

## Molecular characterization and in vitro- Phytochemical Evaluation of two plants extracts against Multidrug-Resistant and Extended Spectrum Beta Lactamase Producing *Salmonella* Isolated from birds

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### Abstract

### Original Research Article

This study aims to know the molecular characterization of multidrug-resistant and extended spectrum  $\beta$ -Lactamase Producing *Salmonella* isolated from birds (chicken faeces) as well as test their susceptibility to two plants extracts namely *Azadirachta indica* and *Ocimum gratissimum*. A total of 55 *Salmonella enterica* isolates were recovered from chicken faeces. *Salmonella enterica* was identified and characterized using standard methods in the microbiology laboratory. Morphological and biochemical analysis were carried out to identify *Salmonella enterica*. 16S rRNA sequencing was carried out to also confirm the identification of *Salmonella enterica*. The Phenotypic and molecular determinants for the production of extended-spectrum  $\beta$ -lactamase was done using the double-disk synergy test and multiplex polymerase chain reaction respectively. Kirby–Bauer disc diffusion method was used to test the antibacterial susceptibility pattern against eight known antibiotics. Phytochemicals were determined by soxhlex extraction method. Aqueous, ethanol and methanol were the solvents used for extraction. Antibacterial activities of *Azadirachta indica* and *Ocimum gratissimum* were determined by agar well diffusion assay. The minimum inhibitory concentration of the plant extracts on *Salmonella enterica* was determined by the agar dilution method. The study showed the presence of *Salmonella enterica* as well as the presence of extended-spectrum  $\beta$ -lactamase genes responsible for varying resistance of *Salmonella enterica* to *Azadirachta indica* and *Ocimum gratissimum* extracts. Antibiotics were sensitive to *Salmonella enterica* while some were resistant to *Salmonella enterica*. The Antibiotic susceptibility test of *Salmonella enterica* isolated from chicken faeces observed that Aztreonam showed highest (54%) resistance while Cefixime (20%) showed the least resistance. The Phytochemicals identified were alkaloids, flavonoids, phenols, saponin, steroids and tannin, showing that the plant extracts contains active secondary metabolites that constitutes to the antimicrobial efficacy of plant extracts. *Ocimum gratissimum* gave more antibacterial activity of Leave extracts (24mm), followed by roots (22mm) then the least antibacterial activity was the stem (20mm) at a concentration of 200mg/ml compared to the antibacterial activity of *Azadirachta indica* Leave extracts (15mm), followed by roots (13mm) then the least antibacterial activity was the stem (10mm) at also a concentration of 200mg/ml. The zone of inhibition for *Salmonella enterica* on the different extracts of *Azadirachta indica* was between 1mm to 15mm while the zone of inhibition for *Salmonella enterica* on the different extracts of *Ocimum gratissimum* was between 2mm to 24mm. The observed antibacterial effects on *Salmonella enterica* can be as a result of the presence of phytochemicals. This confirms that *Azadirachta indica* and *Ocimum gratissimum* have some degree of antibacterial effect and should be used to treat *Salmonella enterica* infections. There is need to study plants extracts in order to develop a good bio-therapeutic agents originating from plants.

**Keywords:** Antibiotics, Antibacterial susceptibility *Azadirachta indica*, Chicken faeces. Extended-spectrum  $\beta$ -lactamase, Phytochemicals, *Salmonella enterica* and *Ocimum gratissimum*.

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## INTRODUCTION

Poultry (Chicken) is an important source of meat and it nourishes the human body with its high protein content. Its meat production has increased from 15 to 30% (FAO, 2006). One of the gram negative bacteria that can infect poultry is *Salmonella*. The morphology of *Salmonella* is that it is a gram negative rod shaped bacilli microorganism. They do not form spores or capsules. They are motile; they move by peritrichous flagella and possess fimbria that enables them to stick to their host. *Salmonella* belong to the family of Enterobacteriaceae. The sub specie known as *Salmonella enterica* has been implicated in most of the infections in humans as well as warm blooded animals (Jajere, 2019). *Salmonella* isolated from poultry birds, chicken faeces carries as asymptomatic reservoirs, which pose a significant zoonotic risk to human infections (Jajere, 2019).

The emergence of multidrug resistant and Extended Spectrum  $\beta$ -Lactamase Producing *Salmonella* infections is as a result of compromised antimicrobial therapy. This results because of indiscriminate over the counter use of antibiotics in human and animal medicine (Founou *et al.*, 2021). This can result in increased morbidity and mortality rates both in humans and animals and can adversely affect public health (WHO, 2023).

Africa is a home to over 45,000 different medicinal plant species and more than 5000 of these plants have medicinal values (Bhattacharya *et al.*, 2024). *Azadirachta indica* (Neem tree) and *Ocimum gratissimum* (African basil) are among the medicinal plants known to treat various ailments like diarrhoea, flu, cold, respiratory diseases among others (Ezeorba *et al.*, 2024). *Azadirachta indica* and *Ocimum gratissimum* are rich in phytochemical contents like alkaloid, flavonoid, phenols, saponin, steroids and tannin. Plants from studies have been known to be friendly and less toxic to man. They have been shown in vitro to exhibit some level of antibacterial and antioxidant properties (Ezeorba *et al.*, 2024).

This study aims to investigate the molecular

characterization of multidrug-resistant and extended spectrum  $\beta$ -Lactamase producing *Salmonella* isolated from birds as well as test their susceptibility to two plants extracts namely *Azadirachta indica* and *Ocimum gratissimum*.

## MATERIALS AND METHODS

### Study area

**This study was done in Owerri, Imo State Nigeria**

### Sample collection

Chicken faeces were aseptically collected from two farms in Owerri, Imo State, Nigeria. Using a sterile spatula, chicken faeces was placed into 20ml sterile sampling containers while observing zoonotic pathogen protocols (Foley *et al.*, 2021). The chicken faeces was transported to Microbiology Laboratory of Imo State University, Owerri for analysis.

### Isolation and Identification of *Salmonella*

Chicken faeces samples were first cultured on the Brain Heart Infusion broth agar after which it was incubated at a temperature of 37°C for a time period of 18–24 h. Then aliquots were streaked onto *Salmonella–Shigella* agar as well as MacConkey agar. The plates were incubated at a temperature of 37°C for a time period of 18–24 h. Pure isolates were obtained from distinct colonies that were subcultured on nutrient agar. Identification of *Salmonella* was obtained from gram staining and from biochemical tests like Catalase, Indole, Methyl Red, Voges-Proskauer and Citrate tests (Cheesbrough, 2019).

### Molecular characterization of the test isolate

The test isolate was subjected to I6S rRNA gene sequencing methods in order to characterize the test isolate. A zymo research bacteria Deoxyribonucleic acid (DNA) mini prep extraction kit was employed to characterize the isolate. Qualitative determination of the qualitative estimation of the genomic DNA was

done using the Agarose gel electrophoresis technique. After the extraction of the estimated DNA, it was subsequently amplified by the amplification protocol of Polymerase chain reaction. The sequencing of the DNA fragments was performed by Nimagen brilliant dye terminator cycle sequencing kit according to the instruction of the manufacturer (Platt, *et al.*, 2007).

### Phenotypic detection of the presence of Extended-Spectrum $\beta$ -Lactamase (ESBL)

The combination disk method and the double-disk synergy test (DDST) according the protocol of Clinical Laboratory Standard Institute was adopted where the initial detection for the ESBL production was done by the reduced susceptibility to cefotaxime and ceftazidime. Subsequently, the amoxicillin–clavulanate disk was placed at the central position, with cephalosporin disks positioned 15–20 mm apart. Conspicuous zone of inhibition toward the clavulanate disk indicated the production of Extended-Spectrum  $\beta$ -Lactamase

### The molecular detection of the presence of ESBL genes

The boiling method of genomic DNA was used in the extraction of the DNA fragments. The fragments concentration and purity were spectrophotometrically assessed. Multiplex Polymerase chain reaction was performed to detect genes responsible for ESBL production (Asgin *et al.*, 2019).

### Antibiotic preparation

Eight known antibiotics from major classes of carbapenems, cephalosporins, fluoroquinolones aminoglycosides, beta-lactam and monobactams was evaluated. These antibiotics includes

Aztreonam (30  $\mu$ g), Cefixime (5  $\mu$ g), Cefuroxime (30  $\mu$ g), Ciprofloxacin (5  $\mu$ g), Gentamicin (10  $\mu$ g), imipenem (10  $\mu$ g), Levofloxacin (5  $\mu$ g), Penicillin (10  $\mu$ g). Antibacterial susceptibility was interpreted based on the breakpoints given by Clinical and

Laboratory Standards Institute guidelines (CLSI, 2023).

### Antibacterial Susceptibility Testing

Kirby–Bauer disk diffusion method was adopted to perform the antibacterial susceptibility test. This test was performed according the protocol of Clinical and Laboratory Standards Institute guidelines were bacterial suspensions was standardized to conform to the 0.5 McFarland turbidity. Aliquot of isolate suspension were inoculated on Mueller-Hinton agar plates, then antibiotics disc were aseptically placed on the plates. The plates were incubated at 37°C for 18–24 h. The resultant zone of inhibitions were measured in millimeters as well as interpreted as being susceptible or resistant (CLSI, 2023).

### Collection and identification of two plant materials

The leaves, stem and bark of the two plants namely *Azadirachta indica* and *Ocimum gratissimum* were aseptically collected from a farm in Owerri, Imo State, Nigeria. The plants were authenticated by a botanist at Imo State University, Owerri. The plants were washed with sterile distilled water, dried under room temperature and processed for subsequent extraction.

### Preparation of plant extracts

The fresh pant parts (leaves, stem and roots) of *Azadirachta indica* and *Ocimum gratissimum* were washed separately with distilled water to avoid contamination. The different plant parts were left to dry on a clean surface at room temperature.

### Extraction solvents.

**The extraction solvents were aqueous, ethanol and methanol**

### Aqueous Extraction

Fine leaves, stems and roots were separately ground into powder using a blender. 200grams of each leaves, stems and roots were measured

into a separate sterile container and soaked with 2000ml each of distilled water (aqueous). These mixtures were allowed to stand for 72 hours with intermittent shaking, and then macerated. Whatman filter paper No.1 was used to filter the leaves, stems and roots separately. The filtrate was concentrated using a water bath centrifuged to obtain the crude extracts. The different extracts obtained were preserved in a refrigerator at 4°C temperature until needed for analysis of phytochemical and antimicrobial susceptibility tests (Nigussie *et al.*, 2021).

### Ethanol / methanol extraction

Fifty grams (50g) of each sample of leaves, stems and roots of *Azadirachta indica* and *Ocimum gratissimum* were separately prepared and used for the extraction. Soxhlex apparatus was used to extract the active ingredients of each plant parts. For the extraction of the active ingredient, 250ml of ethanol and 250ml methanol were used respectively. Sterile filter paper was used to filter the extract. The filtrate was evaporated to dryness using rotary vacuum evaporator at a boiling temperature of the solvent ethanol (79°C) and methanol at (65°C). The extracts remain in the sample holder while the solvent settled at the recovery flask. The extract was stored in the refrigerator at 4 °C. Ultraviolet light was used to sterilize the extract before use (Nigussie *et al.*, 2021).

### Test for the sterility of plant extracts

The sterility of plant extracts were performed by inoculating the different parts of plant extracts (leaves, stems and roots) into nutrient broth and incubating at 37°C for 24 h. Sterility was confirmed by the absence of turbidity (Dalitha, 2008).

### Tests for the presence of Phytochemicals

The qualitative analysis for the presence of different phytochemicals like tannins, saponins, flavonoids, and alkaloids were performed using standard protocols of (Akintelu, & Amoo, 2017).

**Test for Alkaloids** On a steam bath, about 0.5g of each extracts (leaves, stems and roots) were mixed with 5ml of 1% aqueous hydrochloric acid; 1ml of each filtrate was treated with a few drops of Mayer's reagent, and a second 1ml portion was treated similarly with Dragendorff's reagent, turbidity or precipitation with either of these reagents. As preliminary proof of the presence of alkaloids in the extracts, turbidity or precipitation with either of these reagents was detected.

**Test for Flavonoids** A fraction of the aqueous filtrate of each plant extracts (leaves, stems and roots) were mixed with 5 ml of diluted ammonia solution, concentrated sulphuric acid was added. Flavonoids presences were detected by a yellow coloration.

**Test for Phenols.** A few drops of a neutral 5% ferric chloride solution were added to 50 mg of each extracts (leaves, stems and roots) dissolved in 5 ml of distilled water for each sample. Dark green colour indicates the presence of phenols.

**Test for Saponins.** Powdered sample (2g) of each plant extracts (leaves, stems and roots) was cooked in water bath in 20 ml of distilled water, and the filtrate was mixed with 5 ml of distilled water and rapidly shaken for stable persistent foam. Three drops of olive oil was added to the foam observed and was vigorously shaken. Emulsion formation detects the presence of saponins.

**Test for Steroid** was done by the Liberman acid test. A portion of the organic of each extracts (leaves, stems and roots) was treated with three drops of acetic anhydride. Then concentrated H<sub>2</sub>SO<sub>4</sub> was carefully added by the side of the test tube. Steroid was detected by a brown colour formation.

**Tests for Tannin** Two grams of each extracts (leaves, stems and roots) were boiled in 20 ml of 45% ethanol for 5 minutes. The mixtures were cooled and the filtrates were used for further analysis. a) Ferric chloride test: One ml of filtrate was diluted with 2.0 ml of distilled water and 2 drops of ferric chloride solution was added. Greenish to brown colour indicated the presence of tannin.

### Antibacterial activity of the different Plant Extracts

The determination of antibacterial activity against multidrug ESBL-producing *Salmonella* isolates. The agar well diffusion method was adopted. Standardized inoculum was spread on Mueller-Hinton agar. Different well of 6mm in diameter were filled with various concentrations (200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml) of extracts. The inoculated plates were incubated at 37°C for 24 h. Transparent metric ruler was used to measure the zone of inhibition in mm. Control was standardized inoculum plates without the extracts (Balouiri *et al.*, 2016).

### Minimum inhibitory concentration (MIC)

An aliquot of each extract (200mg/ml) of leaves, stems and roots plant parts of *Azadirachta indica* and *Ocimum gratissimum* was dissolved in 2ml of sterile distilled water to obtain concentration of 100mg/ml. Double dilution of the 100mg/ml gave 50mg/ml, which was also double diluted to get 25mg/ml. One milliliter (1ml) of each concentration of extracts of leaves, stems and roots were transferred to petri plates, the 10ml of Mueller-hinton agar was poured into the plates, it was swirled well to mix properly then incubated at 37°C for 24, after which it was observed for bacterial growth. The lowest concentration of the extract (leaves, stems and roots) that inhibited the growth of *Salmonella* isolate was recorded as the minimum inhibitory concentration. Control was standardized inoculum plates without the extracts (Balouiri *et al.*, 2016).

## RESULTS

*Salmonella* isolate was identified both morphologically (gram stain, colour, shape arrangement, surface type and arrangement of colony) and biochemically (catalase, indole test, methyl red test, voges-proskauer test and citrate test). These are showed in Tables 1 and 2 respectively. *Salmonella* isolate was also characterized molecularly using the I6S rRNA gene sequencing methods to characterize and

identify the bacterial isolate as *Salmonella enterica* this is seen in table 3. The frequency of ESBL producing *Salmonella enterica* from different farms as shown in figure 1, where chicken feaces farm A had a frequency of 31 (56%) isolates of *Salmonella enterica* and chicken feaces farm B had a frequency of 24 (44%) isolates of *Salmonella enterica* making a total of 55 isolates of *Salmonella enterica*. Table 4 shows the fragments concentration of ESBL genes done spectrophotometrically. Cefotaximase- Munich (CTX-M) gene had the highest frequency of 2(50%), while Temoneira (TEM) genes and Sulfydryl Variable enzyme (SHV) gene had a frequency of 1 (25%) each.

The Antibiotic susceptibility test of *Salmonella enterica* isolated from chicken feaces as shown in table 5, where the various antibiotics namely: Aztreonam (30 µg), Cefixime (5 µg), Cefuroxime (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), imipenem (10 µg), Levofloxacin (5 µg), Penicillin (10 µg) showed different percentage of resistance having cefixime 11(20%), with the least resistance and Aztreonam had the highest resistance of 30 (54%).

The Antibiotic susceptibility test of *Salmonella enterica* isolated from chicken feaces on table 5 observed that Aztreonam showed highest (54%) resistance while Cefixime (20%) showed the least resistance

Table 6 displaced the qualitative phytochemical constituents screening analysis of *Azadirachta indica* extracts of leaves, stems and roots. *Azadirachta indica* contains the following phytochemicals which are alkaloid, flavonoid, phenols, saponin and tannin. The solvents used for extraction were aqueous, ethanol and methanol. Table 7 shows the qualitative phytochemical constituents screening analysis of *Ocimum gratissimum* extracts of leaves, stems and roots. The phytochemicals detected were alkaloid, flavonoid, phenols, saponin, steroid and tannin. The solvents of extraction were aqueous, ethanol and methanol. The antibacterial activity of the aqueous, ethanolic and methanolic *Azadirachta indica* extracts of leaves, stems and roots on *Salmonella enterica* was shown in

Table 8. The minimum inhibition concentration of the aqueous, ethanolic and methanolic *Azadirachta indica* extracts of leaves, stems and roots on *Salmonella enterica* was between 50mg/ml and 100mg/ml. Ethanol extracts of leaves (15mm), stems (10mm) and roots (13mm) on *Salmonella* gave the highest antibacterial activity followed by methanol extracts of leaves (12mm), stems (5mm) and roots (8mm) while aqueous extracts of leaves (8mm), stems (3mm) and roots (5mm) was the least antibacterial activity at concentration of 200mg/ml. Leave extracts gave the highest antibacterial activity (15mm), followed by roots (13mm) then the least antibacterial activity was the stem (10mm) at a concentration of 200mg/ml.

Table 9 shows the antibacterial activity of the aqueous, ethanolic and methanolic *Ocimum gratissimum* extracts of leaves, stems and roots on *Salmonella enterica*. The minimum inhibition concentration of the aqueous, ethanolic and methanolic extracts of *Ocimum gratissimum* leaves, stems and roots on *Salmonella enterica* was between 25mg/ml and 50mg/ml. Ethanol extracts of leaves (24mm), stems (20mm) and roots (22mm) on *Salmonella enterica* gave the highest antimicrobial activity followed by methanol extracts of leaves

(18mm), stems (11mm) and roots (14mm) then aqueous extracts of leaves (11mm), stems (8mm) and roots (9mm), at concentration of 200mg/ml. Leave extracts gave the highest antibacterial activity (24mm), followed by roots (22mm) then the least antibacterial activity was the stem (20mm) at a concentration of 200mg/ml.

*Ocimum gratissimum* gave more antibacterial activity of Leave extracts (24mm), followed by roots (22mm) then the least antibacterial activity was the stem (20mm) at a concentration of 200mg/ml compared to the antibacterial activity of *Azadirachta indica* Leave extracts (15mm), followed by roots (13mm) then the least antibacterial activity was the stem (10mm) at also a concentration of 200mg/ml.

The antibacterial activities of aqueous, ethanolic and methanolic leaves, stem and root extracts of *Azadirachta indica* and *Ocimum gratissimum* is found on tables 10 and 11. The zone of inhibition for *Salmonella enterica* on the different extracts of *Azadirachta indica* was between 1mm to 15mm while the zone of inhibition for *Salmonella enterica* on the different extracts of *Ocimum gratissimum* was between 2mm to 24mm.

**Table 1: Morphological identification of Salmonella.**

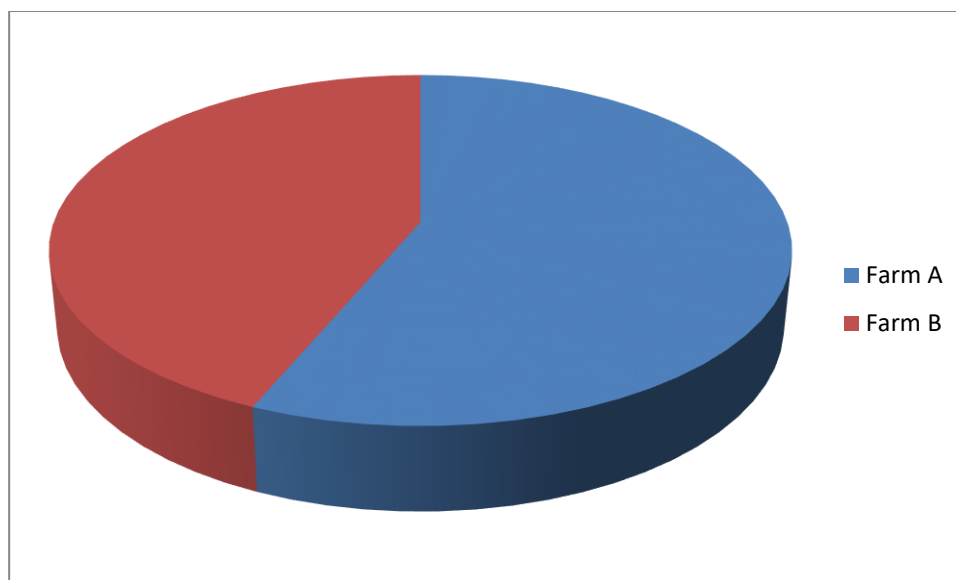
| Sample number | Gram stain | Colour | Shape arrangement | Surface type | Arrangement of colony | Organism          |
|---------------|------------|--------|-------------------|--------------|-----------------------|-------------------|
| 1             | Negative   | pink   | Rod               | Smooth edge  | Single or paired      | <i>Salmonella</i> |

**Table 2: Biochemical tests performed for the identification of Salmonella are: Catalase, Indole test, Methyl Red test, Voges-Proskauer test and Citrate test.**

| Biochemical tests | Catalase | Indole | Methyl | Voges-Proskauer | Citrate | Organism          |
|-------------------|----------|--------|--------|-----------------|---------|-------------------|
| Isolates          | +        | -      | +      | -               | +       | <i>Salmonella</i> |

**Table 3: I6S rRNA gene sequencing methods to characterize Salmonella isolates.**

| Sample | Sequence ID | Percentage | NCBI Match                       | Isolates                   |
|--------|-------------|------------|----------------------------------|----------------------------|
| 1      | NC003197.2  | 98         | <i>Salmonella typhi</i> AE006468 | <i>Salmonella enterica</i> |

**Figure 1: The frequency of ESBL producing *Salmonella* from different farms.**

**Key: Farm A: 31(56%).**

**Farm B: 24 (44%).**

**Table 4: Fragments concentration of ESBL genes done spectrophotometrically.**

| ESBL Genes | Frequency | Percentage (%) |
|------------|-----------|----------------|
| CTX-M      | 2         | 50             |
| TEM        | 1         | 25             |
| SHV        | 1         | 25             |
|            | 4         | 100            |

**KEY WORDS –CTX-M: Cefotaximase- Munich**

**TEM= Temoneira**

**SHV= Sulfhydryl Variable enzyme**

**Table 5: Antibiotic susceptibility test of *Salmonella enterica* isolated from chicken faeces**

| ANTIBIOTICS   | No(%) of Resistant Isolates |                                   |
|---------------|-----------------------------|-----------------------------------|
|               |                             | <i>Salmonella enterica</i> (n=55) |
|               |                             |                                   |
| Aztreonam     |                             | 30 (54)                           |
| Cefixime      |                             | 11(20)                            |
| Cefuroxime    |                             | 13 (24)                           |
| Ciprofloxacin |                             | 17 (31)                           |
| Gentamicin    |                             | 28 (51)                           |
| Imipenem      |                             | 16 (29)                           |
| Levofloxacin  |                             | 20 (36)                           |
| Penicillin    |                             | 25 (45)                           |
|               |                             |                                   |

**Key: Aztreonam (30 µg), Cefixime (5 µg), Cefuroxime (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), imipenem (10 µg), Levofloxacin (5 µg), Penicillin (10 µg).**

**Table 6: Qualitative phytochemical constituents screening analysis of *Azadirachta indica* extracts of leaves, stems and roots.**

| Phytochemicals  | Leaves | Stems | Roots |
|-----------------|--------|-------|-------|
| Aqueous Solvent |        |       |       |
| Alkaloid        | +      | +     | +     |
| Flavonoid       | +      | +     | +     |
| phenols         | +      | -     | -     |
| Saponin         | -      | -     | -     |
| Steroid         | -      | -     | +     |
| Tannin          | +      | +     | +     |
|                 |        |       |       |
| Ethanol solvent |        |       |       |
| Alkaloid        | +      | +     | +     |
| Flavonoid       | +      | +     | +     |
| phenols         | +      | +     | +     |
| Saponin         | +      | -     | +     |
| Steroid         | -      | -     | -     |
| Tannin          | +      | +     | +     |

|                  |   |   |   |
|------------------|---|---|---|
|                  |   |   |   |
| Methanol solvent |   |   |   |
| Alkaloid         | + | + | + |
| Flavonoid        | + | + | + |
| phenols          | - | - | - |
| Saponin          | + | - | + |
| Steroid          | - | - | - |
| Tannin           | + | + | + |

**Key: + present**

**- absent**

**Table 7: Qualitative phytochemical constituents screening analysis of *Ocimum gratissimum* extracts of leaves, stems and roots.**

| Phytochemicals   | Leaves | Stems | Roots |
|------------------|--------|-------|-------|
| Aqueous Solvent  |        |       |       |
| Alkaloid         | +      | +     | +     |
| Flavonoid        | +      | +     | +     |
| phenols          | +      | -     | +     |
| Saponin          | +      | -     | +     |
| Steroid          | -      | +     | -     |
| Tannin           | +      | +     | +     |
|                  |        |       |       |
| Ethanol solvent  |        |       |       |
| Alkaloid         | +      | +     | +     |
| Flavonoid        | +      | +     | +     |
| phenols          | +      | +     | +     |
| Saponin          | +      | -     | +     |
| Steroid          | +      | +     | +     |
| Tannin           | +      | +     | +     |
|                  |        |       |       |
| Methanol solvent |        |       |       |
| Alkaloid         | +      | +     | +     |
| Flavonoid        | +      | +     | +     |

|         |   |   |   |
|---------|---|---|---|
| phenols | + | + | + |
| Saponin | + | - | + |
| Steroid | - | - | - |
| Tannin  | + | + | + |

Key: + present

- absent

**Table 8: Antibacterial activity of the aqueous, ethanolic and methanolic *Azadirachta indica* extracts of leaves, stems and roots on *Salmonella enterica***

|                | Concentration of | different | Plants Extracts. |         |          |
|----------------|------------------|-----------|------------------|---------|----------|
| Solvents/plant | 200mg/ml         | 100mg/ml  | 50mg/ml          | 25mg/ml | MIC      |
| extracts       |                  |           |                  |         |          |
| Control        | 0                | 0         | 0                | 0       |          |
| Aqueous        |                  |           |                  |         |          |
| Leaves         | 8                | 4         | 0                | 0       | 50mg/ml  |
| Stems          | 3                | 0         | 0                | 0       | 100mg/ml |
| Roots          | 5                | 2         | 0                | 0       | 50mg/ml  |
| Ethanol        |                  |           |                  |         |          |
| Leaves         | 15               | 7         | 0                | 0       | 50mg/ml  |
| Stems          | 10               | 3         | 0                | 0       | 50mg/ml  |
| Roots          | 13               | 4         | 0                | 0       | 50mg/ml  |
| Methanol       |                  |           |                  |         |          |
| Leaves         | 12               | 5         | 0                | 0       | 50mg/ml  |
| Stems          | 5                | 2         | 0                | 0       | 50mg/ml  |
| Roots          | 8                | 3         | 0                | 0       | 50mg/ml  |

Key: MIC-Minimum Inhibitory concentration.

**Table 9: Antibacterial activity of the aqueous, ethanolic and methanolic *Ocimum gratissimum* extracts of leaves, stems and roots on *Salmonella enterica***

|                | Concentration of | different | Plants Extracts. |         |     |
|----------------|------------------|-----------|------------------|---------|-----|
| Solvents/plant | 200mg/ml         | 100mg/ml  | 50mg/ml          | 25mg/ml | MIC |
| extracts       |                  |           |                  |         |     |
| Control        | 0                | 0         | 0                | 0       |     |

|          |    |    |    |   |         |
|----------|----|----|----|---|---------|
| Aqueous  |    |    |    |   |         |
| Leaves   | 11 | 8  | 3  | 0 | 25mg/ml |
| Stems    | 8  | 5  | 0  | 0 | 50mg/ml |
| Roots    | 9  | 7  | 0  | 0 | 50mg/ml |
| Ethanol  |    |    |    |   |         |
| Leaves   | 24 | 16 | 10 | 0 | 25mg/ml |
| Stems    | 20 | 11 | 6  | 0 | 25mg/ml |
| Roots    | 22 | 13 | 9  | 0 | 25mg/ml |
| Methanol |    |    |    |   |         |
| Leaves   | 18 | 12 | 8  | 0 | 25mg/ml |
| Stems    | 11 | 8  | 4  | 0 | 25mg/ml |
| Roots    | 14 | 11 | 6  | 0 | 25mg/ml |

**Key: MIC-Minimum Inhibitory concentration.**

## DISCUSSION

According to (Moses, *et al.*, 2016), noted the presence of *Salmonella enterica* in faeces of poultry chicken which may have been as a result of improperly disposed poultry wastes. These poultry wastes may serve as reservoirs for enteric bacteria like *Salmonella*. This subsequently can be a resistance factor for birds like chickens. *Salmonella enterica* isolated were resistant to various antibiotics like Gentamycin, Amoxicillin, Cloxacillin, Erythromycin, Cefuroxime and Ceftazidime. These findings were similar to the findings of this work that showed the presence of multidrug-resistant and extended spectrum  $\beta$ -Lactamase Producing *Salmonella enterica* isolated from chicken faeces. There was resistance of *Salmonella enterica* to the different antibiotics such as Aztreonam, Cefixime, Cefuroxime, Ciprofloxacin, Gentamicin, imipenem, Levofloxacin, Penicillin, used for this study.

The work of (Vlietinck *et al.*, 2005) showed that the Gram-negative bacterial organisms like *Salmonella* are generally regarded more difficult to prevent their growth because they possess a thick murein layer that protects the bacterium accounts for the resistance of

*Salmonella* bacteria. This agreed with this work because of the resistance and presence of multidrug-resistant and extended spectrum  $\beta$ -Lactamase Producing *Salmonella enterica*.

The study of (Azwanida, 2015) showed the greater power of ethanolic extract when compared with methanolic or aqueous extracts, this concurred with this study that showed that antimicrobial zone of inhibition was greatest with ethanolic extracts followed by methanolic extracts while aqueous extract gave the least zone of inhibition.

This work showed that *Ocimum gratissimum* extracts of leaves, stems and roots contains higher presence of phytochemicals than *Azadirachta indica* extracts of leaves, stems and roots. This agreed with the work of (Olajide *et al.*, 2021), that states that there are high values of phytochemicals and food components in *Ocimum gratissimum* than in *Azadirachta indica*. The work of (Mgbeahurike *et al.*, 2022) also showed that *Ocimum gratissimum* exhibited some antimicrobial effects because of their rich contents of alkaloids, flavonoids, tannins and terpenoids. These findings agreed with this work that *Ocimum gratissimum* plant parts which contain phytochemicals responsible

for their various levels of antimicrobial activities.

## CONCLUSION

*Salmonella enterica* isolated was resistant to more than two antibiotics and as such there is the presence of multidrug-resistant and extended spectrum  $\beta$ -Lactamase Producing *Salmonella enterica* isolated from birds (Chicken faeces). This may be attributed to the presence of Munich (CTX-M) gene Temoneira (TEM) genes and Sulfhydryl Variable enzyme (SHV) genes in the bacterium. It can also be as a result of contamination of the chicken faeces. The eight different antibiotics Aztreonam, Cefixime, Cefuroxime, Ciprofloxacin, Gentamicin, imipenem, Levofloxacin, Penicillin, showed various degrees of resistance with the Aztreonam antibiotics showing the highest resistance while Cefixime antibiotics showing the least resistance. *Azadirachta Indica* and *Ocimum gratissimum* contains different phytochemicals. These phytochemicals were responsible for various degree of antimicrobial inhibition of the multidrug-resistant and extended spectrum  $\beta$ -Lactamase Producing *Salmonella enterica* isolated from birds. The therapeutic potential of *Azadirachta Indica* and *Ocimum gratissimum* can be harnessed as an alternative to antimicrobial agents against multidrug-resistant and extended spectrum  $\beta$ -Lactamase Producing *Salmonella enterica*. There is need to study plants extracts in order to develop a good bio-therapeutic agents originating from plants.

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