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COMPARATIVE ASSESSMENT AND ANTIMICROBIAL ACTIVITY OF *Moringa oleifera*, *Cola nitida* AND *Chromolaena odorata* LEAVES AGAINST ENTERIC ORGANISMS FROM POULTRY DROPPINGS

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Abstract

The outbreak of several deadly diseases that cause economic loss and discourage poultry keeping is a major challenge to poultry farming. The rising antimicrobial resistance has led to a renewed interest in medicinal plants as sources of novel antimicrobial agents. The main goal of this research was to isolate and identify different enteric bacteria and to evaluate through a comparative study, the antimicrobial activity of *Moringa oleifera* seeds, *Moringa oleifera* leaves, *Cola nitida* and *Chromolaena odorata* leaves, against the enteric organisms isolated from chicken droppings obtained from different poultry farms in Awka Metropolis. The research study was carried out, using Chicken dropping samples collected from different poultry houses using sterile swab sticks. These poultry droppings were analyzed using standard microbiological protocols. The enteric bacteria in the samples were isolated, identified and characterized biochemically using commercial reagents for biochemical tests, following the standard manufacturer's protocol. The presumptive enteric organisms isolated were. *Escherichia coli, Klebsiella* sp, *Salmonella* sp, *Shigella* sp, *Enterobacter* sp and *Proteus* sp. These organisms showed the highest percentage occurrence with 23% as *Escherichia coli*, while *Shigella* sp had the lowest occurrence of 9%. The ethanol and aqueous extracts were assessed for antimicrobial activity using agar well diffusion method. All extracts exhibited variable antibacterial activity against the test organisms depending on extract type and bacterial isolate. However, the highest spectrum of activity was indicated by *Chromolaena odorata* leaves, with a high antimicrobial activity against *Shigella* sp and *Escherichla coli* (21mm & l5mm) respectively. The results obtained from this research shows the effectiveness of the use of these plants as very efficient sources of antimicrobials, hence its potential in the reduction of infections from microbial pathogens and disease prevention in poultry.

Key words: Antimicrobial activity, *Moringa oleifera*, *Chromoalena odorata*, *Cola nitida*, Poultry droppings, Enteric Organisms

Introduction

The search for natural compounds with antimicrobial properties has become increasingly important in addressing global infectious disease challenges and promoting sustainable agriculture. Agricultural products from poultry provide valuable nutrients such as proteins and minerals (Abd El-Hack *et al*., 2022), but the poultry industry faces major threats from environmental factors like heat stress and infections caused by viruses, bacteria, parasites, and fungi, leading to substantial economic losses (El-Shall *et al*., 2022; Salem *et al*., 2022). Enteric organisms, which inhabit the gastrointestinal tract, can pose risks to both animal and human health if not properly managed. Chicken droppings contain a complex mix of microorganisms, including bacteria harmful to both animals and humans. Enteric bacteria such as *Escherichia coli* and *Salmonella* spp. are common in the intestines of chickens and are excreted in their faeces, contributing to foodborne diseases (Yunying *et al*., 2025). This highlights the role of chicken waste as a reservoir for pathogens that can contaminate the environment and spread through food and water, posing zoonotic risks.

The rise of antimicrobial resistance has intensified the need for effective and environmentally friendly alternatives. Natural products, especially plant extracts, have long contributed to the discovery of antimicrobial agents. Their varied chemical compositions and mechanisms of action make them promising candidates against multidrug-resistant pathogens. The global movement towards using natural alternatives to antibiotics in poultry aims to enhance production and reduce antibiotic residues in food (Rehman *et al*., 2020).

Plant-derived natural products have drawn attention for their potential therapeutic uses. *Cola nitida* (kolanut), *Chromolaena odorata* (Awolowo leaf), and *Moringa oleifera* are plants with traditional and medicinal significance (Olorungbohunmi, 2022). *Moringa oleifera* contains bioactive compounds such as alkaloids, flavonoids, saponins, tannins, and triterpenoids, which have been reported to support growth, act as anti-helmintic, and exhibit antibacterial activity (Fouad *et al*., 2019). *Cola nitida* has also been recognised for its antimicrobial potential, linked to its diverse chemical constituents. Likewise, *Chromolaena odorata*, widely used in African traditional medicine, is known for treating gastrointestinal infections, promoting wound healing, and managing inflammation and diabetes (Owoyele *et al*., 2015)

This study focuses on investigating the antimicrobial activity of extracts from these leaves, against enteric organisms isolated from chicken droppings.

Materials and Methods

Study Area

This research was conducted in Awka, Anambra State. Anambra State is in the South-East Geopolitical Zone of Nigeria and

the research was conducted at the General Microbiology Laboratory of Biosciences at Nnamdi Azikwe University, Awka South Local Government Area of Anambra State. It is located in the south eastern part of Nigeria, latitude 6° 14°34"*N / 6.242889°N and longitude 7°07°06"E / 7.118289°E with a land mass of approximately 4.99 km

(equivalent to 499 Hectares) containing about 50,000 students both undergraduates and post-graduates.

Experimental Design

The experimental research was carried out at the Microbiology Laboratory at, Nnamdi Azikiwe University, Awka. The experimental design employed in this research was the completely randomized design, with the independent variables as: Type of plant extract, concentration of the extracts and the different enteric organisms. The dependent variables however were the antimicrobial activity of the plant extracts and the minimum inhibitory concentration. The controlled variables were the growth media, the incubation conditions, method of antimicrobial testing and the volume of extract used. The analysis was done in triplicates. The positive control used for the antimicrobial activity was the antibiotics discs, while the negative control was normal saline. The standard antibiotics used were beta-lactam antibiotics such as: Azithromycin:15µg, Ofloxacin:5 µg, Pefloxacin: 10µg, Gentamycin:10 µg, Augumentin:5 µg, Ampicillin:5 µg, Ciprofloxacin:5 µg, Streptomycin:30 µg, Cefuroxime:30 µg, Levofloxacin:5 µg.

Collection and Preparation of Plant Materials

Kolanut leaves, *Chromoalena odorata* (Awolowo leaves), *Moringa* seeds and *Moringa* leaves were purchased from Eke Awka Marker, Ifite Market and Eke Nibo market; and different farms in Nibo and Ifite Awka. The samples were wrapped in different sterile poly-ethene bags and labeled appropriately. Taxonomic identification and authentication of the samples were confirmed by an experienced Taxonomist in the department of Botany, Nnamdi Azikiwe University, Awka. The plants were identified as: *Cola nitida* leaves (NAUH-64A), *Chromoalena odorata* (NAUH-73D), *Moringa* seeds and *Moringa* leaves (NAUH-0197). The fresh leaves and seeds selected for antimicrobial analysis studies were washed thoroughly, oven dried at 30-35˚C for several days, while the seed was separated from the husk and grinded coarsely using an electric blender.

Extraction of Plant Metabolite: Maceration method using Ethanol as solvent

The extraction of the plant material followed the method as performed and documented by Owusu *et al*. (2021). Briefly, two hundred grams (200g) of pulverized dried leaves (coarse powder) of the plants and powdered Moringa seeds, were weighed out using an analytical weighing scale. Three and half liters of 70% v/v ethanol was added to it and left to macerate for 4 days at room temperature (28–32 ^{o}C), with occasional stirring. After the 4 days, the 70% ethanol was decanted and filtered through a Whatman No. 1 filter paper. The extracts were then concentrated in an oven with relatively lower heat at 38^{o}C for 24 hours and stored in a refrigerator at 4^{o}C.

Extraction of Plant Metabolite: Maceration method using Water as solvent

Two hundred grams each of the dried leaves of the plants (coarse powder) and powdered *Moringa* seeds were brought to boil in 3.5 L of distilled water on a hot plate, for one hour, thirty minutes and then strained. The aqueous extracts were filtered using Whatman No. 1 filter paper, and subsequently cooled for 45 min. After cooling, the extracts were concentrated using the drying oven with relatively low heat at 38^{o}C for 24 hours and stored in a refrigerator at 4^{o}C. (Abubakar and Haque, 2020)

Collection of Chicken Droppings

Fresh poultry droppings showing physical symptoms of poultry diarrhea were collected from different poultry farms in Awka metropolis using sterile swab sticks and capped. The samples were labeled to avoid errors and were taken to the Microbiology Laboratory for laboratory analysis. Strict hygiene measures were employed throughout the collection process to maintain the integrity of the samples.

Isolation and Characterization of the bacterial Isolates

The poultry droppings diagnostic of diarrhea were subjected to serial dilution, after which inoculation was done using the pour plate technique on Nutrient agar, Eosin Methylene Blue Agar, Simmons citrate agar. These media were all prepared following the manufacturer's instructions. Salmonella-Shigella medium was prepared by boiling with frequent agitation and allowed to cool to 50ºC before dispensing into sterile petri-dishes. The other media were sterilized by autoclaving at 121ºC for 15 minutes. After cooling, the media was dispensed into sterile petri-dishes and allowed to solidify, after which inoculation was done using a pour-plate technique. Incubation was done at 37°C for 24 hours. All other chemicals and reagents used were of analytical grade. Morphological and Biochemical tests were used to identify and characterize the isolates by employing standard procedures (Babu *et al*., 2022; Ezebialu *et al*., 2025).

Antibiotic Susceptibility Testing of the Bacterial Isolates

Identified bacterial colonies were subjected to antibiotic susceptibility testing using various antibiotics. The test organism was emulsified in peptone water until the turbidity was comparable with 0.5% McFarland's standard. A loopful of the suspension was transferred onto a Mueller-Hinton agar plate, and then a sterile cotton swab was used to streak the entire surface of the plate. Sterile forceps were used to apply the antibiotic discs to the surface of the agar plate and incubated at 37ºC for 18 to 24 hours (Thakur *et al*., 2024). Inhibition zone diameters around the antibiotic discs were measured and classified as sensitive or resistant based on the Clinical and Laboratory Standards Institute (CLSI) break point system (CLSI, 2021). Isolates that were resistant to 3 or more antibiotics were labeled as multidrug resistant (MDR) and selected for testing with the plant extracts.

Antimicrobial Activity of Plant Extracts

The already prepared agar plates were inoculated with 0.1mL of standardized microbial suspension using a sterile swab and a micro pipette. Wells of 6mm diameter were punched in the agar using a sterile cork borer. An aliquot of 0.1mL of the plant extract were Innoculated into the wells. Standard antibiotics were used as positive control. The plates were incubated at 37°C for 24 hours. (Romha *et al*.,2018).

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of the crude extract was performed by the macro dilution method described by Odonye *et al*. (2021) with slight modifications. First the extracts were diluted in sterile distilled water to a highest concentration of 1000mg/ml and then five dilutions were performed to obtain concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 36.25mg/ml in a capped tube. To 1ml of the above concentrations, was added 1ml of bacterial inoculum to obtain 2ml as final volume. The first tube containing 1000 mg/ml of the extract, neither broth nor inoculum was added and this served as positive control. A separate tube containing broth and inoculum alone served as negative control. The tubes were then incubated at

37 ℃ for 18 hours and growth was indicated by turbidity. The Minimum inhibitory concentration is the lowest concentration of the extract that didn't produce visible turbidity.

Data Analysis

Mean zone diameters, MIC values were calculated from triplicate observations. The significant differences in antimicrobial efficacy based on extract type and pathogen was examined using a one way ANOVA. The quantitative data were analyzed statistically using SPSS (version 20) statistical software with a significant level of 5%.

Results

Biochemical Characterization for identification of Enteric Isolates

The identification and biochemical characterization of the enteric isolates from the poultry droppings are presented in Table 1. All isolates were negative for gram reaction, and positive for catalase and citrate with different reactions to other identification tests. The results of the characterization gave rise to the presumptive identification of: *Eschericiha coli*, *Klebsiella* sp, *Proteus* sp, *Enterobacter* sp, *Shigella* sp and *Salmonella* sp.

Table 1: Biochemical Characterization of Microorganisms

Test	*Escherichia coli*	*Klebsiella sp*	*Proteus* species	*Enterobacter* species	*Shigella* species	*Salmonella* species
Gram reaction	- Rods	-rods	-Rods	-Rods	-Rods	-Rods
Catalase	+	+	+	+	+	+
Motility	-	-	-	-	-	-
Indole	+	-	-	-	+	+
Citrate	+	+	+	+	+	+
Methyl Red	+	+	+	+	+	+
Voges Proskauer	-	-	+	+	-	-
Oxidase	-	-	-	-	-	-
Urease	+	+	+	+	+	+
Glucose	A+G	A+G	A+G	A+G	A+G	A+G
Fructose	A+G	A+G	A+G	A+G	A+G	A+G
Lactose	A+G	A+G	A+G	A+G	A+G	A+G
Sucrose	A+G	A+G	A+G	A+G	A+G	A+G
Mannitol	A+G	A+G	A+G	A+G	A+G	A+G

Key : + positive -negative

A+G : Acid and Gas production

Percentage occurrence of isolated Enteric Organsisms.

The total percentage occurrence of the bacterial isolates obtained from diarrheal poultry droppings is presented in Fig 1. *Escherichia coli* was observed to have the highest occurrence among the enteric organisms. *Klebsiella* sp and *Proteus* sp also had a high percentage occurrence, while *Shigella* sp had the lowest occurrence among the enteric organisms.

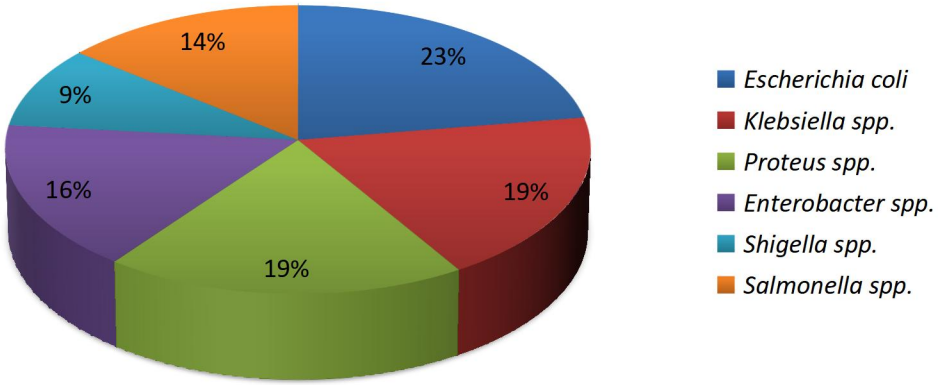


Fig 1: Percentage Occurrence of Bacterial Isolates of Poultry diarrheal droppings

Antibiotic Susceptibility Test Against Isolates

Table 2 presents the results of the antibiotic sensitivity profile of different antibiotics against the isolated enteric organisms. Augmentin and Streptomycin had the high resistance against the isolates. Other antibiotics however, showed different levels of sensitivity to the enteric organisms.

Table 2: Antibiotic Susceptibility on Bacterial Isolates (Zone of Inhibition).

Antibiotics	***Escherichia coli***	***Klebsiella* species**	***Proteus* species**	***Enterobacter* species**	***Shigella* species**	***Salmonella* species**
LEV	29mm	18mm	25mm	21mm	15mm	17mm
CEF	-	20mm	5mm	15mm	11mm	
SP	-	10mm	-	-	-	-
CPX	29mm	35mm	10mm	15mm	22mm	-
AM	-	5mm	9mm	-	11mm	-
AU	-	-	-	-	-	-
CN	18mm	20mm	3mm	10mm	-	6mm
PEF	27mm	27mm	21mm	9mm	15mm	19mm
OFX	18mm	18mm	20mm	18mm	23mm	12mm
AZ	8mm	17mm	7mm	13mm	11mm	5mm

Key:
(-) = No zone of inhibition
AZ = Azithromycin:15µg, OFX =Ofloxacin:5 µg, PEF = Pefloxacin: 10µg, CN = Gentamycin:10 µg, AU = Augumentin:5 µg, AM = Ampicillin:5 µg, CPX = Ciprofloxacin:5 µg, SP = Streptomycin:30 µg, CEF = Cefuroxime:30 µg, LEV = Levofloxacin:5 µg

Antimicrobial Activity of *Cola nitida*

The antimicrobial activity of *Cola nitida* against the enteric organisms isolates is shown in Table 3. The ethanol extracts were all positive for antimicrobial activity with the highest activity observed against *Shigella* sp and the lowest activity observed in *Proteus* sp, while the *Cola nitida* leaf aqueous extracts had no antimicrobial activity against any of the test isolates.

Table 3: Antimicrobial Activity of *Cola nitida* extracts against Enteric Bacteria Isolates

Organisms	**Ethanol kolanut leaf Extract**	**Aqueous kolanut leaf Extract**
Escherichia coli	13mm	-
***Klebsiella* sp.**	11.5mm	-
***Proteus* sp.**	11mm	-
***Enterobacter* sp.**	16mm	-
***Shigella* sp.**	21mm	-
***Salmonella* sp.**	12mm	-

Antimicrobial Activity of *Moringa oleifera* Extracts

Table 4 reports the antimicrobial activity of the *Moringa oleifera* extracts against the isolated enteric organisms. The highest antimicrobial activity was observed in *Moringa* leaf exytracts, while the *Moringa* seed extracts had lower antimicrobial activity. The *Moringa* extracts also showed better antimicrobial activity than the aqueous extracts.

Table 4: Antimicrobial Activity of *Moringa oleifera* extracts against Enteric Bacteria Isolates

Types of extract	*Escherichia coli*	*Klebsiella* species	*Proteus* species	*Enterobacter* species	*Schigella* species	*Salmonella* species
***Moringa* leaf ethanol extraction**	16mm	19mm	23mm	11mm	19mm	17mm
***Moringa* seed ethanol extraction**	9mm	15mm	14mm	12mm	8mm	14mm
***Moringa* leaf aqueous extraction**	7mm	3mm	-	-	-	4mm
***Moringa* seed aqueous extraction**	-		-	-	-	-

Antimicrobial activity of *Chromoalena odorata*

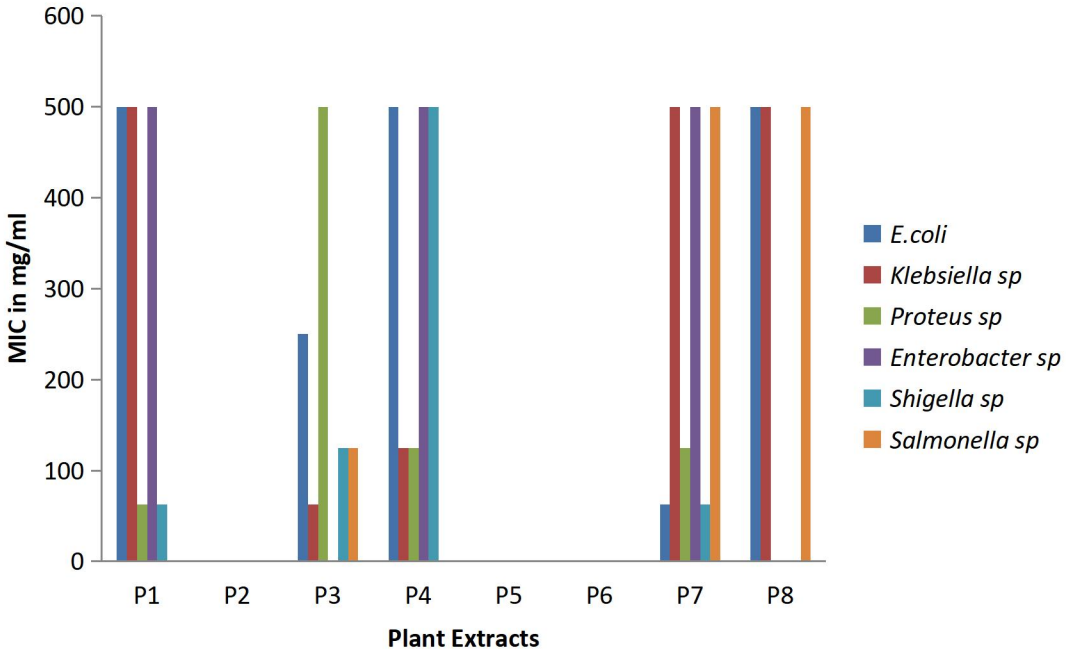
Table 5 reports the antimicrobial activity of *C. odorata* against the isolated enteric organisms, the ethanol extracts were observed to possess a higher antimicrobial activity, with the highest activity reported against *Proteus* sp. (32mm) while, the lowest antimicrobial activity was observed with the aqueous extracts against *E.coli* and *Klebsiella* sp (10mm). However, the other pathogens: Proteus sp, *Enterobacter* sp. and *Salmonella* sp. were completely resistant to the aqueous extract of *C. odorata* antimicrobial activities against all the pathogens.

Table 5: Antimicrobial Activity of *Chromoalena odorata* extracts against Enteric Bacteria Isolates

Organisms	Ethanol extract	Aqueous extract
Escherichia coli	14mm	10mm
***Klebsiella* sp.**	12.5mm	11mm
***Proteus* sp.**	32mm	-
***Enterobacter* sp.**	10mm	-
***Shigella* sp.**	17mm	-
***Salmonella* sp.**	12mm	-

Minimum Inhibitory Concentration (MIC) of the Plant Extracts

The minimum inhibitory concentration of the plant extracts is highlighted in Figure 2. The plant extracts are represented as P1-P8. P1: *Kola nitida* leaf ethanol extracts, P2: kolanut leaf aqueous extract, P3: *Moringa* leaf Ethanol extract, P4: *Moringa* seed ethanol extract, P5:*Moringa* leaf Aqueous, P6:*Moringa* seed aqueous, P7:*C. odorata* ethanol extract, P8: *C. odorata* leaf aqueous extract. *C.odorata* showed the best MIC among the plant extracts, while aqueous extracts of the plants showed mainly resistance by the test isolates and thereby no MIC, with the exception of C. odorata extracts against *E. coli* and *Klebsiella* sp



Key: P1: *Cola nitida* ethanol extract, **P2**: *Cola nitida* aqueous, **P3**: *Moringa* leaf Ethanol, **P4**: *Moringa* seed ethanol, **P5**: *Moringa* leaf Aqueous, **P6**:*Moringa* seed aqueous, **P7**:*C. odorata* ethanol, **P8**: *C. odorata* leaf aqueous.

Fig 2: **Minimum Inhibitory Concentration of plant extracts in mg/ml**

Discussion

The results from the percentage occurrence of the enteric organisms provide valuable

insights into the composition and abundance of microorganisms present in the chicken droppings. The morphological and biochemical identification revealed a variety of enteric organisms such as: *Escherichia coli, Klebsiella* sp*., Proteus* sp*., Enterobacter* sp*., Shigella* sp*.,* and *Salmonella* sp*.* In similar research, Madaki *et al.,* (2019) observed *Proteus mirabilis, Escherichia coli* and *Salmonella* sp*.* as enteric organisms from the samples analyzed.

Some of these organisms are well known pathogens of poultry as well as other farm animals. *E. coli* for example was reportedly implicated in disease conditions such as colibacillosis which occurs in forms such as enteric and septicaemic colibacillosis, whereas *Salmonella* is capable of producing acute and chronic infections in all or most types of birds and animals (Madaki *et al.,* 2019). These organisms have been reported to have a wide host range and can infect both humans and animals (Asfa *et al*., 2025).

This research also provides valuable scientific evidence regarding the antimicrobial efficacy of plant extracts: *Moringa oleifera, Cola nitida* and *Chromolena odorata*, against common enteric pathogens associated with diarrehea in poultry. Positive results as presented in Tables 3, 4, and 5 with a susceptibility pattern ranging from 11mm to 32mm authenticate the use of this plant as effective traditional medicine for the control of gastrointestinal infections in birds. Compared with synthetic antibiotics or inorganic chemicals counterparts, these plant-derived products have proven to be natural, less toxic, residue-free and are thought to be ideal feed additives in animal feed production (Olorungbohunmi, 2022). The leaves and seeds of *M. oleifera* have been reported in previous researches to inhibit pathogens: *Staphylococcus aureus, E. coli, S. typhi, Mucor* sp*, Candida albican*s at low concentrations (Ahmed *et al*., 2023). Ethanol extracts from *M. oleifera* leaves in this research exhibited high antibacterial activity against isolated Enteric organisms. The leaves of *Chromolaena odorata*, in this research possessed the highest antimicrobial activity (32mm) as reported in table 5, amongst other plant extracts used in this research, against the enteric pathogens. Olaeale *et al*. (2022) also reported high antagonistic activity against pathogenic bacteria from clinical isolates by utilizing aqueous and ethanolic extracts of *Chromolaena odorata.*

Cola nitida seeds and leaves have been reported from different researches to possess high antimicrobial activity against bacterial pathogens such as *Klebsiella pneumoniae* and *Escherichia coli* (Abass, 2017). The antimicrobial activity of *Cola nitida* extracts in this research against the enteric organisms, though positive, was the lowest among the other plant extracts.

These plants have been documented through various research to contain various bioactive compounds such as alkaloids, flavonoids, saponins, tannins, and triterpenoids (Twaji and Hassan, 2022) with an impressive range of medicinal uses, including growth promotion, potent anthelmintic activity (Hegazi *et al*., 2018), antibacterial effect (El-Kholy *et al*., 2018; Fouad *et al.,* 2019), antimicrobial and antioxidant effects (Yeshi *et al.,* 2022). The nutritional profile of its fresh and dried leaves is reported to contain high levels of lipids and essential amino acids important in poultry productivity (Ogbuewu and Mbajiorgu*,* 2023).

The antimicrobial sensitivity tests using ethanol and aqueous extracts showed varying degrees of inhibitory effects on the tested enteric organisms. The ethanol extract generally exhibited higher inhibitory effects compared to the aqueous extract, suggesting

that the ethanol extract may contain more bioactive compounds with antimicrobial properties. Notably, *Enterobacter spp.* and *Shigella spp.* were more susceptible to both extracts, with significant zones of inhibition observed. This corroborates several earlier studies reporting broad spectrum potency of *Chromolaena odorata* phytochemicals against diverse bacterial species (Obiefu *et al*., 2021). Furthermore, Kumar *et al.* (2018) reported the antibacterial activity of ethanolic extract of *Moringa* sp against *Salmonella* sp, *Clostridium perfringens* and *Campylobacter jejuni* colonization in broiler chickens (Sahib *et al.,* 2020, Zubair, 2020).

Conclusion

The antimicrobial effect of the plant extracts on enteric organisms isolated from excreta of chickens has provided valuable insights into the microbial population dynamics of enteric organisms of poultry and the influence of *Moringa* extract which can be used as a substitute for conventional antibiotics in the treatment of bacterial infections caused by these enteric organisms. This study shed light on the diversity of bacterial organisms associated with the gastrointestinal tract of chickens, their characteristics and potential implications in the safety of poultry feed. The prevalence of specific microbial genera and species, as revealed through characterization, provides a foundation for understanding the underlying enteric diseases of poultry and effective utilization of *Moringa* extract as a means of control intervention. The extraction methods achieved release of bioactive compounds in *Moringa* and the nutritional properties of the extract highlights ways to utilize these compounds to achieve antimicrobial effect. The detailed examination of microbial traits, including morphological, and biochemical characteristics, along with biochemical compounds in *Moringa* enhances our understanding of the behavior of these compounds against enteric organisms. This knowledge is critical for developing targeted strategies to battle pathogen resistance to antibiotics and mitigate toxicity to the poultry and consumers.

The extracts from this research showed promising natural therapeutic abilities against enteric infections, which may lead to new plant-based drug discoveries to better prevent and treat bacterial gastroenteritis and related issues in poultry and potentially in other livestock. Controlled clinical studies in live animals are the next step to further validate its antimicrobial efficacy and safety.

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