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Activities of Three Nigerian Medicinal Plants Against Plasmid-Carrying Enteric Bacterial Pathogens

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Abstract

Background: Three medicinal plants *Danielliaoliveri, Lophiraalata* and *Lanneawelwitschii* were tested for in-vitro antibacterial activity and their plasmid eviction potentials on enteric pathogens.

Method: Fresh plant materials collected from herbs seller were sun dried and grounded to fine powder. Water and methanol extracts of these plants were obtained by standard methods. Enteric bacterial agents from clinical samples were used. *In-vitro* antimicrobial susceptibility testing of the bacterial isolates was carried out using commercial antibiotics and the crude plant extracts by standard procedures. Phytochemical screening of the plant extracts was carried by the standard procedure. Plasmid DNA extraction was performed using the alkaline lyses method.

Result: The crude methanol extracts of all the plants were effective on MDR strains of *Salmonella enteric serovar Typhi, S. Typhimurium, Escherichia coli, Shigella spp* and *Klebsiella spp* used in this study MIC and MBC values of 0.625 to 10 mcg/ml and 5.0 to 20 mcg/ml respectively were recorded for *L. welwitschii*, 10 to 20 mcg/ml and 20 to40 mcg/ml for D. oliveri, and 10 to 20 mcg/ml and 20 to 40 mcg/ml were recorded for *L. alata* respectively. The plasmid eviction potentials of the crude plants extracts indicated that only crude extract of *L. welwitschii* exerted anti-plasmidic effect on *Shigella* spp, *Klebsiella* spp, *S. Typhimurium*, and *S. Typhi*, as over 60% of these bacterial strains loss their plasmid DNA.

Conclusion: This study revealed that methanol extracts of the three medicinal plants were active against enteric bacterial pathogens. The extracts of *L. alata* and *D. oliveri* were not active against MDR pathogens thus, their continuous usage should be discouraged. The methanol crude extract of *L. welwitschii* exerted anti-plasmidic effect on MDR enteric pathogens. This finding suggests possibility of a new approach in drug formulation using anti-plasmidic plant extracts for effective management of plasmid encoded drug resistant enteric bacteria-associated diseases.

Keywords: *Plant; Extract; MDR; Pathogens; Plasmid; Curing; Phytochemical*

Background

Enteric infection is an increasingly recognized array of bacterial; parasitic and viral pathogens which can profoundly disrupt intestinal function with or without causing overt dehydrating diarrhoea. Some of the organisms implicated in enteric infection include *Salmonella* spp; *Shigella* spp; *Proteus* spp; *Klebsiella* spp; *Staphylococcus aureus* and *Escherichia coli*. Enteric pathogen has been reported as the major causative agent of sporadic and endemic diarrhoea both in children and in adult [1]. Drugs like aminoglycosides; cephalosporins; and fluoroquinolone have antibacterial effects against enteric pathogens [2]. The evolutionary pressure from the use of antibiotics has played roles in the development and the spread of multidrug resistance among different bacteria species. Also; extra-chromosomal genetic elements (plasmids) harboured by some bacterial agents had been responsible for increased resistant development either horizontally or vertically [2]. The use of medicinal plants and herbs in curing diseases has always been part of human culture and has transcended all

social; cultural; economic; religious and other barriers created by man. It has been established that the plants which naturally synthesis and accumulate some secondary metabolites possess medicinal properties [3].

In Nigeria; many hundreds of plants are used in traditional medicine as treatment for bacterial infections; some of these plants have been subjected to an *in-vitro* screening but the efficacy of such herbal medicine has seldom been rigorously tested in controlled clinical trials. Plants have been major source of medicine and plants secondary metabolite has been attributed for most plant therapeutic activities [4,5]. *D. oliveri* is of medicinal important value being potent in treating gastrointestinal ailment [6]; as anti-bioficient agent in pregnancy; pain killer; skin; mucosa and sedative [7]; for the cure of rheumatism pain [8] and active as an antimicrobial agent [9,10]. In traditional African medicine; *L.welwitschii* is used for swellings; oedema; gout; haemorrhoids and emesis [11]; antidiarrheal [12]; and antipurgative [13]. While in South West Nigeria *L.alata* is use for the treatment of typhoid fever [13] and for the treatment of fever; cough; jaundice and gastrointestinal disorder [14]. Therefore; a need to search and develop newer; safer; effective and cheaper antimicrobial agent from natural products such as medicinal plants that will compliment synthetic antimicrobial drugs against multiple drug resistant pathogens is of paramount importance. This study was undertaken with a view to determining the antimicrobial activity of three medicinal plants and their plasmid curing ability on plasmid-mediated multiple drug resistant enteric bacterial pathogens.

Materials and Methods

Plant materials

Fresh plant materials of *L.welwitschii; D.oliveri* and *L.alata* were collected from herbs seller in Itoku market; Abeokuta south local government Area of Ogun state Nigeria. The identification and authentication of these plants were carried out by Mr. O.KOluwa and voucher specimens' numbers were deposited in the herbarium of the Botany department of the Lagos State University; Lagos; Nigeria.

Extraction

Stem bark of *D. oliveri* (A) Rolfe. Hutch & Dalz; *L.welwitschii* (B) Hiern; and *L.alata* (C) Bank ex f. Gaertn.f were sun dried for one week and were grounded to fine powder using pestle and mortar and were stored at room temperature in a clean dry air tight bottle for further use.

Methanol extracts (Cold)

The methanol extractions of these plants were carried as described by Akinyemi., *et al.* [15] In brief; twenty grams of the powdered plant materials A; B; and C were weighed and soaked in separate beakers containing 200 ml of 70% methanol each. The beaker was wrapped with aluminium foil and sealed with tape. The mixture was shaken vigorously and left to stand overnight (24 hours) in a refrigerator maintained at 2 to 8°c. The mixture was filtered using a clean muslin cloth. The filtrate was evaporated to dryness in a hot air oven maintained at 40°c for 72 hours. The crude extracts were scrapped and weighed to determine the yield. The crude extracts yield was dispensed in a sterile container and labelled as methanol extracts and designated as AM (*D. oliveri*); BM (*L. welwitschii*); and CM (*L. alata*) respectively.

Aqueous extract (Cold)

The aqueous extraction method was as described by Akinyemi., *et al* [15]. In brief; twenty grams of the powdered plant materials A; B; and C were weighed and soaked in separate beakers containing 200 ml of distilled water each. The beaker was wrapped with aluminium foil and sealed with tape. The mixture was shaken vigorously and left to stand overnight (24 hours) in a refrigerator maintained at 2 to 8°c. The mixture was filtered using a clean muslin cloth. The filtrate was evaporated to dryness in a hot air oven maintained at 40°c for 72 hours. The crude extracts were scrapped and weighed to determine the yield. The crude extracts yield was dispensed in a sterile container and labelled as water extracts and designated as AW (*D. oliveri*); BW (*L. welwitschii*); and CW (*L. alata*) respectively.

Bacterial cultures

The bacteria used in this work were obtained from the Nigerian Institute of Medical Research (NIMR); Yaba; Lagos; Nigeria and were all subjected to standard microbiological methods maintained on Tryptone soya agar petri dishes at 2 to 8°C for 24 hrs before use. These bacteria include *Salmonella enteric serovar Typhi; S. Typhimurium; Escherichia coli* ATTCC 25922; *Shigella* spp; and *Klebsiella* spp; and were isolated from stool samples of patients diagnosed of gastro intestinal infections.

Antimicrobial susceptibility testing

Agar disc diffusion assay: *In-vitro* susceptibility of the bacterial isolates to the plant extract was tested for lead antimicrobial response by the standard disc diffusion technique using guideline established by the NCCLS 2002 [16]. A standardized Inoculum of 1.0 x 107 with 0.5 McFarland standards was used to evenly inoculate the surface of Mueller-Hinton agar plate (Oxoid; UK). Sterile paper discs previously soaked in known concentration (5; 20; and 40 mcg/ml) of extracts were carefully placed at different spot on agar plate containing the organisms used and the discs were gently pressed down to ensure contact. The plates were incubated aerobically at 37°C for 18 to 24 hours. The diameter of the zones of inhibition were measured with a ruler and interpreted according to the guidelines provided by the National Committee for Clinical Laboratory Standards [16].

Plasmid DNA extraction

Plasmid extraction was performed by a simplified alkaline lyses method described by Birnboim and Doly [17]. Overnight culture (1.5 ml) was centrifuged at 5; 000 rpm for 1 minute to pellet the cells. The supernatant was gently decanted. After washing; the cell pellet was re-suspended in 300 µl Tris (tris(hydroxyl methyl)aminomethane)-EDTA (ethylenediaminetetraacetic acid)-NaOH-SDS (sodium dodecyl sulfate) (TENS) buffer solution and mixed by gentle inversion (five times) within 3 – 5 minutes of incubation on ice to obtain a straw-like; sticky lysate. This was followed by neutralization by adding 200 µl of 5 M potassium acetate buffer (pH 5.2); followed by incubation on ice for 10 minutes. Plasmid DNA solution was recovered as supernatant after centrifugation at 10; 000 rpm for 10 minutes. Plasmid pellets isolated with ice-cold absolute ethanol were washed in 70% ethanol before re-suspension in 20 – 40 µl Tris (10 mM)-EDTA (1 mM) buffer (pH 8.0). The plasmid DNAs were separated by electrophoresis on 0.8% agarose pre-stained with ethidium bromide (0.5µg/ml); the plasmid DNAs were visualized on high performance ultra violet transilluminator and photographed. Molecular weight of the plasmids was calculated based on the DNA maker.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) Broth dilution method

MIC and MBC of the effective extract was determined by broth dilution method. Equal volume of plant extract and basal medium (Tryptone soya broth) was mixed in series of test tubes. Standardized Inoculum 106CFU/ml was added to series of test tubes with the aid of a Micropipette. Incubation was carried out aerobically at 37°C for 18 to 24 hrs. Two control tubes were maintained for each test batch. These include tubes containing plant extract and growth medium without Inoculum (antibiotic control) and tube containing the medium; physiological saline and the Inoculum (organism control) in the test tubes. The lowest concentration (highest dilution) of the extract permitting no visible growth (any turbidity) when compared with the control tube was considered as the MIC. However; the MBC was determined by sub culturing the test dilution on a Tryptone soya agar and was further incubated for 18 - 24 hrs at 37°C. The lowest concentration that is highest dilution of the extract that yields no single bacterial colony on solid medium was regarded as MBC.

Phytochemical screening methods

Test for alkaloids: One ml of 1% Hydrochloric acid was added to 3ml of extract in a test tube. The mixture was heated for 20 minutes. It was cooled and filtered. Two drops of Mayer's reagent was added to the filtrate [18].

Test for tannins: Half a gram of extract was added to 20 ml of distilled water. The suspension was properly mixed and filtered. 3 drops of 5% ferric chloride ($FeCl_3$) was added to the filtrate [18].

Test for flavonoids: One gram of extract was added to 10 ml of ethyl acetate and mixed. 1ml of diluted ammonia was added to the mixture [18].

Test for saponins: One gram of extract was added to 10 ml of distilled water. The mixture was shaken vigorously [18].

Anthraquinones: Five gram of the extracts was added to 10 ml of ether and mixed. 1ml of ammonia solution was added to the filtrate [18].

Results

The profile of the three medicinal plants *D.oliveri*; *L.welwitschii*; and *L.Alata* used in this study is indicated in table 1. Antimicrobial activities of the crude plants extract against the plasmid-carrying MDR bacterial strains are shown in table 2. At 5 to 40 mcg/ml concentration; the methanol extracts of *L.welwitschii;* recorded the highest zone of inhibition against all the strains of organisms used. The highest zone of inhibition recorded was against *S. Typhimurium* with a diameter of 14 mm at 5 mcg/ml; 18.0 mm at 20 mcg/ml and 22 mm at 40 mcg/ml respectively.

Botanical name	Family name	Local name	Plant part used	Voucher number
Daniellia oliveri (Rolfe) hutch &Dalz	Fabaceae	Ogbogbo	Stem	LSH1200
Lannea welwitschii (Hiern)	Anacardiaceae	Orira	Stem	LSH1201
Lophira alata (Banks ex f. Gaertn.f)	Ochnaceae	Ponhan	Stem	LSH1202

Table 1: *Profile of the three medicinal plants used.*

The zones of inhibition of the aqueous extracts against the organisms used were lower than the corresponding methanol extract. At 5mcg/ml; the crude extract of *L. alata* recorded no zone of inhibition against the strains of organisms used except for the standard strain *E.coli* ATCC25922 (Table 2). The MIC recorded in this study ranged from 0.625 to 20.0 mcg/ml and MBC ranged from 5.0 to 40.0 mcg/mcg/ml. *L.welwitschii* recorded the lowest MIC and MBC values against the organisms used; while *L.alata* recorded the highest MIC and MBC values (Table 3).

Plant used	Bacterial pathogens									
	***S. Typhi* (n-12)**		***S. Typhimurium* (n-10)**		***Shigella spp* (n-8)**		***E.coli* ATCC25922**		***Klebsiella spp* (n-10)**	
	AE	ME	AE	ME	AE	ME	AE	ME	AE	ME
5mcgml										
D. oliveri	-	-	-	6.0	6.0	8.0	6.0	8.0	-	-
L. welwitschii	8.0	10. 0	10.0	14.0	-	6.0	8.0	12.0	8.0	10.0
L. alata	-	-	-	8.0	-	-	5.0	8.0	-	-
20mcg/ml										
D. oliveri	6.0	8.0	-	8.0	8.0	12.0	12.0	14.0	-	-
L. welwitschii	12.0	16.0	14.0	18.0	-	8.0	14.0	18.0	10.0	14.0
L. alata	8.0	10.0	-	12.0	-	-	8.0	10.0	-	-
40mcg/ml										
D. oliveri	10.0	12.0	8.0	14.0	12.0	14.0	14.0	18.0	-	10.0
L. welwitschii	14.0	20.0	18.0	22.0	8.0	12.0	18.0	24.0	12.0	18.0

L. alata	10.0	14.0	-	14.0	-	-	10.0	12.0	-	-
Ciprofloxacin 10µl	16.0		14.0		10.0		18.0		12.0	

Table 2: *Antibacterial activities of plants extracts on plasmid-carrying MDR enteric bacterial pathogens.*

Activity key*: figures indicate average diameter of zone of inhibition in mm*

- *= No inhibition; AE = aqueous extract; ME = methanol extract; N = number of isolate tested; Ciprofloxacin = commercial antibiotics used as control; E. coli ATCC 25922 = Standard control strain; MDR= multidrug resistant strain*

Plant	***S. typhi***		***S.typhimurium***		***Shigella spp***		***E.coli* ATTCC25922**		***Klebseilla spp***	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
					In mcg/ml					
D. oliveri	ND	ND	10.0	40.0	20.0	40.0	10.0	20.0	ND	ND
L. welwitschii	0.625	5.0	5.0	10.0	10.0	20.0	5.0	10.0	10.0	20.0
L. alate	20.0	40.0	20.0	ND	ND	ND	10.0	20.0	ND	ND

Table 3: *Inhibitory and bactericidal concentration of plant extract on MDR- strains of enteric bacterial pathogens.*

Key: MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration

ND = not determined; MDR = multidrug resistant strains

The result of the phytochemical analysis of the plant extracts revealed the presence alkaloids; tannins; flavonoids in all the plant extracts used. Saponins were found in *D. oliveri* and *L. alata* while anthraquinone was found in both *D. oliveri* and *L. welwitschii* (Table 4).

Lane	**Isolate**	**Number of plasmid before exposure**	**Sizes, kb**	**Number of plasmid after exposure**	**Sizes, kb**	**Lost rate**
1	10 kb Plasmid DNA Maker	4	10,9,7,6	4	10,9,7,6	
2	*Salmonella Typhi*	1	25	1	25	0%
4	*Salmonella Typhi*	1	15	0	0	100%
6	*Salmonella Typhi*	4	25, 23,6, 5	1	25	75%
8	*Salmonella Typhi*	3	25, 23, 5	1	25	66.7%
9	*Salmonella Typhi*	3	25, 23, 5	2	25,23	33.3%
14	*Salmonella Typhi*	4	10, 9, 7, 6	0	0	100%
17	*Salmonella Typhi*	1	5	0	0	100%
19	*Salmonella Typhi*	2	23,4	1	23	50%
20	*Salmonella Typhi*	1	5	0	0	100%
21	*Salmonella Typhi*	1	5	0	0	100%
3	*Salmonella Typhimurium*	3	10, 9, 8	1	10	75%
4	*Salmonella Typhimurium*	2	10, 9	0	0	100%
5	*Salmonella Typhimurium*	2	9,8	0	0	100

7	*Salmonella Typhimurium*	3	9,8,7	0	0	100%
8	*Salmonella Typhimurium*	2	9,8	0	0	100%
9	*Salmonella Typhimurium*	3	9,8.5	0	0	100%
10	*Salmonella Typhimurium*	3	10,9,8	0	0	100%
12	*Salmonella Typhimurium*	3	9,8,7	0	0	100%
1	*Shigella sp*	2	23,15	1	23	50%
2	*Shigella sp*	1	10	0	0	100%
3	*Shigella sp*	1	9	0	0	100%
5	*Shigella sp*	2	12,15	0	0	100%
7	*Shigella sp*	3	15,10,8	1	15	66.7%
8	*Shigella sp*	1	10	0	0	100%
9	*Shigella sp*	1	7	0	0	100%
11	*Shigella sp*	2	12,10	0	0	100%
12	*Klebsiella sp*	1	8	0	0	100%
13	*Klebsiella sp*	1	15	0	0	100%
14	*Klebsiella sp*	3	25,20,10	2	25,20	33.3%
16	*Klebsiella sp*	2	15,8	0	0	100%
17	*Klebsiella sp*	1	14	0	0	100%
19	*Klebsiella sp*	1	12	0	0	100%
21	*Klebsiella sp*	1	12	0	0	100%
24	*Klebsiella sp*	2	10,8	0	0	100%
25	*Klebsiella sp*	1	15	1	15	0%
26	*Klebsiella sp*	2	23,15	2	23,15	0%

Table 4: *Plasmid analysis of the isolates before and after exposure to methanol extract of Lanneawelwitschiiat 10 mcg/ml (MIC).*

The results of the plasmid eviction potentials of the plants extracts are as shown in table 5. All the strains of *S. Typhimurium* had 100% loss rate. Significant plasmids loss was recorded in all the strains of organisms used except for two strains of *Klebseilla* spp and a strain of *S. Typhi* that had 0% loss rate (Figures 1&2).

Plant screened		Components									
Plant species	Plant part	Alkaloids		Tannins		Flavonoids		Saponins		Anthraquinones	
		ME	AE	ME	AE	ME	AE	ME	AE	ME	AE
D. oliveri	Stem	++	++	+++	+++	+	+	+++	++	++	+
L. welwitschii	Stem	+	+	+++	++	+++	+++	-	-	+++	++
L. alata	Stem	+	+	+++	+++	+++	++	+	+		

Table 5: *Phytochemical analysis of the three-medicinal plant extracts used.*

Key: *+++ Appreciable amount*

++ Moderate amount

+ Trace amount

- Complete absence

ME: Methanol extract

AE: Aqueous extract

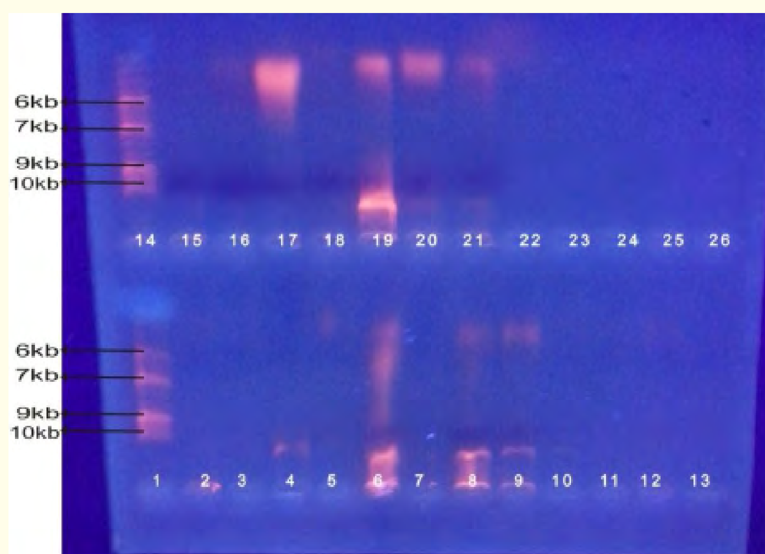

Figure 1: *Representation of Plasmid analysis before the introduction of the extract of Lannea welwitschii Lane 1=10 kb Plasmid DNA Maker; lane 2= 25kb S. Typhi; lane 3 = S.Typhi lane 4=15kb S. Typhi; lane 5= S .Typhi lane 6=25; 23.6; 5 kb S. Typhi; lane 7= S. Typhi Lane 8=25; 23; 5kb STyphi; lane 9= 25; 23; 5kb lane 10= S. TyphiSTyphi; lane 11-13= S. Typhi lane 14=10; 9; 7 S.Typhi; lane 15-16= S.Typhi lane 17= 5kb lane18= S.Typhi lane 19= 23; 4kb S. Typhi; lane 20= 5kb S. Typhi lane21= 5kb S. Typhi.*

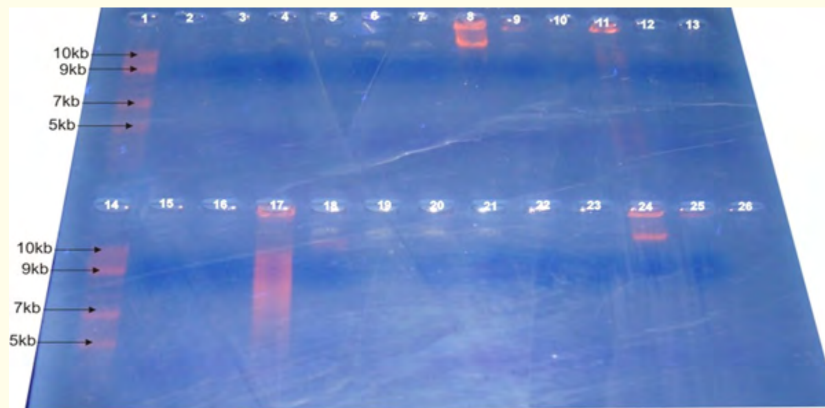

Figure 2: *Representation of plasmid Analysis after the introduction of the extract of Lannea welwitschii Lane1 = 10 kb Plasmid DNA Maker, lane 2 = S. Typhi, lane 3 = S. Typhi, lane 4 = S. Typhi, Lane 5 = Shigella spp, lane 6 = Shigella spp, lane7 = Shigella spp, Lane 8 = 25, 23kb S Typhi, lane 9 = 25kb Klebseilla spp, lane10 = Klebsiella spp, lane11 = 25kb S. Typhi, lane12 = Klebsiella spp, lane13 = Klebsiella spp, lane14 = 10, 9, 7. 5kb S. Typhi, lane15 = Shigella spp, lane16 = Shigella spp, lane17 = 25, 23, 6, 5kb S. Typhi, lane18 = 5kb S. Typhi, lane19 = 5kb S. Typhi, lane 20 = Shigella spp, lane21 = Klebsiella spp, lane 22 = Klebsiella spp, lane 23 = Klebsiella spp, lane 24 = 25, 23kb S. Typhi, lane 25 = 25kb Klebsiella spp, lane 26 = Klebsiella spp.*

Discussion

Medicinal plants are considered as a potential source of new chemotherapeutic drugs because of their diverse phytochemicals. In this study antimicrobial activities of the crude plant extracts against the plasmid-carrying MDR bacterial strains were carried out at 5 to 40 mcg/ml concentration. The methanolic extract of *L. welwitschii* at 10 mcg/ml was found to be active against strains of *S. Typhi*; *S. Typhimurium; E. coli; Shigella* spp and *Klebsiella* spp High zone of inhibition was recorded against *S. Typhimurium* with a diameter of 14mm at 5mcg/ml; 18.0mm at 20 mcg/ml and 22 mm at 40 mcg/ml respectively. This result was consistent with previous studies conducted elsewhere where increase in the antimicrobial activities of the plant extract against the MDR organisms were reported to be attributable to the increase in the concentration and the nature of the active components. Also; that the active components are water soluble and are easily extracted by water [15,19,20]. Traditionally; the plant is soak in water for days; thereafter ready for drinking for the treatment of patients with gastro-intestinal disorder. Our findings therefore further corroborated the preference of water extract of the plants by traditional medical practitioner. Generally; the MIC of the three medicinal plants evaluated in this study ranged from 0.625 to 20.0 mcg/ml and MBC from 5.0 to 40.0 mcg/mcg/ml. The MIC values of the three extracts were lower than the MBC value indicating that; these plants are bacteriostatic at lower concentration and bactericidal at higher concentration; an assertion that had been documented by several workers [9,15,21]. Inactivity of some of extracts at lower concentration was observed in this study. This was probably attributed to the presence of plasmid in most of the organisms used. In this study; no zone of inhibition was recorded against MDR-enteric pathogens tested at 5mcg/ml concentration of the crude extract of *L. alata*. This however; indicated complete inactivity of the extract except for the standard strain of *E.coli* ATCC25922 used; where certain level of activity was recorded. Similarly; both the aqueous and methanol extract of *D. oliveri* and *L. alata* exerted little activity against most of the strains of MDR enteric pathogens tested. It should be noted that *L.alata* is traditionally used for the treatment of typhoid fever and *D. oliveri* for the treatment of dysentery in Abeokuta; Ogun state Nigeria. The implication of this is that; these two medicinal plants are not effective for the treatment of MDR-enteric bacterial–associated disease; thus their continuous usage as infusion for the treatment of typhoid fever patients should be discouraged.

The phytochemical analysis of the aqueous and methanol extracts of *L.welwitschii*; *D. oliveri* and *L. alata* showed the presence of bioactive compound such as alkaloid; saponins; tannins; flavonoids and anthraquinones which had been documented to inhibit intestinal mobility in a dose related manner [12,19,22]. The extract of *L. welwitschii* has also been reported by Amole., *et al.* [12], to produce a movement at standard charcoal meal in the small intestine; suggesting an antispasmodic activity. The effect of *L. welwitschii* against enteric pathogens as observed in this study may be due to the presence of appreciable amount of tannins; anthraquinones and flavonoids as indicated in the result of the phyto-chemistry. Flavonoids had been reported to be hydroxylated phenolic substances linked to an aromatic ring and are known to be synthesized by plants in respond to microbial infection [23]. Their activity is probably due to their ability to complex with extracellular soluble proteins and bacterial cell walls. Flavonoids; also have been reported to disrupt microbial membranes [24].

In this study; plasmid eviction potentials of the plant extracts against MDR- bacterial pathogens were evaluated. All the strains of *S. Typhimurium* had 100% loss rate. For example; *Shigella* spp had 100% loss rate. Seven out of the ten strains of *Klebseilla* spp had 100% loss rate. Generally; significant plasmids loss was recorded in all the strains of organisms used except for two strains of *Klebseilla* spp and *S. Typhi* with no antiplasmidic effect observed because of 0% loss rate of Plasmid DNA (Figures 1 and 2). It was also observed that high molecular weight plasmid DNA was not evicted (Table 4). Nevertheless; antimicrobial activity and DNA intercalation properties of the active constituents had been documented in literature [25]. The curing ability of this plant extract might be due to its active constituent; which may intercalate with the DNA molecule and might have inhibited plasmid replication selectively at sub-MIC concentration (bellow the MIC of 10mcg/ml used).Curing ability of some medicinal plants on plasmid-encoded MDR- pathogens had been reported [26,27,28]. Also; plant derived curing agents; plumbagin; 5-hydroxy-2-methyl-1; 4-nepthoquinone isolated from *Plumbago zeylanica* had been reported to cure R-plasmids in *E. coli*. [29] In another study by Beg., *et al.* [30], the alcoholic extract of *P. zeylanica* cured R-plasmid harbouring *E. coli* with 14 per cent. Recently anti-plasmid activity of essential oils was reported by Schelz., *et al* [31]. The antiplasmidic activity of medicinal plants seems to be a possible novel effective curing agents that may pave the way for further drug research. Therefore the anti-plasmidic effect of *L. welwitschii* observed in this study calls for further investigation; as this plant may be a useful natural resource; for the development of novel drug; that will complement the orthodox antimicrobials against increasing emerging trends of MDR bacterial pathogens .

Conclusion

This study revealed that methanol extracts of the three medicinal plants were active against enteric bacterial pathogens. It was observed that extracts of *L. alata* and *D. oliveri* were not active against MDR-enteric bacteria–associated diseases; thus their continuous usage should be discouraged. Also; crude methanolic extract of *L. welwitschii* exerted anti-plasmidic effect on MDR enteric bacterial pathogens. The present result has offered organic extracts of L. welwitschii as a plasmid curing agent. It also suggests possibility of a new approach in drug formulation using anti-plasmidic plant extracts for effective management of plasmid encoded drug resistant enteric bacteria-associated diseases. Further studies are required using modern methods for characterizing resistance elements to assess the effects of extract of *L. welwitschii* on clinically significant enteric bacteria.

Competing Interests

The Author declared that there is no competing interest.

Autour's Contributions

Christopher O Fakorede: Coordinated the isolation; screening of the enteric pathogens; carried out the plant extraction; participated in the susceptibility testing and drafted the manuscript. Dr Akeeb O Oyefolu participated in antimicrobial susceptibility testing and plant extractions.

Kabir O Akinyemi: Conceived the study; designed; coordinated the study; and the overall manuscript drafting and editing;

Bamidele A Iwalokun: Carried out the phytochemical analysis of the plant extracts and the plasmid analysis of the enteric pathogens used

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