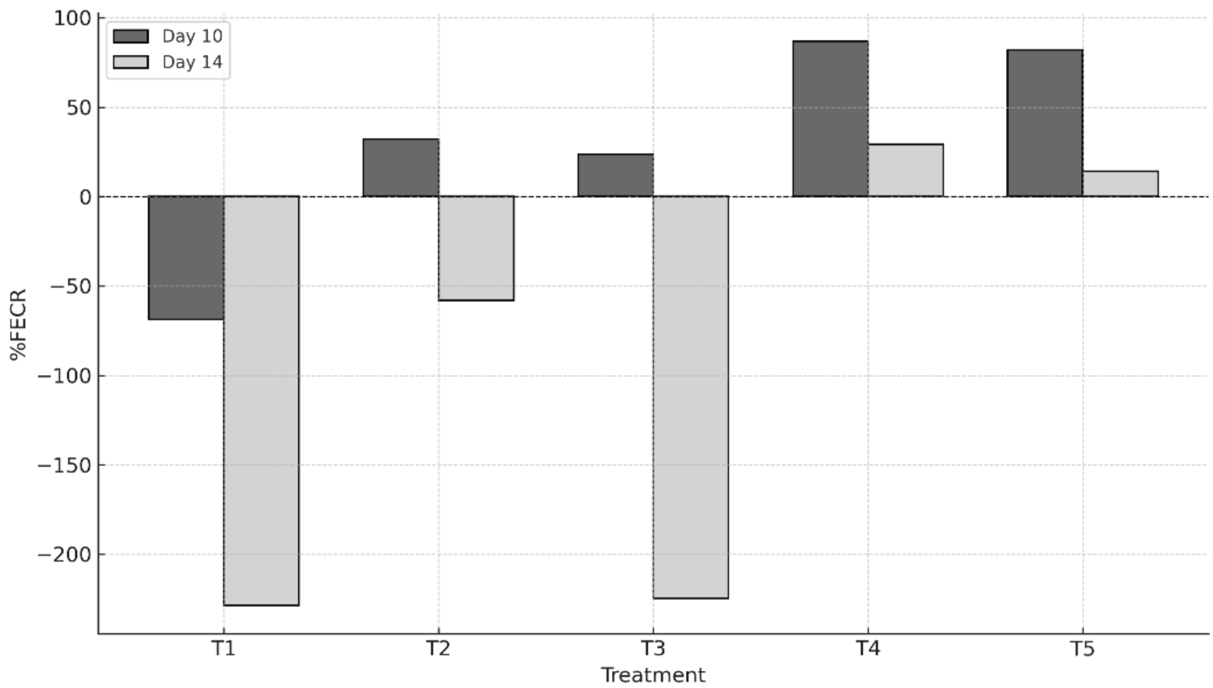

Figure 4. Percentage fecal egg count reduction (%FECR) of treated native chickens at 10- and 14-days post-treatment.

in smallholder poultry systems across tropical regions, including Africa and Asia (Zirintunda *et al.,* 2025). Meanwhile, *B. alba* showed delayed but moderate reductions in EPG, suggesting less potent or slower activity. Its relatively limited efficacy may be attributed to the lower concentration or bioavailability of its saponins and flavonoids, which, despite their recognized membrane-disruptive and oxidative effects on helminths, can vary depending on the extraction techniques employed and the standardization of dosages (Ahmad *et al.,* 2023).

Individual variation and Reliability of Treatment Effects

The EPG distribution patterns revealed varied degrees of consistency across treatment groups. Notably, *C. papaya* (T4) and *A. sativum* (T5) exhibited narrower EPG ranges at day 10, which suggest a more uniform short-term response. This trend aligns with previous reports on fast-acting compounds such as papain and allicin, which exhibit broad-spectrum activity against helminths by disrupting cuticular membranes, metabolic enzymes, or neuromuscular pathways (Velkers *et al.,* 2011; Raza *et al.,* 2015). Likewise, garlic-derived allicin has been credited with multi-site activity, though its variable stability in commercial preparations may explain inconsistencies in longer-term trials (Ayaz *et al.,* 2008; Velkers *et al.,* 2011).

However, treatment groups T2 and T3 displayed broader EPG spread by Day 14, implying variability in host responses or reduced residual efficacy. This variability is also observed in studies showing that levamisole, while initially effective, may fail in subsequent days due to resistance-related receptor mutations in nematodes (Zirintunda *et al.,* 2022; Ahmad *et al.,* 2023). Similarly, plant-based treatments like *B. alba*, despite possessing bioactives such as saponins and tannins, may show fluctuating efficacy due to dose inconsistencies, plant extract stability, or differential host metabolism (Ahmad *et al.,* 2023). Several trials have reported that variability in EPG outcomes may arise from the birds' differing baseline infections, immune responses, or reinfection risks in scavenging environments (Zirintunda *et al.,* 2022). The negative control (T1) showed predictably high and dispersed EPGs, reaffirming its role as a baseline reference.

Temporal Dynamics of EPG and Residual Efficacy

The EPG trends revealed a typical pattern of initial reduction followed by rebound across most treatment groups. The sharp declines by day 10 in *C. papaya* (T4), *A. sativum* (T5), and levamisole (T2) reflect the fast-acting nature of active compounds such as papain and allicin, which disrupt helminth metabolism and neuromuscular function (Ayaz *et al.,* 2008; Raza *et al.,* 2015). Similarly, short-term efficacy of crude extracts has been demonstrated in trials using various ethnomedicinal plants, especially during the first 7–10 days post-treatment (Hazarika *et al.,* 2023).

However, the notable resurgence in EPG at Day 14, most visibly in T2 and T3 underscores the limited residual action of both synthetic and herbal single-dose treatments. This rebound may be attributed to the low bioavailability and rapid degradation of compounds in aqueous herbal extracts, as well as environmental reinfection common in free-range systems (Shifaw *et al.,* 2021; Zirintunda *et al.,* 2022). Furthermore, garlic's active component, allicin, is known to degrade quickly, reducing its long-term efficacy unless repeatedly administered (Ayaz *et al.,* 2008).

On the other hand, the EPG resurgence and fluctuations observed in EPG in T2 may suggest the onset of resistance. This phenomenon could be attributed to mutations in the nicotinic acetylcholine receptor subunits of *A. galli*, particularly resulting from repeated or subtherapeutic dosing (Zirintunda *et al.,* 2022). Similar resistance patterns have been noted among poultry nematodes in Bangladesh and Africa (Beech *et al.,* 2011; Ritu *et al.,* 2024). In contrast, the inconsistent response in *B. alba* (T3) suggests that despite its saponin content, the extract's phytochemical profile or concentration may be insufficient for prolonged suppression. This aligns with findings from studies on comparable leafy extracts, which emphasize that efficacy is strongly influenced by factors such as extraction method, dosage, and parasite burden (Hazarika *et al.,* 2023).

Percentage Fecal Egg Count Reduction (% FECR).

This study confirms the short-term anthelmintic efficacy of *C. papaya* and *A. sativum*, supported by previous reports identifying papain and allicin as key bioactives that disrupt parasite metabolism and structural integrity (Raza *et al.,* 2015; Jamil *et al.,* 2022). Papain hydrolyzes helminth cuticle proteins, and allicin impairs thiol-dependent enzymes essential for parasite survival (Ayaz *et al.,* 2008; Velkers *et al.,* 2011). Moreover, the observed day 10 efficacy aligns with findings from trials using single-dose preparations of papaya or garlic extracts (Raza *et al.,* 2015; Zirintunda *et al.,* 2025). However, both herbal and synthetic treatments show a decline in efficacy by Day 14, suggesting limited residual action. This aspect may be attributed to the rapid degradation and low systemic retention of active compounds in aqueous formulations, which often lack protective carriers or slow-release mechanisms (Velkers *et al.,* 2011; Hazarika *et al.,* 2023). Moreover, reinfection under semi-scavenging poultry conditions likely contributed to the rebound in EPG levels, a pattern frequently reported in ethnoveterinary trials and field-based helminth control (Shifaw *et al.,* 2021).

The commercial drug (levamisole) also showed reduced efficacy at day 14, possibly due to suboptimal dosing or the emergence of resistance. Although some trials report continued levamisole efficacy (Zirintunda *et al.,* 2025), others have observed decreased effectiveness under field conditions where reinfection is rapid and resistance genes may proliferate (Raza *et al.,* 2015).

CONCLUSION

This study highlights the potential of selected medicinal plants as natural alternatives to synthetic anthelmintics for native chickens. The presence of key phytochemicals likely contributed to the observed efficacy, particularly the strong short-term effects of *C. papaya* and *A. sativum*. Although *B. alba* demonstrated -moderate effectiveness, but its consistent performance across time points indicates promising potential for further investigation. Overall, the findings support the integration of scientifically validated phytotherapeutics into sustainable helminth control strategies, particularly in resource-limited rural poultry systems.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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