

## HELMINTH PREVALENCE AND ANTHELMINTHIC EFFICACY OF METHANOL LEAVES EXTRACTS OF COMMON LOCAL PLANT (*VERNONIA AMAGDALYNA* AND *CYMBOPOGUM CITRATUS*) ON FREE RANGED CHICKENS IN LOKOJA, NIGERIA

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### Abstract

The poultry industry is one of the most efficient animal husbandry methods and it provides nutritional security to a significant number of the world population. Using modern intensive farming techniques, global production has reached 133.4 million in 2020, with a steady growth each year. However, this industry greatly threatened by parasitic diseases which affects the productivity, growth and the impact on income, food and nutrition security, and public health and may lead to total extermination of the entire poultry farm. The control of these parasitic infections is necessary for the maintenance of healthy and productive livestock. The aim of this study is to determine the prevalence of intestinal helminths in Lokoja and to determine the comparative anthelmintic efficacy of methanol leaves extracts of common local plant *Vernonia amagdalina* and *Cymbopogon citratus*. To determine prevalence of helminths in Lokoja, eighty free ranged chickens were sacrificed and intestinal contents harvested, dissected and helminths isolated and identified. Local plants *Vernonia amagdalina* and *Cymbopogon citratus* leaves were collected. Overall Helminth prevalence of 78.75% was recorded in the study area. Quantitative analysis by HPLC however revealed 2350.27 mg/100g phenols, 5412.60 mg/100g flavonoids, 1655.10 mg/100g alkaloids, 2955.57 mg/100g saponins and 2949.63 mg/100g tannins in *Vernonia amagdalina*. 852.23 mg/100g phenols, 1021.30 mg/100g flavonoids, 417.33 mg/100g alkaloids, 761.77 mg/100g saponins and 546.27 mg/100g tannins in *Cymbopogon citratus*. Anthelmintic efficacy of methanol extracts of these plants leaves extracts in different doses of 30mg/ml (Group A), 20mg/ml (Group B), 10mg/ml (Group C) was administered to chickens as well as 30 mg/ml Albendazole (positive control) to Group D and water (negative control) to Group E. This was followed by processing fecal samples using sodium chloride floatation method. Fecal Egg Count Reduction of methanol leaves extracts results revealed most significant fecal egg count reduction occurred in chickens treated with 20 mg/ml *Cymbopogon citratus* and *Vernonia amygdalina*. The plant-derived bioactive compounds in *Cymbopogon citratus* and *Vernonia amygdalina*. particularly be potent natural alternatives to synthetic anthelmintics by effectively reducing helminthic infection in free-range poultry and thus offer a sustainable, plant-based supplements for poultry parasite management.

**Keywords:** *Cymbopogon citratus*, *Vernonia amygdalina*, Albendazole, anthelmintic efficacy

## Introduction

The preference for free-range chickens for their perceived superior flavor and nutritional quality and their growth under particularly organic environment has made them of high demand in suburban and urban areas (Ritonga, 2018). This preference has periodically placed a high demand on the ability of breeders to meet the high supply needs (Amelia *et al.*, 2018), since farmers face barriers in scaling up free-range poultry production, due to the challenges of managing these chickens which are free ranged and are exposed to helminthic infections. This has led to low productivity and failure to satisfy consumer expectations (Uhuo *et al.*, 2013).

The fast-growing Nigerian poultry industry at an alarming rate, due to high demand for animal protein is confronted with helminthosis. The free-ranged management system which is the most common poultry management system practiced in Lokoja are prone to the acquisition of helminth parasites due to their feeding habits mainly by scratching and feeding on insects, earthworms and ingesting the eggs from the soil. Helminth parasites prevalence data in free-range chickens in Nigeria according to Amuna *et al.* (2023) revealed *Ascaridia galli*, *Heterakis gallinarum*, *Capillaria* spp., *Gongylonema ingluvicola*, *Strongyloides avium*, *Raillietina echinobothrida*, *Trichuris trichiura*, *Choanotaenia infundibulum* and *Coccidian* spp. as prevalent helminths and *Coccidian* spp. as the dominant protozoan. *Ascaridia galli*, according to Junquera (2014) is one of the most pathogenic helminths, infecting up to 90% of flocks globally and thrives in free-range settings due to their foraging behaviors that facilitate fecal-oral transmission, unlike those under intensive confinement. These infections cause weight loss, reduced feed efficiency, intestinal damage, and mortality, exacerbating supply deficits. The preference of this system of poultry farming is attractive to both the poor and rich farmers. However, most owners of chickens kept under this system are unable to keep up with the high cost of the conventional anthelmintic drugs. It is hoped that the determination of the phytochemical contents of common local plants *Vernonia amygdalina* (bitter leaf), and *Cymbopogon citratus* (lemon grass) and their anthelmintic efficacy on helminth infected free-ranged chickens (*Gallus gallus domesticus*) in Lokoja, Nigeria, will help to address this problem and provide a cheap and effective source of anthelmintic for the local farmers.

Traditional medicines have significantly curbed neglected tropical diseases in endemic areas (Ndjonka *et al.*, 2013). In Kogi State, Nigeria, where free-range poultry is vital for livelihoods, locally sourced *Vernonia amygdalina* (bitter leaf), and *Cymbopogon citratus* (lemon grass) holds promise for solutions to helminth infections. *Vernonia amygdalina* which is

commonly called bitter leaf (English) (Alara *et al.*, 2017). is used as antimalarial, antioxidant, hepatoprotective, anthelmintic and other such health-related pharmacological activities.

(Degu, *et al.*, 2024) In Nigeria, different dialects call it by different local names such as ‘Ewuro’ in Yoruba, ‘Etidot’ in Ibibio, ‘Onuigbo’ in Igbo, ‘Ityuna’ in Tiv, ‘Oriwo’ in Edo and ‘Shuwaka’ in Hausa (Farombi and Owoeye, 2011) belongs to the Classification: Kingdom: Plantae; Division: Angiosperms; Order: Asterales; Family: Asteraceae; Genus: *Vernonia*; Species: *amygdalina*.

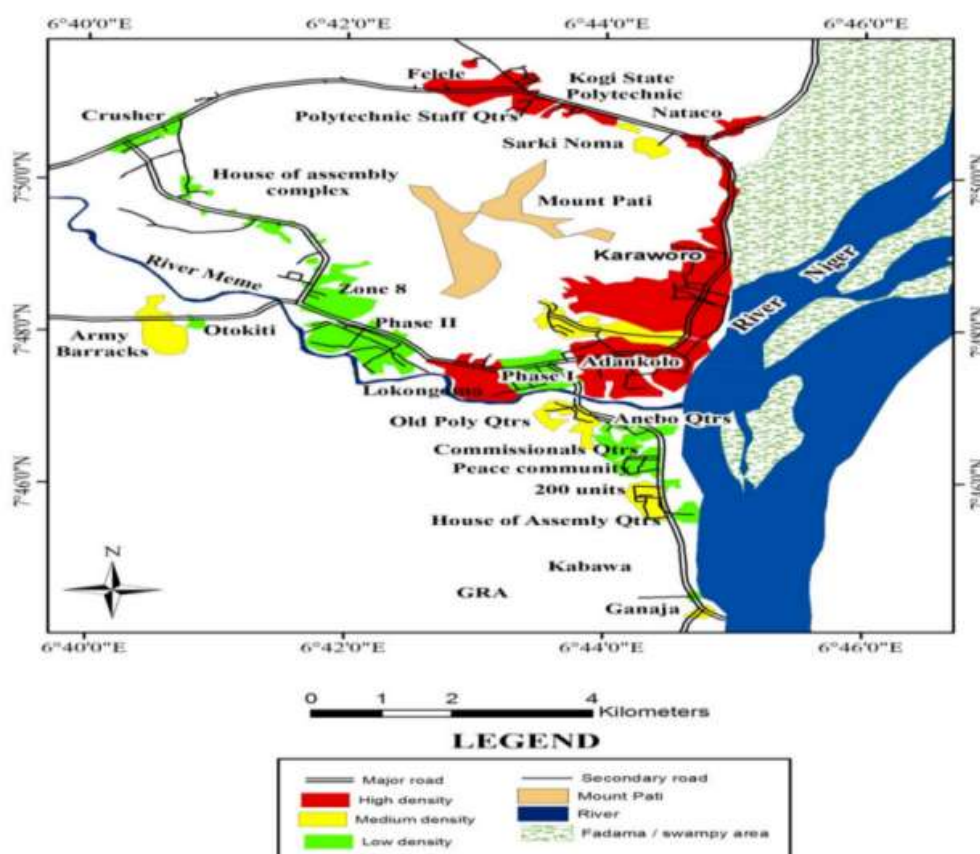
*Cymbopogon citratus* (lemon grass) is a plant in the family *Poaceae*. Its essential oil has been known to increase the productive performance of laying hens because of its anti-inflammatory, immunomodulatory, antioxidant and antimicrobial effects (Alagawany *et al.*, 2021). The use of lemongrass oil and its anthelmintic efficiency was shown in *in vivo* experiments in gerbils (Macedo *et al.*, 2015) and sheep (Macedo *et al.*, 2019).

Information of the phytochemical contents and the antihelmintic efficacy of these plants will go a long way in the pharmaceutical industry production of anthelmintic drugs and thus reducing helminthosis infection both in animals and humans and contribute immensely in the production of cheaper anthelmintic drugs from these locally sourced plants.

## **Materials and Methods**

### *Study Area*

Study area is Lokoja metropolis located on the confluence of River Niger and River Benue. It is the capital of Kogi State Nigeria. The geographical location is between latitude 7° 45' 27.56" - 7° 51' 04.34" N and longitude 6°41' 55.64" - 6°45' 36.58" E of the equator with a total land coverage of about 63.82 sq. km. (Adeoye, 2012). The eastern part of Lokoja is River Niger, while the Mount Patti is located at the western part of the city (Figure 1).



**Figure 1:** Map of Lokoja (Source: Adeoye 2012)

### Sample Size

The sample size was determined by method of Amuna *et al.* (2023) using Slovin's formula  $n = N / (1 + Ne^2)$ .

where  $n$  = sample size,  $N$  = total population = 445, and  $e$  = margin of error = 0.10.

Therefore,  $n = 445 / [1 + 445(0.10)^2] = 80$

### Study Design and Sampling

#### Sample sites

The study sites were selected from neighbourhood and conventional farms in Adankolo, Felele, Cantonment and Gadumo where chickens freely ranged and scavenged on dumpsites. Laboratory analysis was carried out in various laboratories for the HPLC at the Yobe State University and the Phytochemical analysis at Kogi State Polytechnic Chemistry laboratory. Plant identification was conducted at the Federal University Lokoja Herbarium.

### *Sample Size*

#### *Determination of Positivity of Experimental Chickens*

Eighty free-range chickens (40 males and 40 females) were obtained from farms in Lokoja. From the following locations: Cantonment, Adankolo, Felele, and Gadumo (20 birds per location which were then housed in cages and fed with guineacorn and water. They were humanely sacrificed, dissected, and their intestines removed. Each intestine was placed separately in a labeled, covered plastic bow, whichl were then transported to the laboratory. The entire length of each intestine from each labelled plastic was incised longitudinally, and the contents were emptied into sieves positioned over large, clean plastic cups. The contents were washed through the sieve, with the filtrate collected in the cups and the helminths recovered from the residue using the method of Egbunu *et al.* (2025).

Helminths collected were grouped and identified using identification key proposed by Soulsby (1982). Infection prevalence of different parasite species calculated as:

$$\text{Overall \% Prevalence} = \frac{\text{number of infected chickens}}{\text{number of chickens examined}} \times 100$$

#### *Preparation of Plant Extracts for Antihelminthic and Phytochemical Analysis*

Fresh leaves of *Cymbopogon citratus* and *Vernonia amygdalina* were collected from gardens in Lokoja and washed with clean water to remove dirt. The plants were identified using WHO guide lines for medicinal plants (WHO, 2003) and authenticated at the Herbarium of the Department of Botany, Federal University, Lokoja and voucher specimen was deposited there. The fresh leaves collected were cleaned and dried under room temperature. Cold Maceration method was used for the extraction. 75g of leaves were coarsely powdered and the powdered leaves was extracted with 150ml of 99.8% Methanol (Adedapo *et al.*, 2005) by maceration for 48 h after which it was filtered into a beaker using Whatman No. 1 filter paper immersed in a narrow-necked funnel. The solvent was evaporated by immersing the beaker in a water bath at a temperature of 100°C. The resulting gel-like extracts were filtered through an extra-fine sieve, followed by filtration through Whatman No. 1 filter paper, and stored at 4°C for further use.

#### *Determining the LD<sub>50</sub> of Methanol Extracts of Cymbopogon citratus and Vernonia amygdalina Leaves*

The median lethal dose test for the plants studied was calculating as described by Lorke (1983) Using the Formular  $LD_{50} = \sqrt{D_0 \times D_{100}}$  ( $D_0$  = Highest Dose causing No Death,  $D_{100}$  = Highest Dose causing Maximum Death)

### *Exposure of Chicken to the Methanol Extracts of Cymbopogon citratus, and Vernonia amygdalina Leaves*

Free-range chickens were divided into five groups of five birds each (Groups A–E). Groups A, B, and C received oral doses of *Cymbopogon citratus*, methanol extract (test treatment) at 10, 20, and 30 mg/mL, respectively. Group D (positive control) received 30 mg/mL albendazole, and Group E (negative control) received water only. This was also repeated for the *Vernonia amygdalina* methanol extract (test treatment).

Fecal samples were collected in three sterile sample bottles for examination from each group on Day 1 (pre-treatment) and sterile bottles were placed in a cool box, and immediately transported to the Biology Laboratory for processing. The plant extract as well as albendazole was administered orally to the chickens on Day 2, Faecal sample was collected on Day 3. Samples were collected in three well-labeled sterile bottles per group, placed in a cool box, and transported to the laboratory for analysis using the sodium chloride flotation technique. This procedure was repeated three times to obtain average fecal egg count.

### *Processing of Fecal Sample*

Sodium Chloride Flotation test was used for the detection of eggs of different parasitic worms in fecal samples. Two grams of each fecal sample in sample bottles was mixed until thoroughly homogenized with 28ml of saturated sodium chloride solution and filtered (using a tea sieve) into a beaker and carefully poured into a glass test tube on a test tube stand. Ensuring the mixture was filled to the brim (forming a meniscus) with a clear silicone dropper, and a clean coverslip was gently placed on top of the test tube, thereby avoiding spillage. The coverslip was left for about 20 min; afterward, the coverslip (having the harvested eggs) was placed on a clean glass slide and examined with the light microscope using the 10× objective lenses. Egg count per gram was calculated by multiplying by the conversion factor: Multiply the total egg count (N) by a factor (e.g., 50) to obtain the faecal eggs per gram (e.p.g.).

Number of faecal eggs counted (N) x 50= EPG (Ghafar *et al.*, 2021).

### *Percentage Faecal Egg Count Reduction of Leave Extract on Helminth Parasites*

The percentage faecal egg count reduction based on anthelmintic effect of the *Cymbopogon citratus* and *Vernonia amygdalina* extracts was determined by comparing the egg per gram (epg) before treatment of groups A, B, C, D, E and after treatment. The percentage reduction in helminth eggs in the treatment groups was calculated using the method by Kamrun *et al.* (2017).

$$\text{FECRT} = \frac{\text{EPG (pretreatment)} - \text{EPG (post-treatment)}}{\text{EPG (pretreatment)}} \times 100$$

#### *Qualitative Screening of Cymbopogon citratus and Vernonia amygdalina Leaves Extract*

The presence of alkaloids, flavonoids, tannins and saponins were qualitatively screened from the leaves. Alkaloids: Mayer's Test: 1ml of Mayer's reagent was added along the sides of test tube to a 1ml of plant sample extract, Appearance of white creamy precipitate indicated the presence of alkaloids (Banu and Catherine, 2015). Flavonoids: Alkaline reagent test: To about 1ml of extract in a test tube, few drops of sodium hydroxide solution were added and shaken. Yellow color that turned to colorless after adding dilute acid showed the existence of flavonoids (Abdullahi *et al.*, 2020). Tannins: Ferric chloride test: To 0.5 ml of extract solution, 1 ml of distilled water and 1 to 2 drops of ferric chloride solution was added. Blue or green black coloration was observed for tannin presence. (Onwuliri, 2006). Saponins: Froth formation test: 2 ml of distilled water was added to 2 ml of the *Cymbopogon citratus* and *Vernonia amygdalina* leaves Extract solution and shaken very well till frothing was observed (Nayak *et al.*, 2011). Phenols: Ferric chloride test. 5mL distilled water was added to 50mg of plant extract placed in a test tube. To this 2drops of 5% ferric chloride was added and shaken. Formation of dark green color indicated the presence of phenols (Banu and Catherine, 2015).

#### *Data Analysis*

Prevalence of gastrointestinal helminths in relation to helminth species and data analysis of curative effect of 30 mg/ml albendazole, and different concentrations of the plant extracts on helminth percentage faecal egg count of infected free roaming chickens were analyzed using Chi- square. All the results were in triplicates, and mean values and standard deviation were calculated. In Excel software. Faecal Egg count per gram was calculated by multiplying by the conversion factor: Multiply the total egg count (N) by a factor (e.g. 50) to obtain the number of eggs per gram (e.p.g). Number of eggs counted (N) x 50= EPG (Kopp *et al.*, 2008). The percentage faecal egg count reduction (FECRT) in helminth eggs in the treatment groups was calculated using the method by Kamrun *et al.* (2017).

$$\frac{\text{EPG}(\text{pretest}) - \text{EPG}(\text{posttest})}{\text{EPG}(\text{pretest})} \times 100$$

(Stephen *et al.*, 2021; Suleiman *et al.*, 2005).

## Results

Table 1 shows prevalence of helminths in free ranged chickens bred in Lokoja Kogi State. Six Nematode species *Capillaria* spp, *Ascaridia galli*, *Heterakis gallinarum*, *Strongyloides avium*, *Trichuris trichiura* and *Gongylonema ingluvicola*. And two cestode species were *Hymenolepis* spp and *Rallietina echinobothrida* were isolated and identified which was an indication of multihelminthic infection.

This study revealed that the total helminth prevalence in Lokoja was 90%. The highest species prevalence being cestodes 25%, *Hymenolepis* spp and 23.75% *Rallietina echinobothrida*. Nematode prevalence recorded 11.25% *Capillaria* spp, 5% *Ascaridia galli*, 3.75% *Heterakis gallinarum*, 5% *Strongyloides avium*, the least being *Trichuris trichiura* 3.75% and *Gongylonema ingluvicola* 1.25%.

**Table 1: Prevalence of Soil Transmitted Helminth in Free Ranged Chickens in Lokoja Kogi State**

| Study Sites/<br>Chickens examined | Felele<br>20 |                      | Cantonement<br>20 |                      | Ganaja<br>20 |                      | Adankolo<br>20 |                      |                     |
|-----------------------------------|--------------|----------------------|-------------------|----------------------|--------------|----------------------|----------------|----------------------|---------------------|
| STH Chicken Count                 | Egg<br>count | Positive<br>chickens | Egg<br>count      | Positive<br>chickens | Egg<br>count | Positive<br>chickens | Egg<br>count   | Positive<br>chickens | % Prevalence        |
| <b>Nematodes</b>                  |              |                      |                   |                      |              |                      |                |                      |                     |
| <i>Ascaridia galli</i>            | 0            | 0                    | 8                 | 2                    | 0            | 0                    | 9              | 2                    | 5(4/80)             |
| <i>Capillaria</i> spp             | 1            | 1                    | 13                | 2                    | 10           | 3                    | 5              | 3                    | 11.25(9/80)         |
| <i>Gongylonema ingluvicola</i>    | 0            | 0                    | 10                | 1                    | 0            | 0                    | 0              | 0                    | 1.25(1/80)          |
| <i>Heterakis gallinarum</i>       | 17           | 2                    | 4                 | 1                    | 0            | 0                    | 0              | 0                    | 3.75(3/80)          |
| <i>Strongyloides avium</i>        | 2            | 1                    | 0                 | 0                    | 8            | 1                    | 17             | 2                    | 5(4/80)             |
| <i>Trichuris trichiura</i>        | 19           | 1                    | 8                 | 1                    | 13           | 1                    | 0              | 0                    | 3.75(3/80)          |
| <b>Cestodes</b>                   |              |                      |                   |                      |              |                      |                |                      |                     |
| <i>Hymenolepis carioca</i>        | 38           | 7                    | 0                 | 0                    | 8            | 3                    | 70             | 10                   | 25(20/80)           |
| <i>Rallietina echinobothrida</i>  | 7            | 2                    | 43                | 5                    | 0            | 0                    | 51             | 12                   | 23.75(19/80)        |
| <b>Total Chickens Infected</b>    | <b>14</b>    |                      | <b>12</b>         |                      | <b>8</b>     |                      | <b>29</b>      |                      | <b>78.75(63/80)</b> |

Table 2 shows the result of qualitative phytochemical analysis of methanol extracts of leaves of *Cymbopogon citratus*, and *Vernonia amygdalina* which reveals high phenols, alkaloids, saponins and tannins but very low colouration change observed with flavonoid content in *Vernonia amygdalina* and no colouration change observable with flavonoid in *Cymbopogon citratus*.

**Table 2: Qualitative Phytochemical Analysis of Methanol Extracts of Leaves of *Cymbopogon citratus*, and *Vernonia amygdalina***

| Phytochemical | Test            | Observation      | <i>Vernonia amygdalina</i> | <i>Cymbopogon citratus</i> |
|---------------|-----------------|------------------|----------------------------|----------------------------|
| Alkaloids     | Mayer's         | White creamy     | +++                        | +++                        |
| Phenols       | Ferric Chloride | Dark Green       | +++                        | +++                        |
| Flavonoids    | Alkaline        | Colourless       | ++                         | ---                        |
| Tannins       | Ferric Chloride | Blue/green black | +++                        | +++                        |
| Saponnins     | Froth formation | Frothing         | +++                        | +++                        |

Key: +++....High ++...Average +: Low---: Nil

Table 3 shows the qualitative analysis of the methanol extracts of *Vernonia amygdalina* and *Cymbopogon citratus* which revealed that *Vernonia amygdalina* had significant ( $P < 0.05$ ) phytochemical compositions. *Vernonia amygdalina* had Flavonoids contents of  $5412.60 \pm 83.15$  mg/100g which is significantly higher than contents recorded for *Cymbopogon citratus*. The least Flavonoid content was recorded for *Cymbopogon citratus* ( $1021.30 \pm 66.07$  mg/100 g) Similarly, the highest significant Alkaloids content was recorded for *Vernonia amygdalina* ( $1655.10 \pm 16.61$  mg/100 g) while the value of  $417.33 \pm 2.75$  mg/100 g recorded for *Cymbopogon citratus*. For Saponins, *Vernonia amygdalina* had the highest contents of  $2955.57 \pm 20.94$  mg/100 g which is significantly higher than the values recorded for *Cymbopogon citratus* ( $761.77 \pm 24.75$  mg/100 g). The  $2949.63 \pm 313.98$  mg/100 g recorded for the Tannin content of *Vernonia amygdalina* was significantly higher than the values recorded for *Cymbopogon citratus* ( $546.27 \pm 19.13$  mg/100 g) and the Phenols contents followed similar trend with *Vernonia amygdalina* having the highest value of  $2350.27 \pm 23.82$  mg/100 g which was significantly higher than the values recorded for *Cymbopogon citratus* ( $852.23 \pm 17.67$  mg/100 g) (Table 4).

**Table 3: Quantitative Phytochemical Composition of Methanol Extract of *Vernonia amygdalina* and *Cymbopogon citratus***

| Phytochemical Component    | Flavonoids (mg/100 g)        | Alkaloids (mg/100 g)       | Saponins (mg/100 g)         | Tannins (mg/100 g)            | Phenols (mg/100 g)          |
|----------------------------|------------------------------|----------------------------|-----------------------------|-------------------------------|-----------------------------|
| <i>Vernonia amygdalina</i> | 5412.60 ± 83.15 <sup>a</sup> | 1655.10± 6.61 <sup>a</sup> | 2955.57± 20.94 <sup>a</sup> | 2949.63 ± 313.98 <sup>a</sup> | 2350.27± 23.82 <sup>a</sup> |
| <i>Cymbopogon citratus</i> | 1021.30± 66.07 <sup>b</sup>  | 417.33± 2.75 <sup>b</sup>  | 761.77± 24.75 <sup>b</sup>  | 546.27± 19.13 <sup>b</sup>    | 852.23± 17.67 <sup>b</sup>  |
| P-value                    | 0.000                        | 0.000                      | 0.000                       | 0.000                         | 0.000                       |

● Means with the same alphabets in the same column are not statistically different at P<0.05

Table 4 shows the calculated LD<sub>50</sub> recorded for *Vernonia amygdalina* (bitter leaf) and *Cymbopogon citratus* (lemongrass), at the various methanol extract concentrations of the plant leaves was  $\sqrt{D_0 \times D_{100}} = \sqrt{20 \times 30} = 24.49 \text{ mg/ml}$  with maximum survival dose of 20 mg/ml and highest mortality dose of 30 mg/ml.

**Table 4: Determination of LD<sub>50</sub> of Methanol Extracts of The Leaves of *Cymbopogon citratus*, and *Vernonia amygdalina***

| Dose (mg/ml)                                     | % Mortality <i>Vernonia amygdalina</i> | % Mortality <i>Cymbopogon citratus</i> |
|--------------------------------------------------|----------------------------------------|----------------------------------------|
| 10mg/ml                                          | 0%                                     | 0%                                     |
| 20mg/ml                                          | 0%                                     | 0%                                     |
| 30mg/ml                                          | 0%                                     | 100%                                   |
| LD <sub>50</sub> ( $\sqrt{D_0 \times D_{100}}$ ) | Zero mortality                         | 25mg/ml                                |

Table 5 shows that the treatment of free ranged chicken *Gallus gallus domesticus* with 10 mg/ml, 20 mg/ml, 30 mg/ml and 30 mg/ml of Methanol Extracts of The Leaves of *Cymbopogon citratus*, *Vernonia amygdalina* and 30 mg/ml Albendazole. The result indicates highest % egg reduction of 98.71% with 20 mg/ml *Cymbopogon citratus*, and 95.24% with *Vernonia amygdalina*. which is These results for *Cymbopogon citratus* and *Vernonia amygdalina* are higher than percentage reduction of 68.94% recorded with the 30mg/ml of the conventional antihelminthic drug Albendazole. The lowest % reduction for *Cymbopogon citratus* was 2.16% with 30 mg/ml while lowest % reduction recorded was 67.99% for *Vernonia amygdalina*. This result recorded for 30 mg/ml *Vernonia amygdalina* was still higher than the % reduction recorded for 30 mg/ml with the conventional antihelminthic drug Albendazole.

**Table 5a: Efficacy of Different Treatments of Methanol Extracts of The Leaves of *Cymbopogon citratus*, *Vernonia amygdalina* and Albendazole in Reducing Helminth Fecal Egg Count in Infected Free Ranged Chickens**

| Extract                           | <i>Cymbopogon citratus</i> |             |               |             |             |             | <i>Vernonia amygdalina</i> |             |             |            |             |             | Albendazole  |             |
|-----------------------------------|----------------------------|-------------|---------------|-------------|-------------|-------------|----------------------------|-------------|-------------|------------|-------------|-------------|--------------|-------------|
|                                   | 10 mg / ml                 | 20 mg / ml  | 30 mg / ml    | 10 mg / ml  | 20 mg / ml  | 30 mg / ml  | 10 mg / ml                 | 20 mg / ml  | 30 mg / ml  | 10 mg / ml | 20 mg / ml  | 30 mg / ml  | 30 mg / ml   | 30 mg / ml  |
| Test                              | Pre-test                   | Post-test   | Pre-test      | Post-Test   | Pre-Test    | Post-Test   | Pre-test                   | Post-Test   | Pre-test    | Post-Test  | Pre-test    | Post-Test   | Pre-test     | Post-test   |
| <i>Hymenolepis carioca</i>        | 2940                       | 120         | 95800         | 885         | 950         | 0           | 58                         | 850         | 250         | 150        | 5800        | 850         | 1850         | 550         |
| <i>Strongyloides avium</i>        | 140                        | 0           | 450           | 250         | 50          | 50          | 1000                       | 50          | 0           | 0          | 1000        | 50          | 1000         | 450         |
| <i>Capillaruid spp</i>            | 100                        | 0           | 50            | 0           | 0           | 0           | 0                          | 0           | 150         | 0          | 50          | 0           | 350          | 50          |
| <i>Syngamus sp</i>                | 0                          | 0           | 0             | 0           | 50          | 0           | 0                          | 0           | 0           | 0          | 0           | 0           | 0            | 0           |
| <i>Raillietina echinobothrida</i> | 4530                       | 1610        | 13300         | 135         | 2300        | 345         | 1550                       | 800         | 1550        | 0          | 1550        | 800         | 6050         | 2500        |
| <i>Ascaridia galli</i>            | 280                        | 0           | 400           | 150         | 100         | 0           | 350                        | 150         | 150         | 0          | 50          | 0           | 100          | 0           |
| <i>Heterakis gallinarum</i>       | 0                          | 0           | 0             | 0           | 200         | 50          | 0                          | 0           | 0           | 0          | 350         | 150         | 2400         | 300         |
| <b>Total</b>                      | <b>7990</b>                | <b>1730</b> | <b>109950</b> | <b>1420</b> | <b>3650</b> | <b>3550</b> | <b>8600</b>                | <b>1850</b> | <b>2100</b> | <b>150</b> | <b>8800</b> | <b>1850</b> | <b>11750</b> | <b>3650</b> |
| Total %                           | 78.35%                     |             | 998.71%       |             | 2.16%       |             | 67.99%                     |             | 95.24%      |            | 78.98%      |             | 68.94%       |             |
| Curative                          |                            |             |               |             |             |             |                            |             |             |            |             |             |              |             |

p-value= 0.05

**Table 5b: Efficacy of Different Treatments of *Vernonia amygdalina* Methanol Extract in Reducing Helminth Fecal Egg Count in Infected Free Ranged Chickens**

| STH                   | 10mg/ml  |           | 20mg/ml  |           | 30mg/ml  |           | Albendazole 30mg/ml |           |
|-----------------------|----------|-----------|----------|-----------|----------|-----------|---------------------|-----------|
|                       | Pre-test | Post-test | Pre-test | Post test | Pre-test | Post test | Pre-test            | Post Test |
| <i>Hymenolepis</i>    | 5800     | 850       | 250      | 150       | 5800     | 850       | 1850                | 550       |
| <i>Strongyloides</i>  | 1000     | 50        | 0        | 0         | 1000     | 50        | 1000                | 450       |
| <i>Capillaruid</i>    | 0        | 0         | 150      | 0         | 50       | 0         | 350                 | 50        |
| <i>Syngamus sp</i>    | 0        | 0         | 0        | 0         | 0        | 0         | 0                   | 0         |
| <i>Raillietina sp</i> | 1550     | 800       | 1550     | 0         | 1550     | 800       | 6050                | 2300      |
| <i>Heterikea sp</i>   | 350      | 150       | 150      | 0         | 50       | 0         | 100                 | 0         |
| <i>Ascaridia</i>      | 0        | 0         | NIL      | NIL       | 350      | 150       | 2400                | 300       |
| Total                 | 8600     | 1850      | 2100     | 150       | 8800     | 1850      | 11750               | 3650      |
| % Reduction           | 78.49%   |           | 92.86%   |           | 85.35%   |           | 68.94%              |           |

## Discussion

This study (Egbunu, *et al.*, 2025) showed the highest Prevalence of infection of 25%(20/80) in *Hymenolepis carioca*, 23%(19/80) in *Raillietina echinobothrida*, 11.25%(14/80) in *Capillaria spp*; 3.75%(7/80) in *Heterakis gallinarum*; 5%(4/80) in *Ascaridia galli*; 5%(4/80) in *Strongyloides avium*; the least being 3.75%(3/80) *Trichuris trichiura* and 1.25%(1/80) in *Gongylonema ingluvicola* this differed from the finding of Faezeh *et al.* (2024) in Iran where *Raillietina tetragona* was the most prevalent cestode. This difference in prevalence and

intensity of helminth infections may be influenced by factors, such as climatic conditions (temperature and humidity) which may alter the population dynamics of the parasites, resulting in dramatic changes in the prevalence and intensity of helminthic infections (Naphade, 2013). Multi-infection recorded in this study (Egbunu, *et al.*, 2025) confirms other studies (Dauda *et al* 2016; Shifaw *et al* 2021; Abdullahi *et al* 2020). The high prevalence of cestodes such as *Hymenolepis carioca* and *Rallietina echinobothrida* in this study has been attributed to the fact that cestode require intermediate hosts such as houseflies which free ranged chickens easily engulge in their feeding habitat (Katoch *et al.*, 2012)

Qualitative analysis of both Methanol Extracts of the leaves Of *Cymbopogon citratus*, and *Vernonia amygdalina* revealed presence of phenols, flavonoids, alkaloids, saponins and tannins but no noticable flavonoids colouration change in *Cymbopogum citratus*. However quantitative analysis by HPLC revealed 1021.30 mg/100g flavonoids, 852.23 mg/100g phenols, 417.33 mg/100g alkaloids, 761.77 mg/100g saponins and 546.27 mg/100g tannins in *Cymbopogum citratus*. 2350.27 mg/100g phenols, 5412.60 mg/100g flavonoids, 1655.10 mg/100g alkaloids, 2955.57 mg/100g saponins and 2949.63 mg/100g tannins in *Vernonia amagdalina*. Alkaloids, phenols, saponins and tannins were found to be very present in *Vernonia amygdalina* which confirms the review of Egharevba *et al* (2014). The indication of low alkaloid content is however complimented by the very high flavonoids, saponins and tannins content to enhance the antihelminthic efficacy of *Vernonia amygdalina* leaf methanol extract, since Steroidal saponin alters the membrane permeability and inhibits mitochondrial activity resulting in worm death. Both flavonoids and tannins act synergistically and have the same function as praziquantel. Isoflavones inhibit energy generation and calcium utilization causing the death of worms. Isoquinoline alkaloids are neurotoxic to the different helminths. Phytochemicals have an important role in improving growth, enhancing immunity, increasing nutrient absorption, and maintaining gut integrity (Shahid *et al.*, 2023). The high bitter taste of *V. amagdalina* (Bitter leaf) has been inferred to be due to its significant saponins content which accounts for its high anthelmintic effect recorded in this study. Tannin content of *Vernonia amygdalina* was significantly higher than the values recorded for *Cymbopogon citratus*. The phenols contents followed similar trend. Aiswarya *et al.* (2011), reported that flavonoids, saponins, glycosides, tannins and phenolics was an indication of their anthelmintic action as recorded in this study. The qualitative analysis showed the presence of alkaloids, tannin, flavonoid and saponin as dominant phytochemical compounds across the four plant species.

Abdullahi and Mainul (2020), reported these bioactive compounds in other aromatic plant, *Ocimum* species similar to *Cymbopogon citratus* against adult *Haemonchus* sp. The anthelmintic effect of tannins is reported to be the binding to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite (Suleiman, 2017).

The effect of 10mg/ml, 20mg/ml and 30mg/ml dosage of Albendazole, and *Vernonia amygdalina* methanol leaves extract on Percentage Faecal Egg Count Reduction of Soil Transmitted Helminths of infected free-range chickens shows (% curative) of 30mg/ml Albendazole 68.94%. whereas a higher egg count reduction with 30mg/ml dosage of *Vernonia amygdalina* leaf methanol extract was 78.98%. However, with 20mg/ml dosage of *Vernonia amygdalina* 95.24% was recorded while a higher Faecal Egg Count Reduction of 98.71% was recorded for 20 mg/ml *Cymbopogon citratus*. This was an indicator of highest %curative on administration of 20mg/ml dosage of *Vernonia amygdalina* and *Cymbopogon citratus*. On individual parasites the curative percentage showed that *Raillietina* sp, *Ascaridia galli*, *Syngamus* sp. and *Capillaria* sp recorded 100% curative. A lower %curative of 40% was recorded for *Hymenolepsis* sp with *Vernonia amygdalina* but lowest 44% reduction with *Cymbopogon citratus*. With 30mg/ml Albendazole *Heterikis* sp recorded 100% curative. The result indicates highest % egg reduction of 98.71% with 20 mg/ml *Cymbopogon citratus*, and 95.24% with *Vernonia amygdalina*. These results for *Cymbopogon citratus* and *Vernonia amygdalina* are higher than percentage reduction of 68.94% recorded with the conventional antihelminthic drug Albendazole. The lowest % reduction for *Cymbopogon citratus* was 2.16% with 30 mg/ml while lowest percentage reduction recorded was 69.99% with 10mg/ml *Vernonia amygdalina*. This result recorded for 30 mg/ml *Vernonia amygdalina* was still higher than the percentage reduction recorded for 30 mg/ml with the conventional antihelminthic drug Albendazole. According to Asante-Kwatia *et al.* (2021), the *Vernonia amygdalina*, extract and oils of *Cymbopogon citratus* have highest content of flavonoids and phenol which aid antioxidant activities and this might have been responsible for its highest egg reduction count in this study. The result of higher efficacy of methanol extracts of leaves of plants in this study defers from the work by Gasaliyu *et al.* (2022), who reported that deparasitization caused at 200 mg/kg dose of Albendazole was (93.8%), while at 200 mg/kg, 400 mg/kg and 800 mg/kg, of *Vernonia amygdalina* deparasitization was 77.5%, 87.5% and 91.86% respectively. These plants-derived bioactive compounds, may offer potent natural alternatives to synthetic anthelmintic by effectively reducing helminth burdens in free-range poultry and thus offer a sustainable, plant-based alternative for poultry parasite management.

### Author's Contribution

Egbunu, A. A.: Designed the concept, laboratory analysis, data collection, funding and writing of the original manuscript. Audu, P.A: Reviewed and read final copy of the manuscript. Adang, K.L.: Supervised the methodology, validation, review and editing of the manuscript, critical review and the project administration. Alege, G.O.: Supervised and validated the biochemical laboratory analysis and guided the data analysis. Isah, A.O.: Supervised the Identification of the plant of choice for this project

### Conflict of Interest

The authors declare no conflict of interest

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