

Phytochemical Evaluation of *Ocimum gratissimum* as a Potential Therapeutic Agent Against characterized Multidrug-Resistant and Extended Spectrum Beta Lactamase Producing *Salmonella* Isolated from Swine

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Abstract

Original Research Article

This study investigated the phenotypic resistance patterns, molecular determinants of extended-spectrum β -lactamase production, phytochemical composition, and antibacterial potential of *Ocimum gratissimum* leaf extracts against extended-spectrum β -lactamase producing multidrug resistance *Salmonella* isolates recovered from swine. A total of 81 *Salmonella* isolates were recovered from porcine samples and identified using standard microbiological techniques. Antimicrobial susceptibility profiling was performed using the Kirby–Bauer disc diffusion method against ten commonly prescribed antibiotics. Extended-spectrum β -lactamase production was phenotypically confirmed using the double-disc synergy test, while multiplex polymerase chain reaction was employed to detect clinically relevant β -lactamase resistance genes. Crude aqueous and ethanolic leaf extracts of *Ocimum gratissimum* were prepared and evaluated for antibacterial activity against confirmed extended-spectrum β -lactamase producing isolates using the agar well diffusion assay. Minimum inhibitory concentrations were determined by standard broth dilution techniques, and qualitative phytochemical analysis was conducted by gas chromatography-mass spectrometry to identify the major bioactive constituents responsible for antimicrobial activity. The findings revealed a high prevalence of multidrug resistance among the *Salmonella* isolates, characterized by extensive resistance to β -lactam antibiotics and molecular confirmation of extended-spectrum β -lactamase associated resistance genes. Both aqueous and ethanolic extracts of *Ocimum gratissimum* exhibited pronounced inhibitory activity against all tested multidrug resistance extended-spectrum β -lactamase producing isolates, with an average Minimum inhibitory concentration of 1.78 mg/mL, demonstrating broad-spectrum antibacterial efficacy. Phytochemical analysis confirmed the presence of biologically active secondary metabolites, including flavonoids, alkaloids, tannins, and saponins, whose synergistic interactions are likely responsible for the observed antimicrobial effects. The combined phenotypic, molecular, and phytochemical evidence highlights the remarkable therapeutic potential of *Ocimum gratissimum* as a natural antimicrobial agent against drug-resistant *Salmonella*. This study provides compelling evidence supporting the development of *Ocimum gratissimum* which has some antimicrobial potential against multidrug resistance and extended-spectrum β -lactamase producing *Salmonella* isolates. *Ocimum gratissimum* can to an extent serve as a phytotherapeutic compounds for managing infections caused by multidrug resistance and extended spectrum β -latamase producing *Salmonella* within veterinary and public health settings. Further studies involving bioactive compound isolation, toxicity assessment, pharmacokinetic evaluation, and in vivo efficacy are recommended to facilitate its translation into clinically relevant antimicrobial therapeutics.

Keywords: Antimicrobial resistance, Extended-spectrum β -lactamase, Multidrug-resistant *Salmonella*, *Ocimum gratissimum*, Phytotherapy, Swine.

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INTRODUCTION

Salmonella remains one of the most important foodborne pathogens globally, responsible for a wide spectrum of diseases collectively referred to as salmonellosis. Transmission is primarily associated with the consumption of contaminated foods of animal origin, particularly pork and poultry products, which serve as major reservoirs for human infection. Clinically, *Salmonella* infections range from self-limiting gastroenteritis to invasive systemic conditions such as septicemia, meningitis, and focal infections, thereby constituting a significant burden on public health systems worldwide (Eng *et al.*, 2015; Ferrari *et al.*, 2019).

Taxonomically, *Salmonella* is a Gram-negative, rod-shaped bacterium belonging to the family *Enterobacteriaceae*, with *Salmonella enterica* subspecies accounting for the majority of infections in warm-blooded animals and humans. Numerous serovars have been implicated in both zoonotic transmission and host-specific diseases, highlighting the organism's adaptability and epidemiological significance (Grimont & Weill, 2007; Jajere, 2019). In both clinical and veterinary contexts, *Salmonella* has been associated with diverse pathological conditions including gastroenteritis, bacteremia, urinary tract infections, and pneumonia (Eng *et al.*, 2015).

The effectiveness of antimicrobial therapy in managing *Salmonella* infections has been increasingly compromised by the emergence and global spread of multidrug-resistant (MDR) strains. This trend is largely attributed to the extensive and often indiscriminate use of antibiotics in human medicine, animal husbandry, and aquaculture (Founou *et al.*, 2021; Manyi-Loh *et al.*, 2018). Of particular concern is the rise of extended-spectrum β -lactamase (ESBL)-producing *Salmonella* strains, which exhibit resistance to critically important antibiotics such as third-generation cephalosporins, thereby limiting therapeutic options and increasing morbidity and mortality rates (Bush & Bradford, 2020; WHO, 2023).

At the molecular level, antimicrobial resistance in *Salmonella* is frequently mediated by plasmids mobile genetic elements capable of horizontal gene transfer among bacterial populations. These plasmids often harbor multiple resistance determinants, facilitating the rapid dissemination of MDR phenotypes across different ecological niches, including livestock production systems and healthcare environments (Partridge *et al.*, 2018; Carattoli, 2013). The intensification of livestock farming, particularly swine production, further exacerbates this problem by creating conditions conducive to the selection and spread of resistant strains.

Given the interconnectedness of human, animal, and environmental health conceptualized under the One Health framework, food animals such as pigs serve as critical reservoirs for antimicrobial-resistant *Salmonella* strains with zoonotic potential (Robinson *et al.*, 2016; WHO, 2023). Consequently, there is an urgent need to explore alternative antimicrobial strategies that are effective, safe, and sustainable.

Medicinal plants have gained increasing attention as potential sources of novel antimicrobial agents due to their bioactive phytochemicals and relatively low toxicity profiles. *Ocimum gratissimum*, commonly known as African basil, is widely used in traditional medicine and has demonstrated notable antibacterial, antifungal, and antioxidant properties. Its phytochemical constituents, including flavonoids, tannins, alkaloids, and essential oils, are believed to contribute to its antimicrobial efficacy (Prabhu *et al.*, 2009; Akinmoladun *et al.*, 2020).

In this context, the present study investigated the antibacterial potential of *Ocimum gratissimum* against multidrug-resistant and ESBL-producing *Salmonella* isolates of porcine origin. Furthermore, it integrates phytochemical profiling with molecular analysis to elucidate the mechanisms underlying its antimicrobial activity, thereby contributing to the development of alternative therapeutic strategies against resistant *Salmonella* infections.

2.0 Materials and Methods

2.1 Sample Collection and Antibiotics

Salmonella isolates were obtained from pig farms through the collection of rectal swabs and fecal samples using sterile spatulas, which were aseptically transferred into 20 mL sterile sampling bottles. Sampling procedures were adapted from previously described microbiological protocols (Amaechi & Nwankwo, 2015), with modifications aligned to current best practices for zoonotic pathogen surveillance (Foley *et al.*, 2021).

A panel of ten antibiotics representing major classes of aminoglycosides, carbapenems, penicillins, fluoroquinolones, cephalosporins, and monobactams was evaluated. These included gentamicin (10 µg), imipenem (10 µg), ampicillin (10 µg), levofloxacin (5 µg), ceftazidime (30 µg), aztreonam (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), meropenem (10 µg), and ertapenem (10 µg). Interpretation of antimicrobial susceptibility was based on breakpoints established by the Clinical and Laboratory Standards Institute guidelines (CLSI, 2023).

2.2 Isolation and Identification of *Salmonella*.

Samples were pre-enriched in Brain Heart Infusion (BHI) broth and incubated at 37°C for 18–24 h. Subsequently, aliquots were streaked onto MacConkey agar and *Salmonella*–*Shigella* agar for selective isolation. Plates were incubated at 37°C for 24 h. distinct colonies with characteristic morphology were subcultured on nutrient agar to obtain pure isolates. Identification was performed using Gram staining and standard biochemical tests, including **Indole Methyl Red Voges-Proskauer Citrate** (IMViC) reactions, following established microbiological procedures (Cheesbrough, 2019; ISO 6579-1:2017 protocols).

2.3 Antimicrobial Susceptibility Testing.

Antimicrobial susceptibility testing was conducted using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI, 2023). Bacterial suspensions were standardized to 0.5 McFarland turbidity. Sterile swabs were used to inoculate agar plates uniformly, after which antibiotic discs were aseptically applied. Plates were incubated at 35–37°C for 18–24 h. Zones of inhibition were measured in millimeters and interpreted as susceptible, intermediate, or resistant.

2.4 Detection of Extended-Spectrum β -Lactamase (ESBL).

2.4.1 Phenotypic Detection

Initial screening for ESBL production was based on reduced susceptibility to cefotaxime and ceftazidime. Confirmatory testing was performed using the combination disk method and double-disk synergy test (DDST), following CLSI recommendations (CLSI, 2023; Bush & Bradford, 2020). An amoxicillin–clavulanate disk was placed centrally, with cephalosporin disks positioned 15–20 mm apart. Enhancement of inhibition zones toward the clavulanate disk indicated ESBL production.

2.4.2 Molecular Detection of ESBL Genes

Genomic DNA was extracted using the boiling method (Akinyemi *et al.*, 2015). DNA concentration and purity were assessed spectrophotometrically. Multiplex PCR was performed to detect **blaTEM**, **blaSHV**, and **blaCTX-M** genes using previously validated primers (Asgin *et al.*, 2019; Al-Hashimy *et al.*, 2020). PCR conditions included initial denaturation at 94°C, followed by 25 amplification cycles and final extension at 72°C. Amplified products were resolved on 2% agarose gel stained with ethidium bromide and visualized under UV illumination. Band sizes were compared with a 100-bp DNA ladder.

Table 1. Primers used for PCR

amplification genesTarget	of	ESBL Primer Sequence (5'–3')	Product (bp)	
bla_TEM		TEM-F TCCGCTCATGAGACAATAACC	931	Asgin <i>et al</i> .,2019
		TEM-R TTGGTCTGACAGTTACCAATGC		
bla_SHV		SHV-F TGGTTATGCGTTATATTCGCC	868	Asgin <i>et al.</i> , 2019
		SHV-R GGTTAGCGTTGCCAGTGCT		
bla_CTX-M		CTX-F TCTTCCAGAATAAGGAATCCC	909	Al-Hashimy <i>et al.</i> ,2020
		CTX-R CCGTTTCCGCTATTACAAAC		

2.5 Plant Material Collection and Identification

Fresh leaves of *Ocimum gratissimum* were collected from Owerri North Local Government Area, Imo State, Nigeria. The plant was

taxonomically identified by a botanist at Imo State University. Leaves were washed with sterile distilled water, air-dried, and processed for extraction.



Fig 1 *Ocimum gratissimum* leaf

2.6. Methods for Extraction of Plant Samples

Dried plant material was ground into powder. Aqueous and ethanol extractions were carried out via maceration for 72 h (Azwanida, 2015; Nigussie *et al.*, 2021). Filtrates were concentrated using a water bath and centrifuged to obtain crude extracts. Extracts were stored under refrigeration and partitioned for phytochemical, antimicrobial susceptibility test, and Gas Chromatography-Mass Spectrometry GC–MS analyses.

2.7 Sterility Testing of Extracts

Sterility was confirmed by inoculating extracts into nutrient broth and incubating at 37°C for 24 h. Absence of turbidity indicated sterility (Dalitha, 2008).

2.8 Antimicrobial Activity of Plant Extracts

The agar well diffusion method was used to evaluate antimicrobial activity against ESBL-producing isolates (Balouiri *et al.*, 2016). Standardized inocula were spread on Mueller–Hinton agar. Wells (6 mm diameter) were filled with varying concentrations (12.5, 25 mg/ml, 50

mg/ml and 100 mg/mL) of extracts. Plates were incubated at 37°C for 24 h, and inhibition zones were measured.

2.9 Phytochemical Analysis

Qualitative and quantitative analyses of tannins, saponins, flavonoids, and alkaloids were conducted using standard protocols (AOAC, 2019; Akintelu & Amoo, 2017; Fapohunda *et al.*, 2012).

2.10 GC–MS Analysis

GC–MS analysis was performed using a Shimadzu QP2010 Plus system. Compounds were separated on a capillary column and identified by comparison with spectra from the National Institute of Standards and Technology NIST database (El-Sayed *et al.*, 2021).

2.11 Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 23. Descriptive statistics, chi-square, and analysis of variance (ANOVA) were applied. Statistical significance was set at $p < 0.05$.

3.0. RESULTS.

Table 3.1. Antimicrobial Resistant Profile of *Salmonella* Isolated from Pig Rectal Swab Samples

ANTIBIOTICS	No(%) of Resistant Isolates	
		<i>Salmonella</i> (n=41)
Ampicillin	49(98)	
Cefotaxime	49(98)	
Ceftazidime	44(88)	
Ciprofloxacin	24(48)	
Imipenem	48(96)	
Meropenem	44(88)	
Aztreonam	50(100)	

Gentamycin	4(8)	
Ertapenem	44(88)	
Lavofloxacin	20(40)	
SD	15.88	
X²	60.33;p < 0.001	
ANOVA LSD	9.078	

Statistical analysis indicated a highly significant variation in resistance patterns across antibiotics ($\chi^2 = 60.33$; $p < 0.001$). The standard deviation (SD = 15.88) reflects considerable variability among antimicrobial agents, while ANOVA (LSD = 9.078) confirmed significant differences in mean resistance levels.

Table 3.2. Antimicrobial Resistant Profile of *Salmonella* Isolated from Pig Feces Samples.

ANTIBIOTICS	No(%) of Resistant Isolates	
	<i>Salmonella</i> (n=40)	
Ampicillin	49(98)	
Cefotaxime	50(100)	
Ceftazidime	50(100)	
Ciprofloxacin	19(38)	
Imipenem	37(74)	
Meropenem	38(76)	
Aztreonam	45(90)	
Gentamycin	11(22)	
Ertapenem	14(28)	
Lavofloxacin	20(40)	
SD	15.73	
X²	66.91;p < 0.001	
ANOVA LSD	8.997	

Statistical analysis revealed a highly significant variation in resistance patterns across antibiotics ($\chi^2 = 66.91$; $p < 0.001$). The relatively high standard deviation (SD = 15.73) indicates substantial variability in resistance distribution, while ANOVA results (LSD = 8.997) confirm significant differences between mean resistance levels.

Frequency (%) of ESBL Producers from Different Sample Types

Table 3.3: Frequency (%) of Esbl Producers from the Different Sample Types

Sampling Sources	Total
Pig feces (n=50)	11(48)
Pig rectal swab(n=50)	12 (52)
TOTAL (100)	23(100)

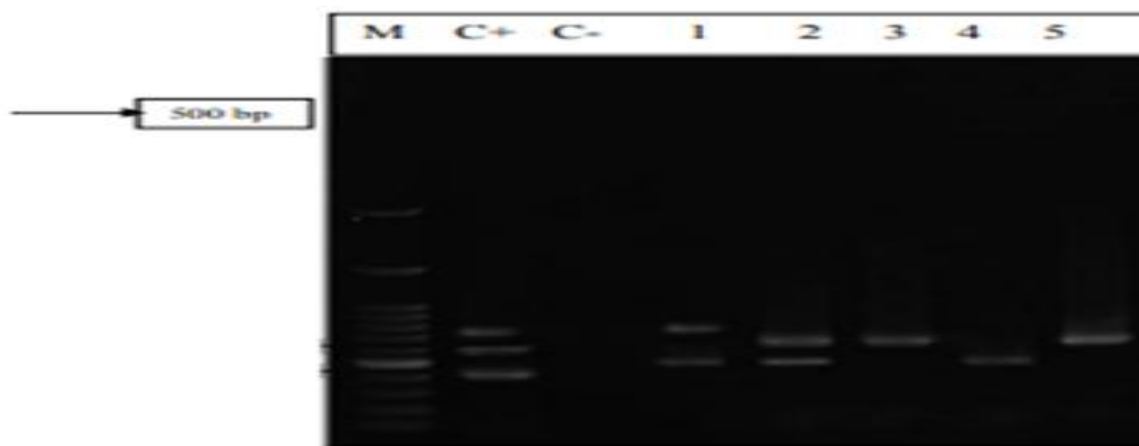
Detection of ESBL Resistance Genes in β -Lactamase-Positive Isolates

Fig3.3. The results of M-PCR from left to right: M; bp 100 ladder, positive control (*Salmonella tphi*), negative control (*Esccherichia coli* 25923 ATCCC), bla SHV (bp 747), bla TEM (bp 455) and bla CTX-M (bp 593), 11-1; veterinary strains of *Salmonella*.

Distribution of ESBL Genotypes in *Salmonella* Isolates (n = 5)Table 3.4: Distribution of TEM, SHV, and CTX-M genes in *Salmonella* isolates (n = 5).

ESBL genotype	Number of isolates (%) <i>Salmonella</i>
TEM	1(20)
CTX-M	2 (40)
TEM and CTX-M	1 (20)
TEM, SHV, and CTX-M	1 (20)
Total	5 (100)
SD	0.5
X²	0.6;p = 0.896
ANOVA LSD	0.333

KEY WORDS –**TEM= Temoneira****CTX-M: Cefotaximase- Munich****SHV= Sulfhydryl Variable enzyme**

Statistical analysis showed no significant difference in genotype distribution ($\chi^2 = 0.6$; $p = 0.896$), indicating a random distribution pattern within the study population.

Antimicrobial Susceptibility and Minimum Inhibitory Concentration of Plant Extract**Table 4.5:** Minimum Inhibitory Concentration (Mic) Of Plant Extracts against *Salmonella* Spp. (Mic Mg/ML).

S/NO	TEST ORGANISM(s)	<i>Salmonella</i>
<i>Ocimumgratissimum</i>		1.78
SD		0.325
X²		0.07;p = 0.794
ANOVA LSD		0.381

Statistical analysis further validated the experimental findings. The low standard deviation (SD = 0.325) indicates minimal variability among replicates, demonstrating high reproducibility of MIC measurements. Chi-square analysis showed no significant difference in antimicrobial activity between extract treatments ($\chi^2 = 0.07$; $p = 0.794$), confirming that observed variations were not statistically meaningful. Similarly, ANOVA (LSD = 0.381)

indicated that differences in MIC values fell within experimental error margins.

Collectively, these results demonstrate that while *O. gratissimum* exhibits some level of antimicrobial inhibition activity against multidrug resistant and ESBL-producing *Salmonella* spp. and therefore supports its potential as a complementary source of antimicrobial agent.

Phytochemical Constituents of Crude Aqueous and Ethanolic Extract**Table 4.6:** Qualitative Phytochemical Screening of Crude Ethanolic and Hot Water Extractions of Scent Leaf (*Ocimum Gratissimum*)

	OE	Ow
Alkaloids	+++	-
Saponins	++	+
Tannins	+++	+
Flavonoids	+	+

OW = Water extract of *O. gratissimum*OE = Ethanol extract of *O. gratissimum*

Overall, the findings demonstrate that ethanolic extraction is more effective than aqueous extraction in isolating key phytochemicals from *O. gratissimum*. This justifies further biological

and pharmacological investigations, as well as potential development of plant-derived therapeutic agents.

Gas chromatography–mass spectrometry analysis of *Ocimum gratissimum* Leaf Extracts.

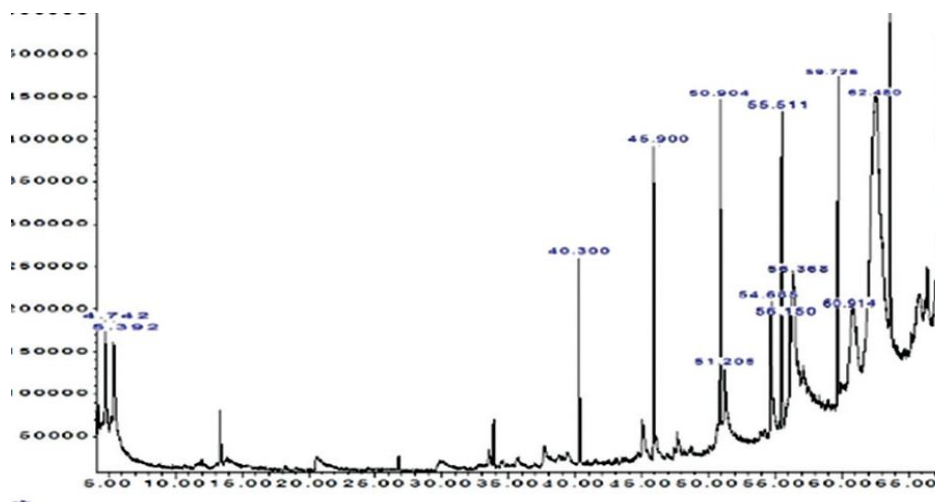


FIG. 3.5: Gas chromatography–mass spectrometry chromatogram ethanol extracts of *Ocimum gratissimum*

GC–MS chromatogram of ethanolic extract of *Ocimum gratissimum* leaf showing multiple peaks corresponding to identified phytochemical constituents.

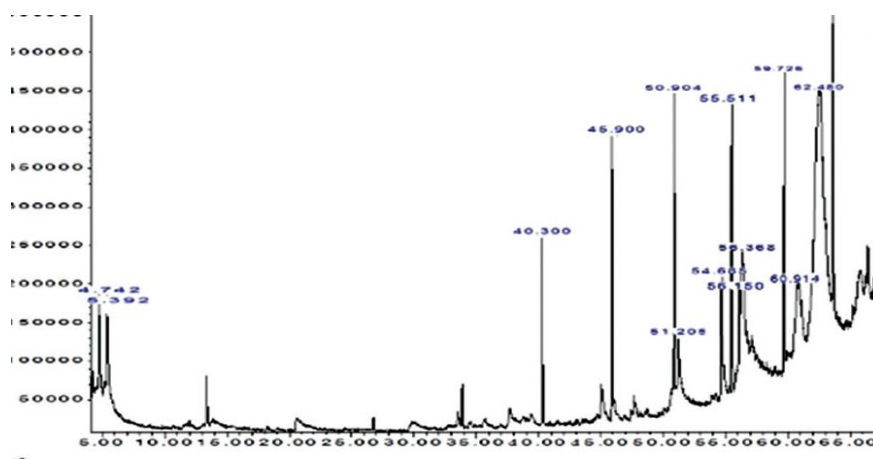


FIG. 3.6: Gas chromatography–mass spectrometry chromatogram water extracts of *Ocimum gratissimum*

GC–MS chromatogram of aqueous extract of *Ocimum gratissimum* leaf showing comparatively fewer peaks and lower signal intensities than the ethanolic extract.

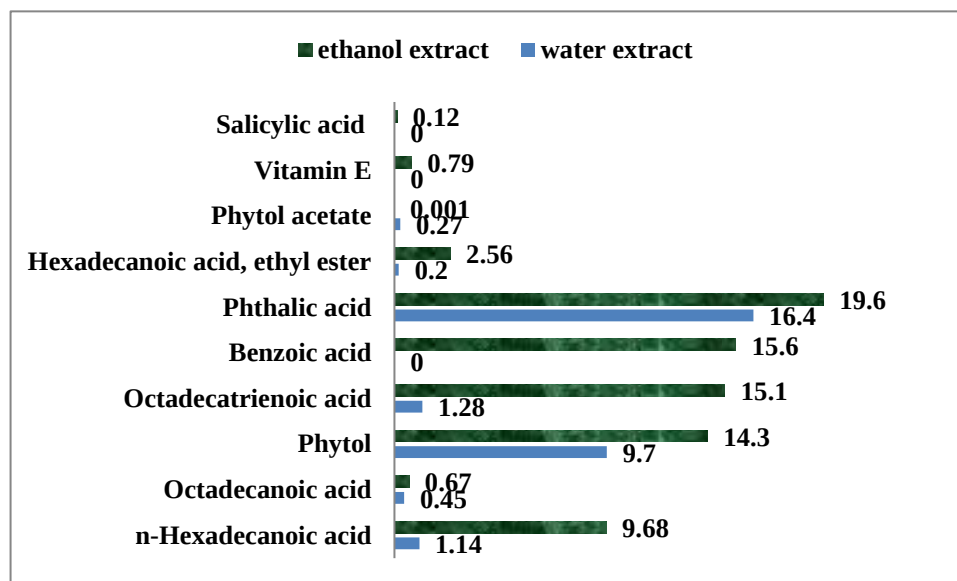


Fig 3.7.: Bar Chart Ethanol Extract And Water Extract Of *Ocimum Gratissimum*.

4.0 DISCUSSION

The present study revealed an alarmingly high prevalence of antimicrobial resistance (AMR) among *Salmonella* isolates recovered from pig rectal swabs and fecal samples, underscoring the critical role of livestock as reservoirs of multidrug-resistant (MDR) pathogens. The near-universal resistance to β -lactam antibiotics—particularly aztreonam (100%), cefotaxime (98–100%), ceftazidime (88–100%), and ampicillin (98%)—strongly suggests widespread dissemination of extended-spectrum β -lactamase (ESBL)-producing *Salmonella* strains. These findings are consistent with recent global reports indicating a surge in ESBL-producing *Enterobacteriales* in food-producing animals, driven largely by indiscriminate antibiotic use in veterinary practice (Effah *et al.*, 2020; Tyson *et al.*, 2021).

The extremely high resistance to carbapenems (imipenem: 74–96%; meropenem: 76–88%; ertapenem: 28–88%) is particularly concerning, as carbapenems are considered last-resort antibiotics for severe infections in humans. The emergence of carbapenem resistance in livestock-associated *Salmonella* suggests possible acquisition of carbapenemase genes, such as *blaKPC*, *blaNDM*, or *blaOXA-48*-like enzymes, which have increasingly been reported

in zoonotic pathogens (Wang *et al.*, 2022). This trend raises significant public health concerns, as it indicates potential transmission of highly resistant pathogens from animals to humans through the food chain, direct contact, or environmental pathways, aligning with the One Health framework (WHO, 2023).

Moderate resistance to fluoroquinolones (ciprofloxacin: 38–48%; levofloxacin: 40%) further supports the hypothesis of selective pressure from frequent antibiotic exposure in pig production systems. Fluoroquinolones are commonly used in both human and veterinary medicine, and resistance often arises from mutations in the *gyrA* and *parC* genes or via plasmid-mediated quinolone resistance determinants (PMQRs) (Hooper & Jacoby, 2016; Rodríguez *et al.*, 2023). The comparatively low resistance to gentamicin (8–22%) suggests that aminoglycosides may still retain therapeutic efficacy, although emerging resistance trends necessitate cautious use.

The significant variation in resistance patterns ($\chi^2 = 60.33$ and 66.91 ; $p < 0.001$) reflects heterogeneous antimicrobial exposure and adaptive responses among *Salmonella* populations. The high standard deviation values further indicate variability in resistance distribution, likely

influenced by differences in farm management practices, antibiotic usage patterns, and environmental factors. These findings corroborate studies highlighting the role of intensive farming systems in accelerating AMR evolution (Manyi-Loh *et al.*, 2018; Van Boeckel *et al.*, 2019).

The identification of 29 and 19 distinct resistance phenotypes in rectal swabs and fecal samples, respectively, demonstrates substantial genetic and phenotypic diversity. This diversity enhances the adaptive fitness of *Salmonella*, facilitating persistence under antimicrobial pressure and increasing the likelihood of horizontal gene transfer (HGT). Mobile genetic elements such as plasmids, integrons, and transposons are known to mediate the dissemination of resistance genes across bacterial populations and environmental niches (Partridge *et al.*, 2018).

The detection of ESBL-producing isolates (38%) further substantiates the widespread occurrence of β -lactam resistance. The predominance of *bla*CTX-M genes aligns with global epidemiological trends, where CTX-M-type enzymes have largely replaced TEM and SHV variants as the dominant ESBLs (Bevan *et al.*, 2017; Castanheira *et al.*, 2021). The co-occurrence of multiple ESBL genes (*bla*TEM, *bla*SHV, *bla*CTX-M) within single isolates highlights the complexity of resistance mechanisms and suggests co-selection driven by multidrug exposure. Although the genotype distribution was not statistically significant ($p = 0.896$), the presence of diverse ESBL combinations indicates ongoing genetic exchange and evolutionary adaptation.

From a One Health perspective, the high prevalence of ESBL-producing *Salmonella* in pig-associated samples underscores the role of animal agriculture in the AMR crisis. Pig feces, in particular, act as environmental reservoirs, facilitating the spread of resistant bacteria through manure application, water contamination, and soil ecosystems (Djordjevic *et al.*, 2021). This environmental dimension is critical, as it enables the persistence and amplification of resistance genes beyond the farm setting.

Importantly, the study also demonstrates the antimicrobial potential of *Ocimum gratissimum* against ESBL-producing *Salmonella*. The observed MIC value (1.78 mg/mL) indicates moderate antibacterial activity, which is noteworthy given the high level of resistance to conventional antibiotics. The efficacy of *O. gratissimum* can be attributed to its rich phytochemical composition, including flavonoids, tannins, alkaloids, and terpenoids, which exert antimicrobial effects through multiple mechanisms such as membrane disruption, enzyme inhibition, and oxidative stress induction (Burt, 2020; Mgbeahuruike *et al.*, 2022).

The GC-MS analysis further confirmed the presence of bioactive compounds such as phytol, hexadecanoic acid, octadecanoic acid, and phenolic derivatives, all of which have documented antimicrobial and anti-inflammatory properties. The synergistic interactions among these compounds likely enhance their efficacy and reduce the likelihood of resistance development, offering a promising alternative or adjunct to conventional antibiotics (Silva *et al.*, 2021).

The superior phytochemical yield observed in the ethanolic extract compared to the aqueous extract highlights the importance of solvent selection in phytochemical extraction. Ethanol's intermediate polarity allows for efficient solubilization of both polar and non-polar compounds, resulting in a broader spectrum of bioactive constituents. This finding is consistent with previous studies demonstrating higher antimicrobial activity in ethanolic plant extracts (Azwanida, 2015).

Overall, the study provides compelling evidence that *Salmonella* isolates from pigs in the study area exhibit high levels of multidrug resistance, including resistance to critically important antibiotics. The coexistence of multiple resistance genes, combined with environmental dissemination pathways, poses a significant threat to public health. However, the demonstrated efficacy of *O. gratissimum* suggests a viable alternative strategy for combating resistant pathogens, warranting further investigation into its

pharmacological applications and potential integration into antimicrobial stewardship programs.

Conclusion

This study demonstrated a high prevalence of multidrug-resistant (MDR) and extended-spectrum β -lactamase (ESBL)-producing *Salmonella* isolates of porcine origin, with pronounced resistance to β -lactam antibiotics including cefotaxime, ceftazidime, aztreonam, and ampicillin. The detection of ESBL-associated genes, particularly *bla*CTX-M, either alone or in combination with *bla*TEM and *bla*SHV, confirms the molecular basis of resistance or highlights the growing dissemination of resistant *Salmonella* strains within pig production systems. The occurrence of carbapenem resistance among several isolates further emphasizes the potential public health threat posed by zoonotic transmission of resistant pathogens through the food chain and environmental contamination.

The findings also established that *Ocimum gratissimum* possesses appreciable antibacterial activity against MDR and ESBL-producing *Salmonella* isolates. Both aqueous and ethanolic extracts exhibited inhibitory effects, although the ethanolic extract demonstrated superior phytochemical yield and stronger antimicrobial activity. The observed antibacterial effects may be attributed to the presence of bioactive phytochemicals such as flavonoids, tannins, alkaloids, saponins, and other compounds identified through GC–MS analysis. These phytoconstituents likely act synergistically to disrupt bacterial growth and survival.

Overall, the study provides scientific evidence supporting the therapeutic potential of *Ocimum gratissimum* as a complementary or alternative antimicrobial agent against resistant *Salmonella* infections. The integration of phytochemical profiling, molecular characterization, and antimicrobial susceptibility testing contributes valuable insights into the development of plant-based strategies for

combating antimicrobial resistance within the One Health framework.

Recommendations.

Continued researches should be carried out for better understanding of exact mechanisms and also pharmacodynamic and pharmacokinetic properties of the molecules and the creation of novel herbal pharmaceuticals that meet GRAS (Generally Recognised as Safe) standards.

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