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Phytotherapeutic Intervention Against MDR Salmonella Enterica Serovar Typhi: Evaluating the Plasmid Curing Potential of Selected Medicinal Plants

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ABSTRACT

Background: Studies have shown that multi-drug resistant Salmonella species is a well-recognized and documented problem worldwide especially in the developing countries, and the gene associated with this resistance are encoded in the plasmid. This study was carried out to evaluate the plasmid eviction potentials of some selected plant extracts against multi-drug resistant (MDR) Salmonella enterica sub sp. enterica serovar Typhi (ST).

Methods: Water samples from rivers, boreholes, and hospital dumping sites were screened for S. Typhi and resistant strains using standard microbiological techniques. Phytochemical constituents of Zingiber officinale, Ocimum gratissimum, Vernonia amygdalina, Azadirachta indica, and Xylopia aethiopica leaf extracts were quantified. Different extract concentrations were tested on isolates, and plasmid analysis assessed their curing potential.

Results: Salmonella enterica serovar Typhi strains STCMCST, WGS1146, STERL12960, ST311189, STCT18, and ST2018K isolated from rivers, boreholes, and hospital dumping sites showed 68.94% resistance to conventional antibiotics. Treatment with Zingiber officinale (ZO), Ocimum gratissimum (OG), and Vernonia amygdalina (VA) extracts significantly ($p \leq 0.05$) reduced resistant strains from 100% to 2%, 10%, and 58%, respectively. Molecular plasmid curing analysis showed no observable band on agarose gel for ZO against the resistant strains, indicating effective plasmid eviction.

Conclusion: From the above study, S. enterica ser. Typhi isolated from the studied samples showed significant resistance to antibiotics, and ZO, OG and VA showed pronounced plasmid eviction potentials by reducing the percentage of resistance to zero or treatable level, of which ZO was most effective-Q3

Keywords: Plasmid, Eviction, Salmonella, Typhi, Extracts
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INTRODUCTION

Salmonella enterica subspecies *enterica* serovar Typhi is an enteric bacterium that causes a great threat to humans and several species of animals, by disrupting the intestinal mucosa.[1,2] The organism is found naturally in water and food products that had been contaminated with feces of infected humans.[3,4] The serovar is a zoonotic bacterium that can be found globally with different host specificity, and the apparent ability to cause disease in those hosts is also serovar dependent. *S. enterica* serovar Typhi is one of the main causes of foodborne diseases, causing gastrointestinal diseases, and is also the causative agent of more serious systematic diseases such as typhoid and paratyphoid fever.[2]

The evolutionary pressure from the use of antibiotics has played a key function in the emergence and the spread of multiple antibiotic resistance among different bacteria species.[5] Also, extra-chromosomal genetic elements harbored by some bacterial agents had been responsible for enhanced resistant development either horizontally or vertically.[5]

Research had revealed that some plants' extracts are capable of evicting plasmids that are responsible for multiple antibiotic resistance in *Salmonella enterica* subspecies *enterica* serovar Typhi. Plasmid eviction is performed so that susceptibility can be restored in MAR serovars.[5] This process can be actualized by targeting the plasmid that is responsible for the antibiotic resistance using a validated screening technology. The plasmid is then removed and the organism eventually loses its resistance potentials as described by.[5]

Several researchers had worked on plasmid curing in *Salmonella* species such as[2,5,6] but little or no information had been documented on molecular analysis of plasmid eviction potential of selected medicinal plants against molecularly characterized MDR *Salmonella enterica* subspecies *enterica* serovar Typhi. This derived the major interest of the research. The result obtained from this work would provide a vital information on method of eliminating multiple antibiotic resistance associated with *Salmonella enterica* subspecies *enterica* serovar Typhi.

MATERIALS AND METHODS

***Sample collection, Handling and transportation*:** The samples used for this study were drawn from the rivers, boreholes and hospital dumping sites. The river sample samples were collected with sterile containers. The containers were thoroughly washed with detergent, rinsed with water, then rinsed with 70% ethanol and final rinsed three times with distilled water. The containers were placed inverted in order to drain the water inside them. The container was inverted and lowered 5 cm below the river water sample, then placed vertically for the water sample to refill the sample container. This sample was covered immediately and kept in a cooler containing ice block, and this transported to the laboratory for immediate analysis. The borehole samples were collected using sterile container sterilized using the above-described procedures. The borehole tap was turned on and allowed to flow for 2 minutes after which the sample was collected using the sterile container. The hospital dumping site samples were collected using the method described in the study published by.[7] The litter from the soil surfaces was carefully scrapped out using sterile spoon. A sterile soil auger was derived to a plough depth of 15 cm in the hospital dumping site, and soil sample was drawn up to 10 samples from each sampling unit into a sterile tray. The samples were thoroughly mixed and foreign materials such as roots, stones, pebbles and gravels were carefully removed. The soil sample was then reduced to half by quartering the sample. Again, quartering was carried out by dividing the soil sample into four equal parts and the two opposite quarters were discarded and the remaining two quarters were mixed. The process was repeated for the rest of soil samples used for this study. The samples were carefully labeled and then kept in a disinfected cooler, to maintain its temperature and stability of the number of the isolates. The samples were transported to the laboratory for analysis.

Culture and Isolation of enteric bacteria: This was carried out using the modified method of.[8] Five grams (5.0 g). The soil sample was aseptically dissolved in 15.0 ml of sterile normal saline in a 50 ml Pyrex beaker, and the volume was adjusted to 50.0 ml before serial dilution. The soil sample was weighed using an electronic weighing scale (DC-300). Ten-fold serial dilutions were prepared up to 10-3 from a millilitre aliquot that had been aseptically placed into a sterile test tube (Pyrex) that contained 9.0 ml of the diluent (sterile normal saline). Likewise, the samples of river water were likewise diluted to 1:10. One millilitre of the processed soil, river, and borehole samples were placed on Petri plates with Deoxycholate citrate agar medium (DCA/Biotech) that were 60 mm OD × 55 mm ID × 13 mm height. We incubated each plate in triplicate for 24 to 48 hours at 37±2°C.[8]

***Characterization and identification of the isolates*:** The isolates underwent a 24-hour inverted incubation

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period at 37°C after being sub cultured on nutrient agar (Biotech). The isolates were described and identified using their colonial and morphological descriptions,[8] biochemical responses[8,9] and molecular characterization.[10] The goal of the colonial description was to determine the isolates' colours on agar medium plates as well as their sizes, edges, consistencies, and optical features.

***Preparation of plant materials*:** The fresh leaves of *Xylopia aethiopica, Ocimum gratissimum* and *Vernonia amygdalina, Azadirachta indica* and rhizomes of *Zingiber officinale* were collected from cultivated land at Uli in Ihiala L.G.A of Anambra State, Nigeria. The samples were appropriately authenticated. The samples were dried under shade at room temperature for 14 days.[11] The dried leaves were blended to powdered form using sterile electric grinder (LXB 242/LE Max). 20 g of the ground leaves each was mixed with distilled water and ethanol respectively for 72 h. The mixture was filtered using Whatman No 1 filter paper. The extracts were concentrated by evaporating to dryness at room temperature in a steady air current.[12]

***Phytochemical analysis of the plant extracts*:** The phytochemical components (alkaloids, glycosides, flavonoids, phenolics, tannins, steroids and saponins) of the plant extracts were determined quantitatively using the methods described by.[13,14,15,16]

***Pre-screening of the resistant isolates against the extracts*:** This was carried out using the method described in the study published by.[17] Each labeled plate was uniformly inoculated with the test organism using pour plate method in Muller Hinton Agar (MHA). A sterile cork borer of 5mm diameter was used to make the wells on the medium. One tenth milliliter of various concentrations of the extracts were dropped into each labeled wells and then incubated at 37°C for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation. Diameter less than 5.50 mm was considered resistant while diameter 5.50 mm and above was considered sensitive.

Plasmid curing: This was carried out following the modified method described by.[18] A 24 h broth culture of the test isolates was prepared in test tubes (Pyrex). Then the test isolates were exposed to different concentrations (1%, 5%, 10%, 15% 20%, 25%, 30%, 35%, 40%, 45%, 50%) of the plant extracts, and incubated for 24 h at 37±2°C in order to determine the sub lethal concentrations of the agents. After 24 h of incubation, 1 millimeter aliquot from each test tube was inoculated into nutrient agar plate and incubated, after which colonies were selected and counted. Then the susceptibility of the cured isolates was carried out using disk diffusion technique. The cured isolates were inoculated into freshly prepared Muller Hinton agar plate. Subsequently, aseptically inserted antibiotic discs with a history of resistance to the plate, ensuring that the discs made contact with the agar surface in the appropriate locations. Following a 24-hour incubation period at 37±2oC, the plates were analysed.

Statistical analysis: The results of the data generated were expressed in percentage, tables and figures. The significance of the sample source, resistance and degree of resistance were ascertained using Chi square at 95% confidence level. The significance of the prevalence and plasmid eviction potential were determined using Analysis of variance (ANOVA) at 95% confidence level. Pairwise comparison was carried out in an excel sheet using student "t" test.[6]

RESULTS

Characteristics of the Isolates: The sequence analysis of the bacterial isolates showed 100% identify for all the six isolates and the identified isolates were: *Salmonella enterica* subspecies *enterica* serovar Typhi strain CMST (STCMST), *Salmonella enterica* subspecies *enterica* serovar Typhi strain WG-S1146 (STWG-S1146),*Salmonella enterica* subspecies *enterica* serovar Typhi strain ERL12960 (STERL12960), *Salmonella enterica* subspecies *entrica* serovar Typhi strain 311189 (ST311189), *Salmonella enterica* subspecies *enterica* serovar Typhi strain CT18 (STCT18) and *Salmonella enterica* subspecies *enterica* serovar Typhi strain 2018K (ST2018K) as shown in Table 1.

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Table 1: Characteristics and identities of the isolates

Isolate Code	Max Score	Total Score	Query Cover (%)	E-value	Percent Identity	Accession Number	Description of the Isolates
15	3620	3620	100	0.0	100	CP053702.1	*Salmonella enterica* subspecies *entrica* serovar Typhi strain CMST (STCMST)
16	3620	3620	100	0.0	100	CP040575.1	*Salmonella enterica* subspecies *entrica* serovar Typhi strain WG-S1146 (STWG-S1146)
17	3620	3620	100	0.0	100	LT904894.1	*Salmonella enterica* subspecies *entrica* serovar Typhi strain ERL12960 (STERL12960)
18	6874	7660	100	0.0	100	CP029645.1	*Salmonella enterica* subspecies *entrica* serovar Typhi strain 311189 (ST311189)
19	6096	11283	100	0.0	100	AL513383.1	*Salmonella enterica* subspecies *entrica* serovar Typhi strain CT18 (STCT18)
20	4257	4257	100	0.0	100	CP044007.1	*Salmonella enterica* subspecies *entrica* serovar Typhi strain 2018K (ST2018K)

Susceptibility of the Isolates to Conventional Antibiotics

The total percentage of resistant strains detected among isolates was 91%, of which STCMCST recorded the highest resistant strains (Table 2)

Table 2: Susceptibility of the isolates to conventional antibiotics

Isolate	Number (%)	Susceptible Strain (%)	Resistance Strain (%)	Implicated Antibiotics
STCMCST	88 (66.67)	21 (23.86)	67 (76.14)	AMX, AU, S, PN, CEP, SXT, CPX
STWGS1146	7 (5.30)	5 (71.43)	2 (28.57)	AMX, AU, S, PN
STERL12960	2 (1.52)	1 (50.00)	1 (50.00)	CEP, AMX, AU, S, PN
ST311189	4 (3.03)	3 (75.00)	1 (25.00)	AMX, AU, S, CPX, PN, CEP
STCT18	30 (22.73)	11 (36.67)	19 (63.33)	AMX, AU, S, PN, CEP
ST2018K	1 (0.76)	0 (0.00)	1 (100.00)	AMX, AU, S, PN
Total	132 (100)	41 (31.06)	91 (68.94)	

Phytochemical Constituents of the Plant Extracts

The study revealed the presence of cardiac glycosides, steroids, alkaloids, tannins, flavonoids, plenolics and saponins in the leaves extracts of *Xylopia aethiopica* (XA), *Zinger officinale* (ZO), *Azadirachta indica* (AI), *Veronica amygdalina* (VA) and *Ocimum gratissimum* (OG). Steroids, tannins, phenolics and saponins has highest activity in VA whereas alkaloids and flavonoids have highest activity in ZO. Cardiac glycosides have highest activity in OG. The data obtained from this study showed that the phytochemical constituents were significantly ($p \leq 0.05$) present in the studied leaves extracts. Also, there was significance difference ($p \leq 0.05$) between the phytochemical constituents present in XA and ZO, XA and VA, ZO and AI, AI and VA, AI and OG and VA and OG as shown in Table 3.

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Table 3: Phytochemical constituents of the medical plant leave extract

Parameter	*Xylopia aethiopica* (g/100g)	*Zingiber officinale* (g/100g)	*Azadirachta indica* (g/100g)	*Vernonia amygdalina* (g/100g)	*Ocimum gratissimum* (g/100g)
Cardiacglycosides	0.29±0.00	1.01±0.01	0.21±0.00	0.94±0.01	1.02±0.03
Steroids	0.11±0.00	0.02±0.00	0.18±0.00	0.36±0.01	0.29±0.01
Alkaloids	2.10±0.07	6.10±0.11	1.42±0.03	2.28±0.01	1.16±0.01
Tannins	0.62±0.01	4.48±0.14	0.27±0.01	7.56±0.14	2.46±0.11
Flavonoids	0.53±0.01	5.56±0.14	0.47±0.01	4.52±0.21	1.71±0.03
Phenolics	1.58±0.03	1.34±0.03	0.21±0.00	3.41±0.11	0.77±0.01
Saponins	0.28±0.00	0.81±0.01	0.83±0.01	5.12±0.14	3.22±0.21

Susceptibility of the Isolates to the Extracts

The study showed that 9, 29, and 37 of the resistant isolates were not susceptible to VA, XA and AI extracts. The activity of the extracts against the resistant strains was highest in ZO extracts, followed by OG and VA extracts. Hence ZO, OG and VA exhibited significant activity, with zones of inhibition ranging from 21.00 mm and above as shown in Table 4.

Table 4: Diameter zone of inibition of pre-screened extracts against the test resistant strains (**N=91)**

Diameter zone of inhibition (mm)	XA (%)	ZO (%)	AI (%)	VA (%)	OG (%)
Below 6	29(31.87)	0(0.00)	37(40.66)	9(9.89)	0(0.00)
6 – 8	37(40.66)	18(19.79)	22(24.18)	26(28.57)	19(20.88)
9 – 11	16(17.58)	19(20.88)	14(15.38)	27(29.67)	22(24.18)
12 – 14	6(6.59)	21(23.08)	11(12.09)	11(12.09)	19(20.88)
15 – 17	3(3.30)	15(16.48)	7(7.69)	14(15.38)	17(18.68)
18 – 20	0(0.00)	9(9.89)	0(0.00)	3(3.30)	11(12.09)
21 – 23	0(0.00)	6(6.59)	0(0.00)	1(1.10)	2(2.20)
24 and above	0(0.00)	3(3.30)	0(0.00)	0(0.00)	1(1.10)

XA – *Xylopia aethiopica,* ZO – *Zingiber officinale,* AI – *Azadirachta indica,* VA – *Vernonia amygdalina,* OG – *Ocimum gratissimum*

Viable counts of the Bacterial Isolates after Post-Plasmid Curing Activity

The study showed that minimal lethal concentrations of ZO, OG and VA extracts against STCMCST, STWGS1146, STERL12960, ST311189, STCT18 and ST2018K ranges from 70%-90%, 90%-100% and 90%-above 100% respectively. It was observed that the MLC for VA could not be determined against STWGS1146, STERL12960 and ST2018K. The optimum concentration of the extracts that yielded viable counts within the stipulated standard plate counts (30-300 CFu/ml) ranges from 50%-70% for ZO extract, 70%-80% for OG extract and 70%-90% for VA extract (Table 5).

Table 5: Viable counts of the bacterial isolates after post-plasmid curing activity

Isolate	ZO			OG			VA		
	MLC (%)	Opt conc (%)	Cfu/ml	MLC (%)	Opt conc (%)	Cfu/ml	MLC	Opt conc %)	Cfu/ml
STCMCST	35	30	64	40	35	49	50	35	36
STWGS1146	40	30	51	50	35	56	60	40	47
STERL12960	40	25	48	50	30	41	70	40	51
ST311189	40	30	69	40	30	39	50	35	37
STCT18	30	20	57	40	25	48	40	30	44
ST2018K	35	15	76	40	35	41	60	35	37

ZO – *Zingiber officinale*; OG – *Ocimum gratissimum*; VA – *Vernonia amygdalina*
MLC – Minimal Lethal Concentration; Opt Conc – Optimum Concentration;
CFU/ml – Colony Forming Unit per millimetre;

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Post Plasmid Curing Potentials of the Studied Extracts

The study showed that ZO, OG and VA extracts significantly ($p \leq 0.05$) reduced the resistant strains from 100% to 2%, 10% and 58% respectively. The data obtained showed that ZO was non-significantly ($p \geq 0.05$) reduced the resistant strains most, followed by OG whereas VA showed the least reduction. It was also observed that ZO revert all the resistant plasmid present in the studied isolates except strain of STC18 that was not completely reverted. Also, complete reversion was shown by OG against STERL12960 and ST2018K. The study also revealed that OG and acridine orange solution (control) exhibited similar result against STWGS1146, STERL12960, ST311189 and ST2018K whereas the reversion potential of ZO was non-significantly ($p \leq 0.05$) higher than that of OG and acridine orange solution (Table 6).

Table 6: Post plasmid curing potentials of the studied extracts

Isolate	Resistant Strain (%)	ZO (%)	OG (%)	VA (%)	AO (%)
STCMST	67(73.63)	0(0.00)	4(4.40)	41(45.05)	7(7.69)
STWGS1146	2(2.20)	0(0.00)	1(1.10)	1(1.10)	1(1.10)
STERL12960	1(1.10)	0(0.00)	0(0.00)	1(1.10)	1(1.10)
ST311189	1(1.10)	0(0.00)	1(1.10)	1(1.10)	1(1.10)
STCT18	19(20.88)	2(2.20)	4(4.40)	13(14.29)	11(12.09)
ST2018K	1(1.10)	0(0.00)	0(0.00)	1(1.10)	0(0.00)
Total	91(100.00)	2(2.20)	10(10.99)	58(63.74)	21(23.08)

Molecular Evaluation of Plasmid Curing Potential of the Extracts

The molecular evaluation of the plasmid curing potentials of the extracts was evaluated by observing the presence or absence of bands on the electrophoretic gels, measuring the reduction in molecular weight of the bands and measuring the size of the bands. In the present study, there was no observable band seen on the agarose gel for ZO against STCMST, STWGS1146, STERL12960, ST311189 and ST2018K. Significant reduction in size and in weight was also observed on ZO against STCT18. OG showed absence of bands against STERL12960 and ST2018K, whereas against STCMCST, STWGS1146, ST311189 and STC18, there were observable reduction in sizes and weights of the bands. VA showed reduction in size and weight of the bands which were not pronounced like ZO and OG extracts. The acridine orange (AO) solution used as control exhibited similar curing potentials with OG but with slight variation against STCMCST and STCT18 (Plates 1, 2 and 3).

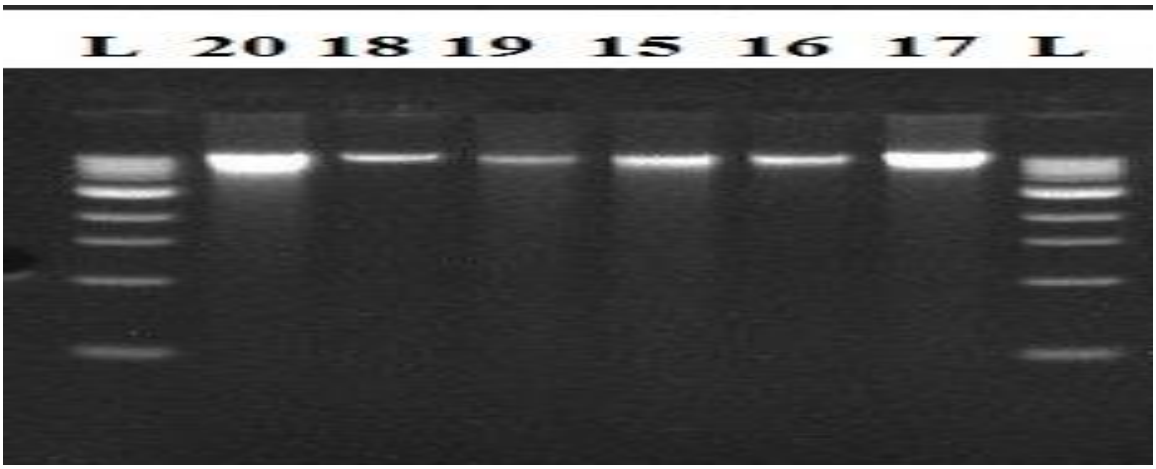

Plate 1: Plasmid Analysis of the Resistant Isolates before Plasmid Eviction
20 = STCMCST; 17 = STCT18; 19 = STWGS1146; 15 = ST311189;18 = STERL12960; 16 = ST2018K

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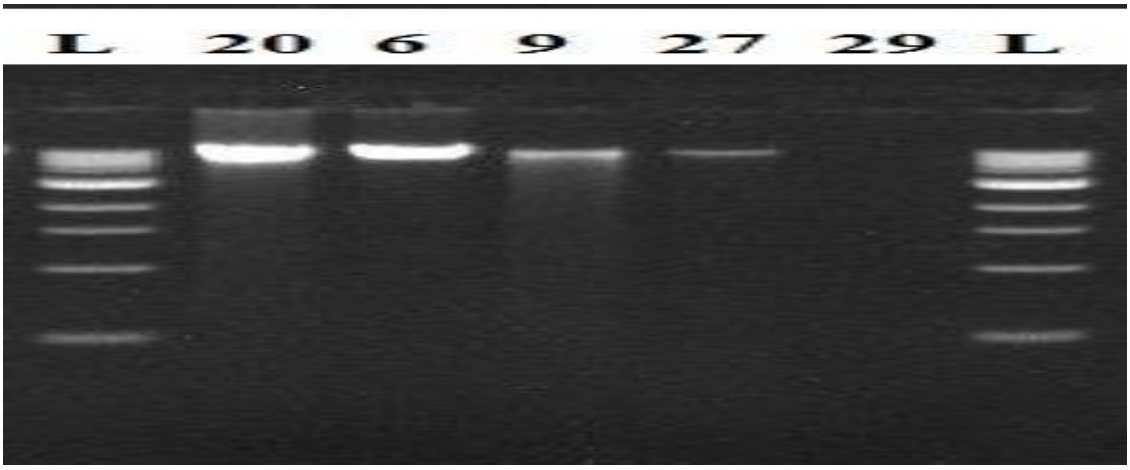

Plate 2: Eviction Potentials of the Extracts against STCMCST
20 = STCMCST; 6 = VA; 9 = AO, 27 = OG; 29 = ZO

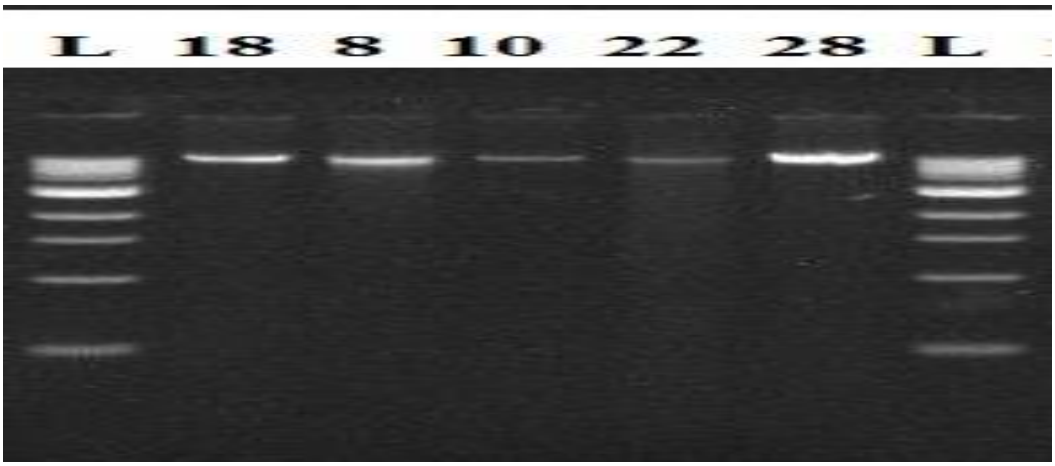

Plate 3: Eviction potentials of the extracts against STERL12960
18 = STERL12960; 8 = AI, 10 = OG; 22 = ZO; 28 = VA

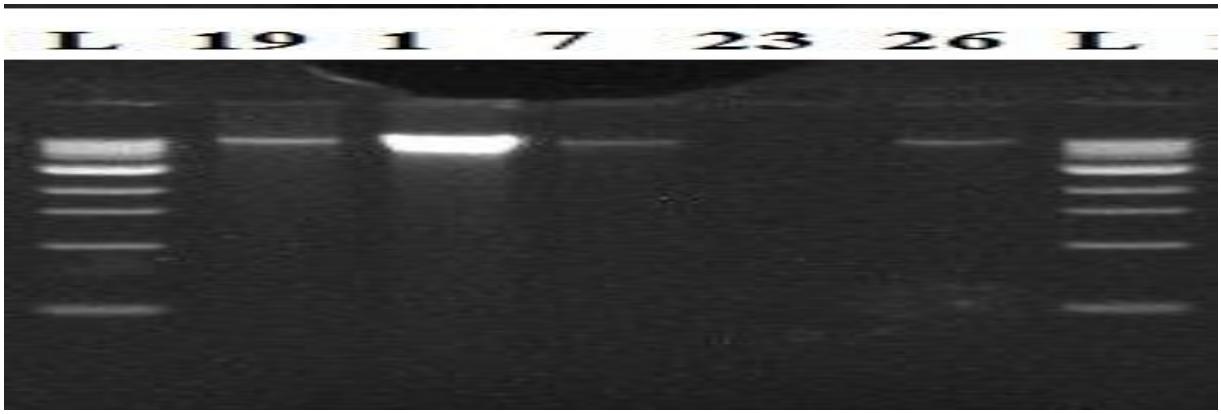

Plate 4: Eviction Potentials of the Extracts against STWGS1146
19 = STWGS1146; 1 = VA; 7 = OG; 23 = ZO; 26 = AO

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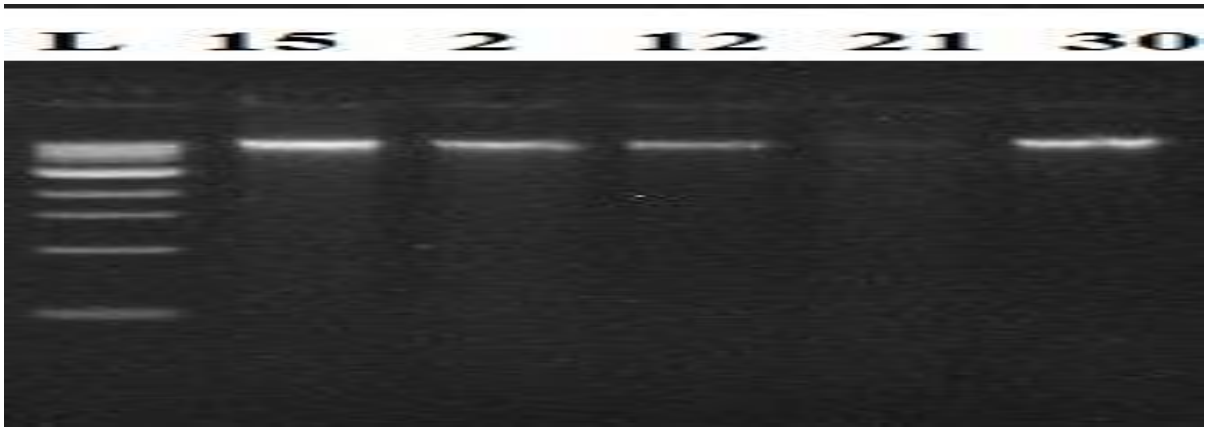

Plate 5: Eviction Potentials of the Extracts against ST311189
15 = ST311189; 2 = AO; 12 = OG; 21 = ZO; 30 = VA

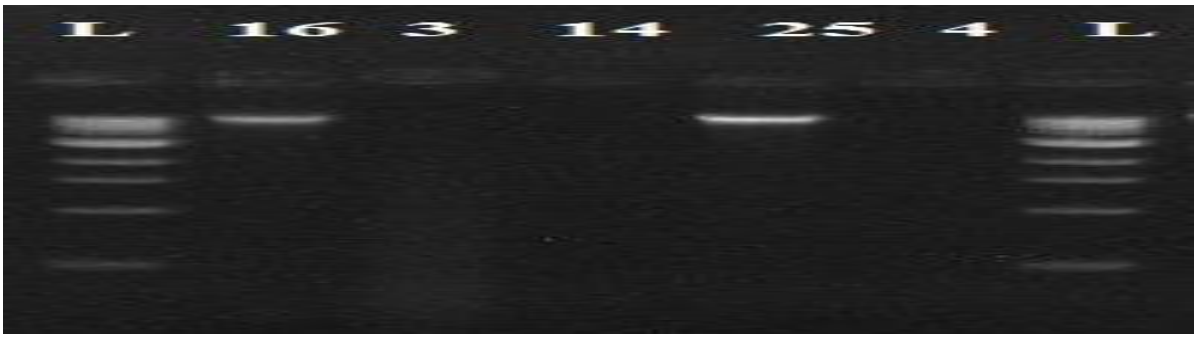

Plate 6: Eviction Potentials of the Extracts against ST2018K
16 = ST2018K; 3 = AO; 14 = OG; 25 = VA; 4 = ZO

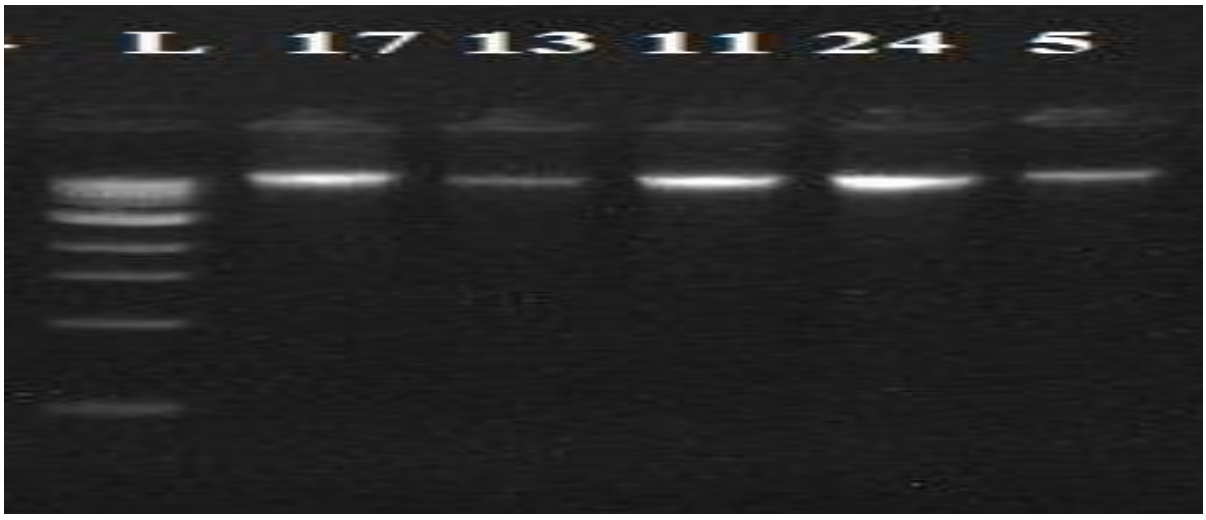

Plate 7: Eviction Potentials the Extracts against STCT18
17 = STCT18; 13 = ZO; 11 = AO; 24 = VA; 5 = OG

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DISCUSSION

The resistance of *Salmonella enterica* subsp.*enterica* serovar Typhi strain CMCST (STCMCST) and WGS1146 (STWGS1146), *Salmonella enterica* subsp.*enterica* serovar Typhi strain ERL12960 (STERL12960), *Salmonella enterica* subsp.*enterica* serovar Typhi strain 311189 (ST311189) and *Salmonella enterica* subsp.*enterica* serovar Typhi strain CT18 (STCT18) isolated from the river, borehole and hospital dumping site to conventional antibiotics agrees with the findings of [4,6,19,20,21] but disagrees with the findings of [22] who isolated a non-resistant *Salmonella* species from recreational aquatic environments.[6] isolated citrate-negative motile Salmonella species and also observed that 68% of the bacterial isolates were resistant to move three antibiotics. The study further pointed out that the resistant antibiotics were mainly AMX, AU, S, PN, CEP, SXT and CPX and similar antibiotics were pointed out in the findings of [6,19] reported high resistance of the isolates to the first line of conventional antibiotics and intermediate susceptibility to CPX.[21] reported that S. *enterica* serovar Typhi was most resistance to penicillin but susceptible to CPX. The multi-drug resistance could be attributed to inappropriate drug prescriptions, indiscriminate antibiotic use among farmers, and patients' failure to complete prescribed treatment regimens.

The study revealed the presence of cardiac glycosides, steroids, alkaloids, tannins, flavonoids, phenolics and saponins from the leaves extracts of *Xylopia aethiopica* (XA), *Zingiber officiale* (ZO), *Azadirachta indica* (AI), *Vernonia amygdalina* (VA) and *Ocimum gratissimum* (OG) and these agree with the findings of many researchers.[6,15,24,25,26,27,28,29,30,31] The researchers also pointed that these phytochemical constituents could be responsible for the activities and other ethno medical potentials attributed to the above studied extracts. Also, the variations in the amount of the phytochemical constituents associated with each extract could be attributed to source and species of the plant involved. The pronounced activities of ZO, OG and VA against the resistant strains of STCMCST, STWGS1146, STERL12960, ST311189, STCT18 and ST2018K could be attributed to the activities of the phytochemical constituents present in the extracts. The significant reduction in resistant strains observed in this study corroborates the findings of previous studies (6, 32–40). Several studies have reported the horizontal spread of antibiotic-resistant genes among bacteria is driven by bacterial plasmid, which promotes evolution of resistance.

Reference 35 reported that multi-drug resistance (MDR) in some Gram-negative bacterial strains may result from the acquisition of mobile genetic elements or intrinsic host-induced mechanisms. In the present study, the plant extracts majorly ZO was able to evict completely or partially the plasmid DNA from the studied isolates. This indicates that most resistance genes are plasmid-encoded rather than chromosomal. [39] reported that the curing ability of the plant extract might be due to its active constituents which may intercalate with the plasmid DNA molecule and inhibit their replication. [6] so reported that the ability of certain plant extracts to significantly reduce resistant Salmonella strains can be attributed to their bioactive constituents. [41] reported that alkaloids are naturally occurring small ligands that can intercalate plasmid DNA or chromosomal DNA and distort their replication depends on potency, concentration, ionic strength, size and structural orientation of the ligand. [42] also reported that phytochemicals in the group of alkaloids, flavonoids and others have multi-drug resistance (MDR)- reversing activity, and these phytochemicals can reverse MDR via affecting the expression or activity of ABC transports and inhibition of replication of plasmid DNA. Also, the phytochemicals can form synergistic interaction with antibiotics which can address the problems of other molecular targets.

CONCLUSION

The study has revealed that *Salmonella enterica* sub sp. *enterica* serovar Typhi strain CMCST (CMCST), WGS1146 (STWGS1146), ERL12960 (STERL12960), CT18 (STCT18) and 2018K (ST2018K) isolated from rivers, boreholes, and hospital dumping sites in Uli, Anambra State, Nigeria, showed significant resistance to conventional antibiotics. The study also showed that *Zingiber officinale* (ZO), *Ocimum gratissimum* (OG), *Vernonia amygdalina* (VA) extracts which contain varying quantities of phytochemicals were able to reduce the number of resistant strains of the studied isolates to zero or an insignificant level, of which ZO was observed to be most effective.

Declarations

***Conflicting Interest Statement*:** We declare that we have no conflict of interest

Ethical Approval: This not applicable in this research

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REFERENCES

1 Iheukwumere IH, Ejike CE. Comparative study of the inhibitory activities of *Ocimum gratissimum* and *Nepeta cataria* against *Salmonella enterica* serovar Typhi and their larvicidal effect against *Anopheles gambiae*. *Afr J Educ Sci Technol.* 2016;5(22):112–118.
2 Jajere SM. A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. *Vet World.* 2019;12(2):9–16. doi:10.14202/vetworld.2019.504-521
3 Ejike CC, Iheukwumere IH, Amadi RE. The effects of *Allium sativum* and *Zingiber officinale* extracts on *Shigella dysenteriae* isolated from ready-to-eat fried chicken sold in Ihiala L.G.A, Anambra State. *J Nat Sci Res.* 2017;7(1):1–5.
4 Iheukwumere IH, Uneze BC, Ejike CE. Efficacy of some selected antimicrobial substances in prevention of enteric bacteria in broiler chicks. *J Biol Agric Healthc.* 2017a;7(4):58–67.
5 Kirby JE. Plasmid eviction to restore susceptibility in carbapenem resistant Enterobacteriaceae. *J Antimicrob Chemother.* 2017;5(1):3–9.
6 Iheukwumere IH, Dimejesi SA, Iheukwumere CM, Chude CO, Egbe PA, Nwaolisa CN, Amutaigwe EU, Nwakoby NE, Egbuna C, Olisah MC, Ifemeje JC. Plasmid curing potentials of some medicinal plants against citrate negative motile *Salmonella* species. *Eur J Biomed Pharm Sci.* 2020;7(5):40–47.
7 Iheukwumere IH, Opara GR, Iheukwumere MC, Okafor CF, Nwakoby NE. Prophylactic potential of essential cream produced from *Chromolaena odorata* leaf extract against *Cladosphialophora bantiana* strain D12E. *IPS J Appl Microbiol Biotechnol.* 2021;1(1):1–11.
8 Cheesebrough M. *District Laboratory Practice in Tropical Countries.* 2nd ed. Part 1. Cambridge: Cambridge University Press; 2010. pp. 132–135. doi:10.1017/CBO9780511581304
9 Iheukwumere IH, Amadi RE, Unaeze BC. Enterotoxigenicity profile of *Salmonella enterica* serovar Typhimurium in suckling albino mice. *J Nat Sci Res.* 2017b;7(14):16–20.
10 Iheukwumere IH, Olusola TO, Chude C. Molecular characterization and diversity of enteric bacteria isolated from chicken feeds. *J Nat Sci Res.* 2018a;8(3):21–33.
11 Iheukwumere IH, Chude C, Unaeze BC. Toxicological study and antibacterial activities of effectively validated medicinal plants against enteric bacteria isolated from chicken feeds. *J Health Med Nurs.* 2018b;7:19–34.
12 Iheukwumere IH, Chukwura EI, Chude C. *In vivo* activities of some selected antimicrobial agents against enteric bacteria isolated from chicken feeds on broiler layers. *J Biol Agric Healthc.* 2018c;9(1):21–36.
13 Chukwura EI, Iheukwumere IH. Larvicidal activity of *Ocimum gratissimum* and *Solenostemon monostachyus* leaves on *Anopheles gambiae*. *J Sci Ind Res.* 2013;72:577–580. *No DOI found.*
14 Arewande JO, Akinnusolu A, Alademeyin JO. Extractive value and phytochemical screening of *Zingiber officinale* and *Curcuma longa* using different solvents. *Int J Tradit Nat Med.* 2018;8(1):13–22.
15 Lawal AR, Olayinka BU, Murtadha RA, Ayinla A, Etejere EO. Comparative analysis of phytochemical and proximate composition of *Allium sativum* and *Zingiber officinale*. *Niger J Basic Appl Sci.* 2018;26(2):82–87. doi:10.4314/njbas.v26i2.12
16 Virshette SJ, Patil MK, Desmukh AA, Shaikh JR. Phytochemical analysis of different extracts of *Azadirachta indica* leaves. *Int J Pharm Sci Rev Res.* 2019;59(1):161–165.
17 Iheukwumere IH, Umedum CU. Effect of *Gossypium hirsutum* leaf extracts on Gram negative bacteria isolated from cervix of females with unexplained infertility. *Afr J Sci.* 2013;14:3261–3270.
18 Iheukwumere IH, Ejike CE. Comparative study of the inhibitory activities of *Ocimum gratissimum* and *Nepeta cataria* against *Salmonella enterica* serovar Typhi and their larvicidal effect against *Anopheles gambiae*. *Afr J Educ Sci Technol.* 2016;5(22):112–118.
19 Phoon YW, Chan YYC, Koh TH. Isolation of multidrug resistant *Salmonella* in Singapore. *Singapore Med J.* 2015;56(8):142–144. doi:10.11622/smedj.2015117
20 Bhetwal A, Maharjan A, Shakya S, Satyal D, Ghinuire S, Khanal PR, Parejuli NP. Isolation of potential phages against multidrug resistant bacterial isolates: promising agents in the rivers of Kathmandu, Nepal. *BioMed Res Int.* 2017;2017:1–8. doi:10.1155/2017/3723254
21 Ekelozie IS, Ekejindu IM, Ochiabuto OM, Obi MC, Onwuasonya UF, Obeagu EI. Evaluation of *Salmonella* species in water sources in two local

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government areas of Anambra state. *Cohesive J Microbiol Infect Dis.* 2018;1(1):1–9. doi:10.31031/CJMI.2018.01.000503

22Tracogna MF, Losch LS, Alonso JM, Merino LA. Detection and characterization of *Salmonella* spp. in recreational aquatic environments in the Northeast of Argentina. *Ambi-Agua.* 2015;8(2):18–26. doi:10.4136/ambi-agua.1566

23 Osabar VN, Baseey FI, Umoh UU. Phytochemical screening and quantitative evaluation of nutritional values of *Zingiber officinale*. *Am Chem Sci J*. 2015;8(4):1–6. doi:10.9734/ACSJ/2015/18365

24 Alexander P. Phytochemical screening and mineral composition of the leaves of *Ocimum gratissimum*. *Int J Appl Sci Technol.* 2016;4(2):161–165.

25 Ebana RUB, Ekanemesang UM, Edet UO, Omoruyi EF. Phytochemical screening and antimicrobial activity of *Xylopia aethiopica* and *Gongronema latifolium* on common pathogens. *J Adv Biol Biotechnol.* 2016;9(4):1–7. doi:10.9734/JABB/2016/27698

26 Ajayi A, Ude AN, Balogun OJ. Quantitative and qualitative analysis of *Moringa oleifera* and *Vernonia amygdalina*. *Agric Biol Sci.* 2017;3(2):51–59.

27 Uzoh CV, Iheukwumere IH, Onyewenjo SC. Prevalence of malaria among registered pregnant women attending antenatal centre at Federal Medical Centre Yenagoa, South south Nigeria. *Int J Adv Res.* 2015;3(12):933–938.

28 Sharma A, Patel S. Preliminary phytochemical screening and quantitative analysis of secondary metabolites of *Mentha arvensis* and *Azadirachta indica*. *Int J Adv Res Dev.* 2018;3(1):114–118.

29 Jumare FM. Quantitative and qualitative phytochemical of Nigerian indigenous *Ocimum gratissimum*. *J Sci Technol Educ.* 2019;6(4):338–344.

30 Owoyale ADN, Galadimma M, Duniyan SY, Adabara N. Quantitative phytochemical analysis and antifungal susceptibility of *Azadirachta indica* against some strains of *Candida albicans*. *J Adv Med Pharm Sci.* 2019;21(4):1–14. doi:10.9734/JAMPS/2019/v21i430133

31 Virshette SJ, Patil MK, Desmukh AA, Shaikh JR. Phytochemical analysis of different extracts of *Azadirachta indica* leaves. *Int J Pharm Sci Rev Res.* 2019;59(1):161–165.

32 Jangle SN, Pattan SR. Effects of PRA-5 on elimination of antibiotic resistance in methicillin resistance *S. aureus* (hospital strain). *Pharmacol Online.* 2010;3:222–228.

33 Khider AK, Marolud SQ. Potentials of aqueous extracts of *Allium sativum*, *Mentha spicata*, *Myrtus communis* and *Thymus vulgaris* as antimicrobials and curing of antibiotic-resistant gene in *Klebsiella pneumoniae*. *J Academia.* 2012;2:16–22.

34 Kumar V, Shriram V, Mulla J. Antibiotic resistance reversal of multiple drug-resistant bacteria using *Piper longum* fruit extract. *J Appl Pharm Sci.* 2013;3(3):112–116. doi:10.7324/JAPS.2013.3320

35 Shriram V, Kumar V, Mulla J, Latha C. Curing of plasmid-mediated antibiotic resistance in multidrug resistant pathogens using *Alpinia galanga* rhizome extract. *Adv Biotechnol.* 2013;13(1):1–6.

36 Srivstara P, Wagh RS, Puranik NV, Puntanbekar HM, Jahagirdar SS, Dharkephalkar PK. *In vitro* plasmid curing activity of aqueous extract of *Terminalia chebula* fruit against plasmids of *Bacillus subtilis* and *Shigella sonnei*. *Int J Pharm Sci Rev Res.* 2014;7(4):298–301.

37 Mohite JA, Soman YP, Mujumdar SS. Herbal extraction and plasmid curing of multiple drug resistance in bacteria using herbal extracts. *World J Pharm Res.* 2015;5(1):1554–1565.

38 Abdurahman ZFA, Barzani KKN, Rasheed AA. Plasmid profile, curing analysis and antibacterial activity of *Alcea arbelensis* plant against multiple drug resistance *Staphylococcus aureus*. *Tikrit J Pure Sci.* 2016;21(6):32–40. *No DOI found.*

39 Akinyemi KO, Fakurede CO, Iwaloku BA, Oyetolu AO. Activities of three Nigerian medicinal plants against plasmid carrying enteric bacterial pathogens. *Ecronicon Microbiol.* 2017;5(1):10–21.

40 Orhue PO, Okoebur FO, Momoh MA. Pre and post plasmid curing effect on *Pseudomonas aeruginosa* susceptibility to antibiotics. *Am J Curr Microbiol.* 2017;5(1):1–9. doi:10.9734/AJRC/2017/35294

41 Correa YM, Nino J, Mosquera OM. DNA interaction of plant extracts from Colombian flora. *Pharm Biol.* 2007;45(2):111–115. doi:10.1080/13880200601113084

42 Tinoush B, Shirdel I, Wink M. Phytochemicals: potential lead molecules for MDR reversal. *Front Pharmacol.* 2020;11:1–35. doi:10.3389/fphar.2020.00832

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