INVESTIGATIONOF THE ACUTE TOXICITY AND *In vivo* ANTIDIARRHOEAL ACTIVITY OF THE ETHANOL EXTRACT AND FRACTIONS OF *Ipomoea triloba* BY MODEL INFECTION AND PROTECTION TESTS IN MICE AGAINST SELECTED CLINICAL DIARRHOEAGENIC BACTERIAL ISOLATES

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ABSTRACT

Aim: This research work was aimed at investigating the acute toxicity and *in vivo* antidiarrhoeal activity of the extracts and fractionsof *Ipomoea triloba*, a plant used locally for the treatment of acute infectious diarrhoea in order to investigate its safety profile.
Study design:20 diarrhoeaicClinical stool samples were collected and implicating organisms were isolated.
Place and Duration of Study: Department of Pharmaceutical Microbiology and Biotechnology, University of Uyo AkwaIbom State, South-South Nigeria between May 2021-December, 2022.
Methodology: Antibiogram, Plasmid curing and Intraperitoneal assay of acute toxicity and mouse protection test of the ethanol extract of plant were carried out on the bacterial-diarrhoeaic isolates.
Results: Antibiogram by the agar-diffusion technique indicated a predominant resistance by the isolates. Plasmid curing assessment for antidiarrhoeal activity indicated a predominantly plasmid-borne resistance. Combined antidiarrhoeal activity of the ethanol extract and fractions of *Ipomoea triloba* with standard antibiotic (tetracycline), assessed by the activity index profile (AIP), indicated a reduction in the predominance of antagonistic, 76 (95 %), indifference, 2(2.5 %), additive, 1 (1.25 %) and synergistic, 1 (1.25 %) after curing. Acute toxicity (LD_{50}) assay in albino mice (15.0-18-0 g body weight) indicated that the ethanol extract of *Ipomoea triloba* showed relative non-toxicity with an estimated value of 946.68 mg/kg body weight. *In vivo* antidiarrhoeal activity and protection efficacy of the ethanol extract assayed by mouse protection test (MPT) infection model offered appreciable (50.0 - 83.3 %) protection to the bacterial-diarrhoeaic isolates challenged mice, compared withthe relatively moderate (33.3 - 50.0 %) protection by the control.
Conclusion: There is need for further studies to elucidate the active principles responsible for the antidiarrhoeal activity of *Ipomoea triloba,* this then can be introduced into the treatment regimen as a plausible remedy against acute hypersecretory infectious diarrhoea because of its safety profile which has been established in this work.

Keywords: Bacterial-diarrhoeaic isolates, plasmid curing, antibiogram, mouse protection test

1. INTRODUCTION

Background

Ipomoea triloba is one of the plants used in folklore in Nigeria to stop acute diarrhoeaic episodes. Its leaves are cooked and eaten as a vegetable and a decoction of the leaves is used against stomach ache in Benin, West Africa. (Achigan-Dako *et al.,*2010). It is used in peninsula Malaysia as a poultice against headache (Dibiyantoro, 2001).

Due to the resistance built up by microorganisms to conventional medications and the high cost of medical care as well as access to health care facilities, the World Health organization has given calls to access and validate herbal remedies and eventually incorporate them into the treatment regimen so as to combat the rising resistance by microorganisms. This will then call for safety experiments to be carried out both *in vitro* and *in vivo.* Hence, this research was targeted in this direction.

Acute toxicity (LD_{50}) test refers to the lethal dose of a drug that can cause the death of 50% of the experimental animals used for the study. Acute toxicity tests are used to determine the lethal dose/concentration of a substance that causes death in 50% of the test population (LD/LC50) during short-term exposure. They are the only standardized toxicity tests where death is the intended endpoint.

Model Mouse protection test involves the use of albino mice to investigate the potential of plant extracts or fractions to protect the animals which had a prior exposure to the infectious agent- parasite, bacteria etc. using doses between the minimum inhibitory concentration (MIC) and the acute toxicity (LD_{50}) test value.

Plasmid curing refers to the removal of plasmids which are generally known to confer resistance to antibacterial agents to bacteria. There are three types of antibiotic resistance namely natural (intrinsic or innate), acquired and mutational (Ashraf and Shah, 2011). Most of the genetic determinants that confer resistance to antibiotics are located on plasmids. Acquired antibiotic resistance is generally mediated by extrachromosomal plasmids and transferable to other bacteria in the environment with the aid of vertical gene transfer. (Manjusha and Sarita, 2011)

When treating infectious diseases, one of the greatest challenges encountered is plasmid-mediated resistance. Plasmid-curing agents aid in the elimination of plasmids thereby reducing the spread of antibiotic resistance encoded by antibiotic-resistance plasmids (R-plasmids) (Molnar *et al.*, 2003). Resistance is usually classified as “chromosomal” when unaffected by plasmid curing and as “plasmidial” when affected.

Several methods can be employed to achieve plasmid curing (eliminate plasmids). Plasmid curing can occur naturally by cell division or by treating the cells with any physical or chemical agents

such as acridine orange, ethidium bromide, sodium dodecyl sulphate (Elias *et al*., 2013). The mechanism of plasmid curing starts from the inhibition of plasmid replication which resulted from a single nick, outside of the replication origin of the superhelical structure. The process leads to further relaxation of plasmid DNA, an increase in melting point and circular dichroism. The intercalating agents would then break the superhelical form of plasmid DNA subsequently forming an open circular or linear form plasmid DNA (Spengler *et al*.,2006).

Acridine orange and ethidium bromide are intercalating agents and have been employed successfully in curing bacterial plasmids whose mechanism of action is based on preferential inhibition of plasmid replication. It involves the use of overnight bacterial cultures inoculated into enrichment broth, tryptic soy broth or Luria Bertani broth. The concentration of these curing agents which is added to the culture broth must range from 0.1 to 0.5 mg/ml; the concentration being dependent on the type of organism and the curing agent used. These cultures are then incubated overnight at 35 ^{o}C or 37 ^{o}C under constant agitation. After the treatment, antibiogram assays will be performed again to determine the phenotypes of the antibiotic resistance (Vengadesh *et al.,*2015)

Objectives

a. To evaluate possible antibacterial potential of the ethanol extract and fractions on plasmid-borne resistant diarrhoeal isolates.
b. To evaluate the combined antibacterial activity of ethanol extract and fractions of *Ipomoea triloba* with standard antibiotics on both cured and non-cured diarrhoeal isolates.
c. To determine the acute toxicity (LD_{50}) of the ethanol extract of *Ipomoea triloba* and *in vivo* antidiarrheal protection efficacy using albino mice.

Research Hypotheses

i. There is no significant difference in the inhibition zone diameters before and after plasmid curing of the bacterial-diarrhoeal isolates.
ii. There is no significant difference in the nature of interactions between the ethanol extract and fractions of *Ipomoea triloba* and tetracycline in combination on the non-cured isolates.

Statement of the Problem

Diarrhoea is a very deadly disease in children, neonates and the elderly causing about 10% of all hospital admissions and one of the three most common causes of pediatric morbidity and mortality (Njume and Goduka, 2012). The development of resistance of the causative organisms to conventional medication, beside adverse effects or toxicity of the conventional agents has given rise to the need to find alternative remedies from herbal sources.

Justification of the Research

Several methods to manage diarrhoea have been employed globally and locally yet there proves to be some level of resistance to existing agents. Hence, the need and exigency to search for herbal remedies with broad spectrum of activity against resistant strains and little or no toxic effects on the body and its organs.

Scope and limitation of the Study

The various agents responsible for the condition of acute infectious diarrhoea include bacteria, viruses and protozoans and these give a very wide scope of study. Hence, this study is limited to bacteria as the causative agents of acute- secretive/exudative infectious diarrhoea.

2. MATERIAL AND METHODS

2.1 Antibiogram of Non-Cured and Cured Isolates

The susceptibility or resistance of the isolated bacterial cultures to standard antibiotics was separately tested with antibiotic susceptibility discs on MHA plates, incubated at 37 ^{o}C for 24 h, following the agar disc diffusion technique (ADD), (Collins and Lyne, 1979; Cheesebrough, 2006). Ten microlitre (10 µL) of each bacterial isolate prepared directly from overnight agar plates adjusted to 0.5 McFarland Nephelometer standard, was inoculated onto each of the petri dishes containing Mueller-Hinton agar. The antibiotics tested present on antibiotic multidiscs (Optudisc® Tissue level) were Ciprofloxacin (CPX 10µg), Norfloxacin (NB 10 µg), Gentamycin (CN 10 µg), Amoxicillin (AML 20 µg), Streptomycin (S 30 µg), Rifampicin (RD 20 µg), Erythromycin (E 30 µg), Chloramphenicol (CH 30 µg), Ampicloxacillin (APX 20 µg) and Levofloxacin (LEV 20 µg) for Gram positive bacterial isolates. Then, Ofloxacin (OFX 10 µg), Pefloxacin (PEF 10 µg), Ciprofloxacin (CPX 10 µg), Augmentin (AUG 30µg), Streptomycin (S 10 µg), Cephalothin (CEP 10 µg), Nalidixic acid (NA 30 µg), Trimethoprim-Sulphamethoxazole (SXT 30 µg) and Ampicillin (PN 30 µg) were present on the antibiotic discs for Gram negative bacterial isolates (Optudisc® Urine level).

These antibiotic discs were aseptically placed on the surfaces of the culture plates with a sterile forceps, gently pressed to ensure even contact and incubated aerobically at 37 °C for 18 h. The plates were then observed for the presence or absence of inhibition zones around the discs and the diameter of the zones

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(in millimetres) accordingly measured using a meter rule. The interpretation of the measurements as susceptible or resistance was made (CLSI, 2018).

2.2 Plasmid Curing of Bacterial Diarrhoeal Cultures

Plasmid Curing of the isolated bacterial diarrhoeaic cultures was carried out based on the method earlier described by Ekong and Ibezim (2015). The curing of the bacterial diarrhoeal isolates was carried out using sub-inhibitory concentration of acridine orange dye (0.1 g/mL). To 9 mL of sterile peptone broth in sterile test tubes containing inoculated freshly prepared isolates was added 1.0 mL of the curing agent. All the test tubes were incubated at 37 °C for 24 h. The broth after incubation were checked for susceptibility to antibiotics using antibiotic susceptibility discs. The data obtained (susceptible or resistance) were compared with the susceptibility profile of the non-cured isolates.

2.3 Evaluation of Combined Antibacterial Activity of ethanol extract and fractions of *Ipomoea triloba* and Standard Antibiotics

Evaluation of the combined antibacterial activity of ethanolextract and fractions of *Ipomoea triloba* with standard antibiotics on non-cured and cured isolates was carried out at 1 x MIC of the extracts and antibiotics in equimolar ratio ($1:1^{v}/_{v}$) (Ekong *et al.,* 2015), by the agar well diffusion technique (Collins and Lyne 1979). The assay plates were held at 4 ^{o}C for 1 h to allow for pre-diffusion to take place, before incubation at 37 ^{o}C for 24 h after which the plates were observed for the presence or absence of inhibition zones. The nature of the interactions in the combinations of the agents against the isolates were determined by the activity-index profile (AIP) (Ekong andIbezim, 2015) and interpreted from the specifications of Okore (2009).

2.4 Experimental Animals

The animals (Swiss albino mice 15.0- 21.0 g) were obtained from and kept at the Department of Pharmacology and Toxicology Animal House of the Faculty of Pharmacy, University of Uyo, Uyo, Nigeria. The animals were maintained under standard environmental conditions and fed with standard Pfizer-branded rodent feed (Livestock Feed, Nigeria Ltd) and given access to water *adlibitum*. All animals were kept at room temperature in cross-ventilated rooms, without illumination at night to achieve the 12 h light/ 12 h dark period. The animals were acclimatized to the laboratory condition for at least 7 days prior to the experiment, during which they were given access to food and water *ad libitum*. The experimental animals were albino mice of both sexes of weight range.

2.5 Determination of Acute Toxicity (LD_{50}) of Ethanol Extract of *Ipomoea triloba*

The acute toxicity (LD_{50}) was assayed in six groups of six albino mice (15.0-21.0 g) per group and each group respectively dosed intraperitoneally with graded concentrations of the extract (250 mg/kg to 5000 mg/kg) following the method of 'O'Callaghan (1983) and Lorke (1983). The animals in the control group were dosed only with distilled water or normal saline. All the animals were allowed access to food and water *ad libitum.* The animals were observed for clinical signs of toxicity, including death for 24 h. Thereafter, the LD_{50} of the extract was determined from the formula described by Lorke (1983), expressed as the square root of the product of the minimum dose showing 0% mortality (a) and the maximum dose, showing 100% mortality (b), as $LD_{50} = \sqrt{ab}$.

2.6 *In vivo* Antibacterial Activity Assay by Model Infection and Protection Test

The *in vivo* antibacterial activity and protection efficacy of ethanol extract of *Ipomoea triloba* against the establishment of infection by the isolates was conducted in a model infection with albino mice (15.0 – 18.0 g body weight) following the Mouse Protection Test (MPT) model (O' Callaghan, 1983), with modifications (Ekong *et al.*, 2004).

The efficacy of the ethanol leaf extract of *Ipomoea triloba* to protect experimental animals against infections was investigated using albino mice (15.0-18.0 g). The protection assay was achieved following animal protection method as earlier described by Ekong *et al.* (2004). The mice were grouped into six sets of six mice per group. Each group was challenged intraperitoneally with 0.5 mL of fresh suspensions of bacteria previously sensitive to the extract to establish infection. The animals were injected with the ethanol extract of *Ipomoea triloba* at the dose equivalent to the MIC of a given bacteria, but less than the LD_{50} at an interval of one and five hours respectively (Ekong *et al.*, 2004). The animals were allowed

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access to food and water *ad libitum* throughout the five-day period. The number of deaths and other observations per group of the challenged mice were recorded.

The negative control consisted of six groups with six animals each. Each group was administered 0.5 mL of each standardized test culture intraperitoneally. Distilled water was administered to all the animals after 1 h and 5 h intervals. The animals were allowed access to food and water *ad libitum* and daily observations including deaths were recorded within the five-day study period.

2.7 Statistical Analysis and Data Evaluation

Data obtained from this work were analysed statistically using Student t-test, ANOVA (one – or two- way) followed by a post-test (Tukev – Kramer multiple comparison test). Differences between means were considered significant at 1% and 5% level of significance i.e. $p \leq 0.01$ and 0.05.

2.8 Ethical Issues

All necessary ethical considerations as regard the use of animals and humans in research were satisfactorily met. The principle of beneficence and non-maleficence was employed and the identity of subjects from whose stools the bacterial isolates were obtained was kept confidential. The care and use of animals was conducted in accordance with the National Institute of Health Guide for the Use of Laboratory Animals (NIH, 1996). Moreover, ethical approval for animal use was obtained from the Experimental Ethics Committee on Animal Use of the Faculty of Pharmacy, University of Uyo, Nigeria.

3. RESULTS AND DISCUSSION

Table 1: Antibiogram of Selected Non-Cured isolates

		Antibiogram									
SN	**Isolates**	**OFX**	**PEF**	**CPX**	**AU**	**CN**	**S**	**CEP**	**NA**	**SXT**	**PN**
	E. coli										
A	EC_1	S	I	I	I	I	S	R	-	I	-
	EC_2	I	-	I	I	I	R	-	-	I	-
	EC_3	I	I	I	I	I	I	-	-	S	S
		CPX	**NB**	**EN**	**AML**	**S**	**RD**	**E**	**CH**	**APX**	**LEV**
	Staph. aureus	I	I	I	I	I	I	I	I	I	I
B	SA_1	I	I	I	I	I	I	I	I	I	I
	SA_2	I	I	I	I	I	I	I	I	I	I
		OFX	**PEF**	**CPX**	**AU**	**CN**	**S**	**CEP**	**NA**	**SXT**	**PN**
	P. aeruginosa										
C	PA_1	-	I	I	-	I	-	-	-	R	-
	PA_2	R	-	I	I	I	-	R	-	I	-
	Shigella dysenteriae										
D	SD_1	I	R	I	R	S	I	R	R	R	R
	SD_2	I	I	I	I	I	I	I	I	I	I
	SD_3	R	R	I	I	I	R	R	R	R	R
	Salmonella typhi										
E	ST_1	R	R	I	R	R	R	R	R	R	R
	ST_2	I	R	I	I	R	R	R	R	R	R
	ST_3	I	I	I	I	R	R	I	R	R	I
	Vibrio cholerae										
F	VC_1	I	I	I	I	I	R	R	R	I	R
	VC_2	I	I	R	I	I	I	R	R	I	R
	VC_3	R	I	I	I	R	I	R	R	I	R

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Key - S- Sensitive, I- Intermediate, R- Resistant OFX: Ofloxacin; PEF: Pefloxacin CPX: Ciprofloxacin; AU: Augmentin; CN: Gentamycin; S: Streptomycin; CEP: Cephalothin; NA: Nalidixic acid; SXT: Trimethoprim-Sulfamethoxazole; PN: Ampicillin

Table 2: Antibiogram of Selected Cured Isolates

		Antibiogram									
SN	**Isolates**	**OFX**	**PEF**	**CPX**	**AU**	**CN**	**S**	**CEP**	**NA**	**SXT**	**PN**
	E. coli										
A	EC_1	I	S	I	I	R	I	-	I	-	I
	EC_2	I	-	I	I	I		-	-	-	I
	EC_3	-	R	R	R	I	-	-	-	-	-
		CPX	**NB**	**EN**	**AML**	**S**	**RD**	**E**	**CH**	**APX**	**LEV**
	Staph. aureus										
B	SA_1	I	I	I	I	I	I	I	I	I	I
	SA_2	I	I	I	I	I	I	I	I	I	I
		OFX	**PEF**	**CPX**	**AU**	**CN**	**S**	**CEP**	**NA**	**SXT**	**PN**
	P. aeruginosa										
C	PA_1	R	-	R	-	I	R	-	I	I	R
	PA_2	I	I	R	-	I	I	I	I	I	I
	Shigella dysenteriae										
D	SD_1	I	-	R	-	I	I	I	I	I	I
	SD_2	I	I	I	I	I	I	I	I	I	I
	SD_3	I	I	I	-	I	I	I	I	-	I
	Salmonella typhi										
	ST_1	I	I	I	I	I	I	I	I	I	I
E	ST_2	R	-	I	-	I	-	-	-	-	R
	ST_3	I	I	I	I	I	I	I	I	I	I
	Vibrio cholerae										
	VC_1	I	R	I	I	I	I	I	I	I	I
F	VC_2	I	I	I	I	I	I	I	I	I	I
	VC_3	R	R	-	-	I	R	R	R	R	R

Key - S- Sensitive, I- Intermediate, R- Resistant OFX: Ofloxacin; PEF: Pefloxacin CPX: Ciprofloxacin; AU: Augmentin; CN: Gentamycin; S: Streptomycin; CEP: Cephalothin; NA: Nalidixic acid; SXT: Trimethoprim-Sulfamethoxazole; PN: Ampicillin

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Table 3: Summary of the nature of interactions of ethanol extract and fractions of *Ipomoea triloba* with tetracycline (standard) on non-cured isolates

Combined agents	Organisms (non- Cured)														
	EC_1	EC_2	EC_3	SA_1	SA_2	PA_1	PA_2	SD_1	SD_2	SD_3	ST_1	ST_2	ST_3	VC_1	VC_2	VC_3
EE+TCN	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT
HF+TCN	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT
DF+TCN	ANT	ANT	ANT	IND	ANT	ANT	ADD	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT
BF+TCN	ANT	ANT	ANT	ADD	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT
AF+TCN	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT

Key- ANT- Antagonism, IND- Indifference, SYN- Synergism, ADD- Additive, AF- Aqueous fraction, BF- Butanol fraction, DF- Dichloromethane fraction, EE- Ethanol extract, EF- Ethyl acetate fraction, HF- n-hexane fraction; TCN- Tetracycline; EC- *Escherichia coli*, SA- *Staphylococcus aureus*, PA- *Pseudomonas aeruginosa,* SD- *Shigella dysenteriae,* ST- *Salmonella typhi,* VC- *Vibrio cholerae*.

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Table 4: Summary of the nature of interactions of ethanol extract and fractions *Ipomoea triloba* with tetracycline (standard) on cured isolates

Combined agents	Test organisms (cured)														
	EC_1	EC_2	EC_3	SA_1	SA_2	PA_1	PA_2	SD_1	SD_2	SD_3	ST_1	ST_2	ST_3	VC_1	VC_2	VC_3
EE+TCN	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT
HF+TCN	ANT	ANT	ANT	IDF	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT
DF+TCN	ANT	ANT	ANT	IDF	ANT	ANT	SYN	ANT	ANT	ANT	ADD	ANT	ANT	ANT	ANT	ANT
BF+TCN	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT
AF+TCN	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT	ANT

Key- ANT- Antagonism, IND- Indifference, SYN- Synergism, ADD- Additive, AF- Aqueous fraction, BF- Butanol fraction, DF- Dichloromethane fraction, EE- Ethanol extract, EF- Ethyl acetate fraction, HF- n-hexane fraction; : EC- *Escherichia coli*, SA- *Staphylococcus aureus*, PA- *Pseudomonas aeruginosa,* SD- *Shigella dysenteriae,* ST- *Salmonella typhi,* VC- *Vibrio cholerae*.

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Table 5: Acute toxicity (LD_{50}) of *I. triloba* ethanol extract

Test groups	Dose (mg/kg)	No. of animals	No. of deaths within 24 h	% mortality in 24 h
1	5000	3	3	100
2	3000	3	3	100
3	1000	3	3	100
4	950	3	2	67
5	900	3	0	0
6	850	3	0	0
7	800	3	0	0
8	500	3	0	0

Route of administration-intraperitoneally n=24

LD_{50} (mg/kg) = $\sqrt{D_a \times D_b}$

Table 6: Model infection, *in vivo* antibacterial activity and protection efficacy of ethanol extract of *Ipomoea triloba* against establishment of infections by bacterial diarrhoeal isolates

Test isolates	Number of animals used per group	No of deaths within five days												No. of survivor within five days		Percentage death per group		Percentage protection per group	
		Test						Control						Test	Control	Test	Control	Test	Control
		1	2	3	4	5	Total	1	2	3	4	5	Total						
EC	6	1	1	-	-	-	**2**	-	1	3	-	-	**4**	4	2	33.33	66.67	66.67	33.33
SA	6	1	2	-	-	-	**3**	-	1	1	1	-	**3**	3	3	50.00	50.00	50.00	50.00
PA	6	2	1	-	-	-	**3**	-	1	3	-	-	**4**	3	2	50.00	66.67	50.00	33.33
SD	6	1	-	-	-	-	**1**	-	2	2	-	-	**4**	5	2	16.67	66.67	83.33	33.33
ST	6	0	1	1	1	-	**3**	-	2	2	-	1	**4**	3	2	50.00	66.67	50.00	33.33
VC	6	1	1	-	-	-	**2**	-	1	2	-	-	**3**	4	3	33.33	50.00	66.67	50.00

Key: EC- *Escherichia coli*, SA- *Staphylococcus aureus*, PA- *Pseudomonas aeruginosa,* SD- *Shigella dysenteriae,* ST- *Salmonella typhi,* VC- *Vibrio cholerae*, -= No death

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DISCUSSION

Plasmid-mediated multidrug resistance is one of the most pressing challenges encountered when treating infectious diseases. Hence, the use of plasmid-curing agents may serve as one of the ways to eliminate plasmids thereby reducing the spread of antibiotic resistance from the fecal route to the environment (Molnar *et al.,* 2003). In this research, the antibiogram of the isolates (pre-curing and post-curing) illustrates that some strains of the selected bacterial isolates showed susceptibility after the exposure to the curing agent (acridine orange 10 µg/mL). This indicates that the resistance of the bacterial diarrhoeaic isolates were plasmid-mediated. Also, most of the isolates of *E. coli*, *P. aeruginosa, Shigella, Salmonella* and *Vibrio* were resistant to Nalidixic acid (NA) before curing (Table 1) but after curing, (Table 2), susceptibility was increased for other organisms but resistance was retained with *E. coli*; SA_2 (RD and APX); VC_2 (NA), retained their resistance to these antibiotics even after exposure to the curing agent. This indicated that the resistance was not plasmid-mediated but chromosomal-mediated.

The summary of the nature of interactions of *Ipomoea triloba* ethanol extract and fractions with sub-inhibitory concentrations of tetracycline (standard antibiotic) on non-cured bacterial diarrhoeaic cultures with total number of interactive outcomes, 80 (100 %), antagonism, 77 (96.25 %), indifference, 1 (1.25 %), additive, 2 (2.5 %) and synergism, 0 (0 %) while that of the cured bacterial diarrhoeaic cultures with total number of outcomes being 80 (100 %), antagonistic, 76 (95 %), indifference, 2 (2.5 %), additive, 1 (1.25 %) and synergistic, 1 (1.25 %) indicates a very slim difference in the effect of curing on the outcomes of the combined effect of the standard antibiotic agent (tetracycline) with the ethanol extract and fractions of *I. triloba* on the cured isolates indicating a predominant resistance of the isolates (Tables 3 and 4).

The median lethal dose (LD_{50}) of the ethanol extract of *Ipomoea triloba* was estimated using albino mice by intraperitoneal (i.p) route using the method of Lorke (1983). The estimated value of 948.68 mg/kg indicatesd a relative non-toxicity profile of the plant giving assurance of its safety profile, achievement of clinical efficacy as well as adequate calculation of doses (Table 5).

From the Model infection and protection test results (Table 6), the protection offered by the extract at the dose less than LD_{50} but greater than MIC (900 mg/kg body weight) was appreciable (50.0- 83.3 %). The standard drug tetracycline protected the mice against all the isolates for up to 72 h giving a moderate protection (33.33-50.00 %). This gives an implication of the safety of the extract when administered both orally and intraperitoneally.

4. CONCLUSION

Conclusively, the safety profile of *Ipomoea triloba* extract having been established in this research, further studies have to be carried out in order to obtain the active principles responsible for its antidiarrhoeal activity then it can be introduced into the treatment regimen as a plausible remedy against acute hypersecretory infectious diarrhoea.

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CONSENT (WHERE EVER APPLICABLE)

No human subjects were used for this study.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

“All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

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