Original Research Article

Journal of Biological Research

& Biotechnology

Bio-Research Vol. 20 No.1; pp. 1398-1408 (2022). ISSN (print):1596-7409; eISSN (online):2705-3822

Evaluation of the antibacterial properties of the extracts and fractions of *Ipomoea triloba* l. (Convolvulaceae) on selected enteric diarrheagenic bacteria

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Abstract

Diarrhoea is a leading killer of young children accounting for approximately 8% of all deaths among children < 5 years worldwide and causes neonatal mortality and hospitalization in geriatrics. *Ipomoea triloba* L. has been claimed to have antidiarrheal properties. This study evaluated antibacterial properties of the ethanol / aqueous extracts and fractions of *I. triloba* on diarrheagenic bacteria to validate its use in trado-medical treatment of diarrhoea. Aqueous and ethanol extracts of pulverized *I. triloba* were prepared by cold maceration and phytochemical screening was performed using standard procedures. Diarrheagenic bacteria were isolated from twenty (20) composite diarrhoeal stool samples by community bioprospecting using appropriate selective and differential media. *In vitro* antibacterial activity of extracts and fractions of *I. triloba* was determined by the modified agar-well diffusion technique, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determined by reference standard agar-dilution technique (ADT) after re-incubation of MIC samples at 37º C for 24h. A total of 74 isolates, belonging to six genera, were identified with their numbers and percentages of occurrence as follows: *Escherichia coli,* 26(35.1%)*, Staphylococcus aureus,* 4(5.4 %)*, Pseudomonas aeruginosa*, 9(12.2%)*, Shigella dysenteriae*, 18(24.3%)*, Salmonella typhi*, 8(10.8%) and *Vibrio cholera,* 9(12.2%). Flavonoids, saponins, terpenes, carbohydrates and steroids were detected in both extracts. Ethanol extracts (≥30 mm) showed more potent broad-spectrum antibacterial activity than aqueous extract (≥18 mm). The MIC and MBC values ranged from 250 to 500 mg/mL and 500 to 1000 mg/mL respectively, thus establishing a time-dependent bactericidal mode of antibacterial activity. The best antibacterial activity was elicited by dichloromethane fraction. From the study, *I. triloba* possesses antibacterial potentials and may be exploited in the chemotherapy of bacterial diarrhoea.

Keywords: ***Ipomoea triloba,*** **Diarrhoea, Diarrheagenic bacteria, Antibacterial, Fractions, Extracts**

Received July 29, 2021**; Revised** December 12, 2021**; Accepted** February 1, 2022

https://dx.doi.org/10.4314/br.v20i1.2
Journal Homepage: ***http://www.bioresearch.com.ng.***
Publisher***: Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.***

INTRODUCTION

Diarrhoea is a leading killer of young children accounting for approximately 8% of all deaths among children below 5 years of age worldwide despite the availability of a simple treatment plan to control diarrhoea by UNICEF in 2017 (WHO and UNICEF, 2017). Infants and children below 5 years of age are prone to about four bouts of diarrhoea both in developing and developed countries and are highly susceptible to its dehydrating effects. According to lots of researchers, there is a high incidence of diarrhoea in Sub-Saharan African mostly due to poverty, poor personal hygiene, unhygienic food preparation and storage patterns leading to high morbidity and mortality rates in these rural low-income communities (Njume and Goduka, 2012).

Childhood diarrhoea is rated the fourth leading cause of mortality among children below 5 years of age in Nigeria making Nigeria the second largest contributor to the under-five mortality rate in the world (Oloruntoba *et al.,* 2014; Onanuga *et al.,* 2014) and the prevalence rate of diarrhoeal cases in Nigeria is 18.8 % making it the second leading cause of death among Nigerian children and a major contributor to childhood morbidity and mortality (Joseph *et al.,*2017). According to the latest WHO data published in 2017, deaths caused by diarrhoeal diseases in Nigeria 9.16 % of total deaths (WLE, 2017).

The development of resistance of the causative organisms to conventional medication such as tetracycline which is mediated through one of several mechanisms which include tetracycline efflux, protection to the tetracycline binding site by binding of specific cytoplasmic proteins to the ribosome; metronidazole whose mechanisms of resistance are decreased drug uptake or increased efflux, decreased drug activation or change in the biological target, increased oxygen scavenging capabilities (SOD/catalase/peroxidase) and enhanced activity of DNA repair enzymes (Dhand and Syndman, 2009) and Bacteria resist erythromycin, other macrolides and lincosamide antibiotics in three (3) ways: a) Ribosomal modification- This occurs through target-site modification by methylation or mutation that prevents the binding of the antibiotic to its ribosomal target leading to cross or broad-spectrum resistance to macrolides and lincosamides. b). Through efflux of the antibiotic and c). Through drug inactivation. The multiplicity of these mechanisms of resistance results in a variety of phenotypes of resistance. (Leclerg, 2002) All these beside adverse effects or toxicity of the conventional agents on body organs has given rise to the need to find alternative remedies from herbal sources. This study is limited to bacteria as the causative agents of acute- secretive/exudative infectious diarrhoea.

Ipomoea is the largest genus in the flowering plant family Convolvulaceae with over 600 species of shrubs (Nimmakayala *et al*., 2011). Humans use *Ipomoea* because they contain medical and psychoactive compounds, mainly alkaloids (Nagai *et al*.,2011). *Ipomoea triloba* is a hairless annual herb, or often trailing on the ground and reproducing from seeds (Plate 1). It is used in Malaysia as a poultice for headache (Burkhill, 1985). It is commonly called little bell, pink convolvulus, potato vine and three-lobe morning glory (Plate 1). The Ibibios call it 'Ediamikot' or 'Udia Ekrok' while the Yorubas call it 'Otito' (Etukudo, 2000).

This research work aimed at evaluating the antibacterial properties of extracts and fractions of *Ipomoea triloba* on bacterial infectious diarrhoeal isolates. The objectives were thus: To isolate, characterize and identify diarrhoeaic organisms from composite stool samples by community bioprospecting and to evaluate the *in vitro* antibacterial activity and determine the mode of activity of extracts and fractions of *Ipomoea triloba* on the diarrheagenic bacteria.

MATERIALS AND METHODS

Plant Collection and Identification

Fresh leaves of *Ipomoea triloba* (Plate 1) used in the study were collected from Abak-Ishiett, a

Plate 1: *Ipomoea triloba leaves* in its Habitat

village in Ibiono-Ibom Local government, Akwa Ibom State, Nigeria in October 2019. The plant leaves were identified and authenticated by a taxonomist, Prof. Margaret Bassey, Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. UUPF 25(f) was assigned to the collection and a leaf press was deposited in the Department of Pharmacognosy, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

Extraction Procedure

The leaves were air-dried at room temperature for 72 h and then pulverised. The coarse leaf powder was weighed and divided into two proportions. Each part was macerated in 3 L of distilled water and 70% ethanol, respectively. After 24 h, the aqueous part was filtered using sterile muslin cloth, cotton wool and filter paper (Whatman No.1) to eliminate the marc and after 72 h, the ethanol extract was obtained using the same materials. The solvents were completely removed using a water bath at 40 °C and dried in a vacuum desiccator. The crude extracts obtained were accurately weighed, sealed with aluminium foil and then stored in the freezer compartment at 4 °C for further tests.

Fractionation of *Ipomoea triloba* Ethanol Extract

Partial purification of the ethanol extracts of *Ipomoea triloba* leaves was carried - out by partitioning technique using four different solvents system in the order of increasing polarity: n-hexane, dichloromethane, ethyl-acetate, and n-butanol respectively. The partitioned fractions obtained were concentrated to dryness in a water bath at 40 ^{o}C and weighed to determine their yields (Trease and Evans, 2009). Each fraction was assayed for antibacterial activity. Fractions that showed activity were further tested to determine the MIC and MBC for each susceptible organism.

Phytochemical Screening

The aqueous and ethanol extracts of *Ipomoea triloba* were reconstituted in the respective solvents used for the extraction and tested for the presence of alkaloids, tannins, saponins, glycosides, carbohydrates and other secondary metabolites using standard phytochemical methods (Trease and Evans, 2009).

Stool Samples Collection

Twenty (20) stool samples were collected in sample-stoppered Bijou bottles containing 10 mL normal saline solution from an epidemic infectious diarrhoeal infested community in Akwa Ibom State and from diarrhoeagenic patients attending the University of Uyo Health Centre after obtaining Ethical Clearance. The stool samples were immediately transported to the Pharmaceutical Microbiology Laboratory, University of Uyo, for bacteriological analysis.

Cultivation, Isolation, Purification and Maintenance of Bacterial-Diarrhoeaic Cultures

The bacterial-diarrhoeaic organisms were isolated using the standard pour plate method (Collins and Lyne, 1979), by aseptically inoculating 1 mL aliquots of ten-fold serially diluted samples in Nutrient Agar (NA); Mannitol Salt Agar (MSA); MacConkey Agar (MCA); Eosin methylene-blue Agar (EMBA); Salmonella-Shigella Agar (SSA); Triple Sugar Iron Agar (TSI); Thiosulphate-Citrate-Sucrose bile salt Agar (TCBS) and Cetrimide Agar (CA), as described by Ekong *et al.* (2015a; 2015b) and incubated at 37 ^{o}C for 48 h. The isolates obtained were aseptically purified by twice-repeated streak-subculturing onto nutrient agar and were also maintained as nutrient agar slant cultures at 4 ^{o}C (Ekong *et al*., 2014)

Characterization and Identification of Diarrheagenic Bacteria

Characterization and identification of the isolates were based on standard microbiological, biochemical, and physiological procedures as described by Collins and Lyne, (Collins and

Lyne, 1979; Konemann *et al.,* 1984; Ekong and Ubulom, 2016a & 2016b).

Standardization of Inoculum Density

Standard inoculum density of the bacterial cultures was prepared by aseptically adjusting the turbidity of the broth cultures by ten-fold serial dilutions with sterile distilled water to that of 0.5 McFarland Nephelometer standard with an approximated cell density of 1.0 x 10^8 CFU/ml, following the methods of Tilton and Howard (1987), Baron and Finegold (1990); with modifications (Ekong *et al.*,2015a).Cultures of Gram positive bacteria were standardized to factor 3; while those of Gram negative bacteria were standardized to factor 5 (Ekong *et al.*, 2014). The purity of the standardized inocula was checked by spread plating 0.1 mL of each suspension on the respective isolation culture media (Ekong *et al.*, 2014).

***In vitro* anti-diarrheal activity of *Ipomoea triloba* extracts**

In vitro antidiarrhoeal activity of *I. triloba* extracts was evaluated by spread-plating aliquots of the standardized isolated bacterial diarrhoeaic cultures on Mueller Hinton agar (MHA) with a sterile glass spreader following the agar well diffusion technique (Collins and Lyne,1979), with modifications by keeping the assay plates [for pre-diffusion] on the bench for 5h before incubation at 37ºC for 24 h. Wells of equal diameter (5 mm) like the assay plates were filled with aliquots of Metronidazole, (MET 40 mg/ml), tetracycline, TCN (25 mg/ml) and erythromycin ERY (50 mg/mL) on MHA plates seeded with the respective bacterial isolates following the same assay conditions as the test plates to serve as control. The inhibition zone diameter (IZD) obtained that was less than, equal to, or greater than the well-diameter (5 mm); indicates inactivity (resistance, R); indifference (intermediate, I) or activity (sensitivity, S) respectively. Potency of the *I. triloba* extracts was evaluated by activity-index, calculated as the ratio of the extracts' activity to those of the positive controls (Ekong *et al.*, 2015a & 2015b).

Determination of minimum inhibitory concentration (MIC) of *Ipomoea triloba* on diarrhoeaic isolates

Minimum Inhibitory Concentration (MIC) of crude leaves (aqueous and ethanol extracts) and fractions of *I.triloba* for the diarrheagenic bacteria was determined following the reference standard agar dilution technique (ADT) and assay plates incubated at 37 ºC for 24 h (Tilton and Howard, 1987; Baron and Finegold, 1990). Inoculated MHA plates without the extracts served as negative control. The MICs were taken as the least concentrations that inhibited growth.

Determination of minimum bactericidal concentration (MBC) and mode of activity of *Ipomoea triloba* on diarrhoeic isolates

The Minimum Bactericidal Concentration (MBC) of the crude leaves (aqueous and ethanol extracts) and fractions of *I. triloba* was determined after re-incubating the plates from the overnight agar dilution test with no visible growth at 37 ºC for 24 h (Tilton and Howard, 1987; Baron and Finegold, 1990). The MBCs were taken as the least concentrations that did not show growth (kill the cultures). The mode of activity of the extracts was determined as either –static or–cidal (Ekong *et al.*, 2014).

Ethical issues

All necessary ethical considerations with regard the use of animals and humans in research were satisfactorily met. The principle of beneficence and nonmaleficence was employed and the identity of subjects, diarrhoeic patients, whose bacterial isolates were obtained from their stool samples was kept confidential. Ethical approval for animals and humans was obtained from the Experimental Ethics Committee of the Faculty of Pharmacy and University of Uyo Health Centre.

RESULTS

Percentage yield of extracts and fractions of ethanol extracts of *Ipomoea triloba*

The percentage yield of the aqueous extract was 15.08 $\%^{w}/_{w}$ while that of the ethanol extracts was 14.66 $\%^{w}/_{w}$ (Table 1). The different fractions obtained from the partial purification of the ethanol extract of *I. triloba* using four different solvent systems in the order of increasing polarity: n-hexane, dichloromethane, ethyl acetate and n-butanol had their yields as presented in Table 2 with dichloromethane

fraction giving highest yield and ethyl acetate fraction having the least yield.

Table 1: Percentage yield of extracts of *Ipomoea triloba*

Weight	Aqueous extract	Ethanol extract
Weight of dry leaves (g)	136.00	269.00
Weight of extract obtained (g)	20.51	39.44
% $^{w}/_{w}$ yield	15.08	14.66

Table 2: Percentage yields of partitioned fractions of *Ipomoea triloba*

S/No	Ethanol Fraction	Weight (g)	%$^{w}/_{w}$ Yield
A	n-hexane	2.24	8.96
B	Dichloromethane	10.61	42.44
C	Ethyl acetate	1.37	5.48
D	n-butanol	3.46	13.84
E	Aqueous	3.70	14.80

Key: A-E: order of increasing polarity

Phytochemical constituents

The qualitative phytochemical screening of the aqueous and ethanol extracts of *I. triloba* showed the presence of the following phytochemical constituents: saponins, tannins, flavonoids, cardiac glycosides, carbohydrates, de-oxy sugars, steroids, and terpenes, whereas alkaloids and glycosides were not detected (Table 3).

Community bioprospecting and antimicrobial activity spectra

The results for the community bioprospecting of bacteria from stool samples obtained from patients in the community and those attending the University of Uyo Health Centre produced a total of 74 isolates as presented in Table 4 and Plate 2.

Antibacterial activity of extracts of *Ipomoea triloba* on bacterial isolates

Antibacterial activity of the aqueous and ethanol extracts of *I. triloba* on diarrhoeal isolates is presented in Table 5. The activity-based selection of susceptible isolates was based on their inhibition zone diameters when exposed to the aqueous and ethanol extracts of *I. triloba* as presented in Table 5.

***In vitro* antibacterial activity of ethanol extracts and fractions of *Ipomoea triloba* on diarrheagenic bacteria**

The result of *in vitro* antibacterial activity of ethanol extracts and fractions of *I. triloba* on selected diarrheagenic bacterial isolates is presented in Table 6 and Plate 3. The results showed that dichloromethane fraction elicited best antibacterial activity whereas the aqueous fraction of *I. triloba* had the least antibacterial activity.

Minimum inhibitory concentration (mic) of ethanol extracts and fractions of *Ipomoea triloba* on diarrheagenic bacteria

The results of the MIC of ethanol extracts and fractions of *I. triloba* on diarrheagenic bacteria as presented in Table 7 showed that some of the fractions (aqueous extract, butanol extract) with respect to the specific organisms had no antibacterial activity while others had antibacterial activity (dichloromethane fraction)

Determination of minimum bactericidal concentration of ethanol extract and fractions of *Ipomoea triloba* on selected diarrheagenic bacteria

The result of minimum bactericidal concentration of ethanol extract and fractions of *Ipomoea triloba* on bacterial isolates as presented in Table 8. Some of the fractions (aqueous extract, butanol extract) with respect to the specific organism; did not elucidate antimicrobial activity while others did (dichloromethane fraction).

Table 3: Phytochemical constituents in extracts of *Ipomoea triloba*

S/No.	Phytochemical constituents	Inference AE	Inference EE
1	Alkaloids	-	-
2	Saponins	+	+
3	Flavonoids	+	+
4	Tannins	+	+
5	Cardiac glycosides	+	+
6	De-oxy sugars	+	+
7	Glycosides	-	-
8	Carbohydrates	+	+
9	Steroids and terpenes	+	+

Key: +: present; - : Absent; AE: aqueous extracts; EE: ethanol extracts

Table 4: Occurrence of isolates from community bioprospecting of bacteria from composite stool samples

Bacterial isolates	No of occurrence	Percentage of occurrence
E. coli	26	35.1
S. aureus	4	5.1
P. aeruginosa	9	12.2
S. dysenteriae	18	24.3
S. typhi	8	10.8
V. cholerae	9	12.2
Total	74	100.0

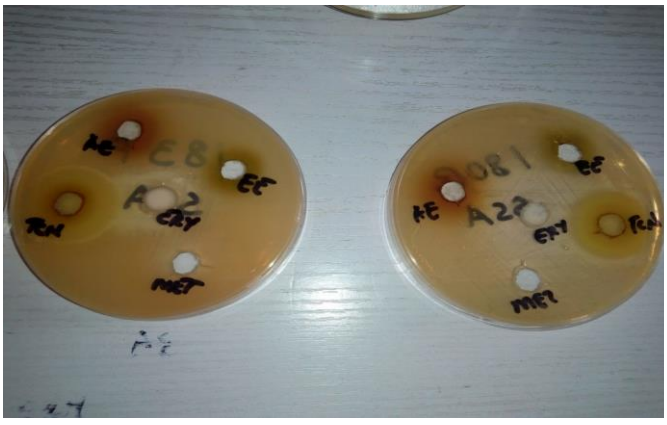

Plate 2: *In vitro* antibacterial activity of extracts of *I. triloba* and standard drugs on diarrhoeal bacterial isolates

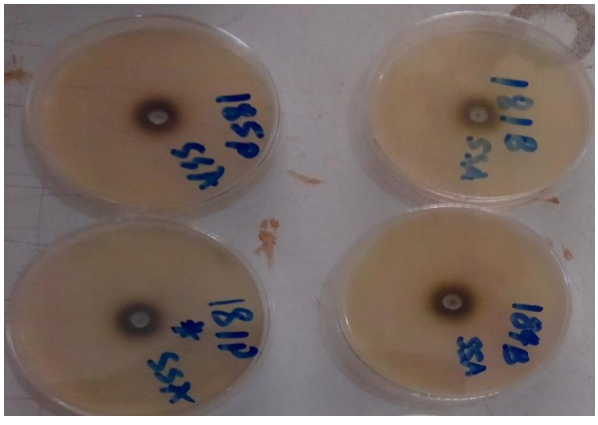

Plate 3: *In vitro* antibacterial activity of *ipomoea triloba* on diarrheagenic bacteria

Test Organisms	Inhibition Zone Diameter (mm ± S.D)					
	Code	AE	EE	MET	TCN	ERY
E. coli	EC001	16.0 ± 2.0	20.0 ± 5.0	7.50 ± 3.5	30.00 ±2.0	34.33 ±2.5
S. aureus	SA001	10.0 ± 2.0	22.0 ± 2.0	NZ	24.00 ±2.8	39.00 ±1.4
P. aeruginosa	PA001	12.0 ± 4.0	20.0 ± 2.0	7.50 ± 3.5	28.50 ±0.7	33.00 ±9.9
S. dysenteriae	SH001	14.0 ± 4.0	20.0 ± 2.0	NZ	24.67 ±0.6	20.67 ±6.4
S. typhi	SAL001	18.0 ± 0.0	12.0 ± 8.0	NZ	26.00 ±2.0	22.00 ±2.0
V. cholerae	VB001	15.0 ± 3.0	25.0 ± 5.0	NZ	28.67 ±1.2	26.33 ±1.5

Keys: AE: Aqueous extracts; EE: Ethanol extracts; MET: Metronidazole; TCN: Tetracycline; ERY- Erythromycin; NZ: No Inhibition Zone

Table 6: Antibacterial activity of ethanol extracts and fractions of *I. triloba* on diarrheagenic bacteria

Test Organisms	Inhibition Zone Diameter (mm ± S.D)							
	Code	AE	AF	BF	DF	EF	HF	TCN
E. coli	EC001	16.0 ± 1.2	NZ	NZ	20± 0.00	15± 0.0	10± 0.0	32±3.5
S. aureus	SA001	15.0 ± 0.7	NZ	NZ	23± 15.6	20± 5.0	15± 0.7	20±1.4
P. aeruginosa	PA001	50.0 ± 7.1	NZ	NZ	20± 0.0	12± 0.0	10±0.0	28±1.0
S. dysenteriae	SH001	26.0 ± 1.5	NZ	8.0 ± 0.0	20± 0.0	NZ	6± 0.0	26±2.0
S. typhi	SAL001	22.0 ± 5.3	NZ	NZ	25± 1.0	20±1.5	18± 0.0	28±1.5
V. cholerae	VB001	20.0 ± 5.0	NZ	NZ	25± 0.0	20±0.0	NZ	28±3.1

Keys: EE: Ethanol Extract; AF: Aqueous fraction; BF: Butanol fraction; DF: Dichloromethane fraction; EF: Ethyl acetate fraction; HF: Hexane fraction; TCN: tetracycline; NZ: No zone of Inhibition; each value represents the mean of three values and standard deviation.

Table 7: Minimum inhibitory concentration of ethanol extracts and fractions of *I. triloba* on diarrheagenic bacteria

Test Organisms	Inhibition Zone Diameter (mm ± S.D)							
	Code	AE	AF	BF	DF	EF	HF	TCN
E. coli	EC001	500± 0.0	500±0.0	250± 0.0	500± 0.0	62.5± 0.0	500± 0.0	32±3.5
S. aureus	SA001	62.5± 0.7	500±12.5	250±25.0	62.5±13.4	62.5±30.9	125±26.5	20±1.4
P. aeruginosa	PA001	62.5±44.2	500±12.5	500±12.5	500±12.5	500±12.5	500±12.5	28±1.0
S. dysenteriae	SH001	500±12.5	500±12.5	500±12.5	500±12.5	500±12.5	500±12.5	26±2.0
S. typhi	SAL001	500±12.5	500±0.0	62.5±44	125±12.5	500±12.5	500±12.5	28±1.5
V. cholerae	VB001	500±14.4	500±12.5	500±12.5	500±12.5	500±12.5	500±12.5	28±3.1

Key: AF-Aqueous fraction, BF-Butanol fraction DF- Dichloromethane fraction, EE- Ethanol extract, EF- Ethyl acetate fraction HF- Hexane fraction, TCN- Tetracycline. Each value represents the mean of three values and standard deviation.

Table 8: Minimum inhibitory concentration of ethanol extracts and fractions of *Ipomoea triloba* on diarrheagenic bacteria

Test Organisms	Code	Inhibition Zone Diameter (mm ± S.D)						
		AE	AF	BF	DF	EF	HF	TCN
E. coli	EC001	500±12.5	500± 0.0	500± 0.0	500±0.0	62.5±38.0	500±12.5	32±3.5
S. aureus	SA001	125±33.6	500±0.0	500±12.5	62.5±13.2	62.5±38.0	250±24.7	20±1.4
P. aeruginosa	PA001	500±12.5	500±12.5	500±12.5	500±12.5	500±12.5	500±12.5	28±1.0
S. dysenteriae	SH001	500±12.5	500±12.5	500 ±12.5	500±12.5	500±12.5	500±12.5	26±2.0
S. typhi	SAL001	500±12.5	500±12.5	500±12.5	125±12.5	500±12.5	500±12.5	28±1.5
V. cholerae	VB001	500±14.4	500±12.5	500±12.5	500±12.5	500±12.5	500±12.5	28±3.1

Key: AF: Aqueous fraction; BF: Butanol fraction; DF: Dichloromethane fraction; EE: Ethanol extract; EF: Ethyl acetate fraction; HF: Hexane fraction; TCN: Tetracycline. Each value represents the mean of three values and standard deviation.

DISCUSSION

The genus *Ipomoea* with its numerous species is widely used in traditional medicine as powerful cathartics and is reported to have anti-inflammatory, antihypertensive, antidiabetic and anticancer properties (Parekh *et al*.,2012) and more specifically, methanol extracts of *Ipomoea batatas, Ipomoea pes-caprae,* and *Ipomoea nil* are reported to show the presence of glycosides, alkaloids, flavonoids, carbohydrates and tannins (Parekh *et al*., 2012) whereas results from the phytochemical screening of *Ipomoea triloba* has revealed the absence of alkaloids.

In this study, the results of phytochemical screening of aqueous and ethanol extracts of *Ipomoea triloba* showed the presence of tannins and saponins as well as other secondary metabolites or phytochemical constituents. These secondary metabolites such as tannins and saponins exert antimicrobial activity through different mechanisms (Akinjogunla *et al.,* 2011; 2012). Tannins has been reported to form irreversible complexes with proline rich protein, causing the inhibition of cell protein synthesis (Olowusulu and Ibrahim, 2006). Flavonoids complex with extracellular-soluble proteins and bacterial cell wall proteins, while lipophilic flavonoids disrupt microbial cells membranes (Olowusulu and Ibrahim, 2006). Our results showed that both aqueous and ethanolic extracts of *Ipomoea triloba* and their fractions have antimicrobial activities on enteric diarrheagenic bacteria, and this corroborated the previous results by Essiett and Obioboho (2014) on antimicrobial activities of *Ipomoea triloba* and also their reports that secondary metabolites of *Ipomoea triloba* were responsible for antidiarrhoeal activity of *Ipomoea triloba*

The activity-based selection of the sensitive diarrheagenic bacteria was due to their inhibition zone diameters being larger than others when exposed to both the aqueous and ethanol extracts as well as the standard first-line antibiotic for acute diarrhoeaic episodes, with tetracycline giving the best inhibition zone diameters for all the isolates thus validating its use as a first line antidiarrhoeal agent in conventional use (Lacy *et al*.,2015).

The *E. coli*, *Shigella* spp. *P. aeruginosa* and *Salmonella* spp in this study were highly sensitive to growth inhibition by the ethanolic and aqueous *Ipomoea triloba* and this substantiates the findings of Ejimadu and Ogbiede (2001) that ethanol extracts of *Ipomoea* had antimicrobial effect on *Klebsiella* sp*, E. coli*, *P. aeruginosa* and *Staphylococcus* sp. The aqueous extract of *Ipomoea reniformis* C. was reported to have antibacterial activity against *Staphylococcus aureus* while its chloroform and ethanol extracts elucidate antifungal activity against *Aspergillus niger* (Andrews (2001).

Both Gram positive and Gram-negative bacteria tested were sensitive to growth inhibition of ethanol and aqueous extracts of *Ipomoea triloba in our study* and this agreed with the results of Ejimadu and Ogbiede (2001) and Ajayi *et al.* (2012) that *Ipomoea* exhibited significant activity against a number of Gram positive and Gram-

negative bacteria. However, the ethanol extracts of *Ipomoea triloba* exhibited greater antimicrobial activity than the aqueous extracts, most especially against Gram negative organisms (*V. cholera*).

The DCM fraction (500-625 mg/mL) of the ethanol extract exhibited the highest antimicrobial activity while the aqueous (375-500 mg/mL) and n-hexane (125-625 mg/mL) fractions exhibited least antimicrobial activity. Thus, DCM fractions is most likely to elicit antidiarrhoeal effect if the diarrhoeal is caused by the test isolates. The MIC was in the range of 250-500 mg/mL while the MBC was in the range of 500-1000 mg/mL showing a concentration dependent mode of activity.

CONCLUSION

Diarrhoea can be caused by bacteria, viruses, protozoans, and helminths. This study was limited to diarrheagenic bacteria and the usefulness of *Ipomoea triloba* trado-medically as an alternative source of therapy in treating diarrhoea caused by bacterial agents. It can thus be concluded from the research findings that aqueous and ethanol extracts of *Ipomoea triloba* contain some phytochemical compounds such flavonoids, tannins, saponins which are known to be responsible for its antidiarrheal activity. The ethanol extract and fractions also possess antimicrobial activity against diarrhoeal isolates with dichloromethane fraction elucidating the best activity thus serving as a pointer that the active principle responsible for the antidiarrhoeal property could be obtained from there using more specialized techniques.

Conflict of interest

The authors report no conflict of interest regarding the research work.

Acknowledgement

The authors wish to acknowledge the assistance from the University of Uyo Health Centre laboratory staff in obtaining stool samples from patients attending the clinic.

AUTHOR CONTRIBUTIONS:

AMF and EUS designed and carried out the research work. EDE, AOJ and UEJ did the literature search and analyzed the data. AMF wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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