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Original article

Antimicrobial activity of extracts and fractions of *Euphorbia lateriflora* (Schum. and Thonn) on microbial isolates of the urinary tract

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ABSTRACT

Euphorbia lateriflora is used in ethnomedicine for treating several conditions, including genital and urinary tract infections (UTI). Although ethnobotanical claims support its use in therapy, there is limited evidence on its effect on UTI, even though UTI remains a public health problem in Nigeria especially due to increasing antimicrobial resistance. We investigated the activity of *E. lateriflora* extracts and fractions on bacterial and fungal isolates from symptomatic urinary tract infections and vaginosis respectively. Qualitative phytochemical screening was conducted on dried pulverised leaves. Successive gradient extraction was carried out with the aid of a soxhlet extractor with n-Hexane, ethyl acetate and methanol respectively. Bioactivity guided fractionation was conducted on the ethyl acetate extract using Vacuum Liquid Chromatography. Antimicrobial susceptibility testing by disc diffusion was conducted on test isolates. Antimicrobial susceptibility of isolates to extracts and fractions was done using the agar well diffusion technique. Minimum Inhibitory Concentrations (MIC) and Minimum Biocidal Concentrations (MBC) were determined by agar and broth dilutions respectively. Time-kill assay of the ethyl acetate extract was conducted using the viable count technique. Phytochemicals present include saponins, tannins and flavonoids. The majority of isolates used in this study were multidrug resistant. Extracts and fractions of *E. lateriflora* produced appreciable zones of inhibition on both antibiotic susceptible and resistant bacteria with MICs of 6.25 mg/mL and MBC ranging from 6.25–50 mg/mL. Bactericidal activity of the ethyl acetate extract was concentration and time dependent with 100% kill at 25 mg/mL after 6 h for *E. coli* and 2 h for *C. albicans*. *Euphorbia lateriflora* contains phytochemicals which possess antimicrobial activity on antibiotic resistant bacteria and has potential in the development of chemotherapeutics for bacterial and fungal infections.

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1. Introduction

Natural antimicrobials of plant origin have been reported to be effective against multi-drug resistant pathogens in a number of studies (Egharevba et al., 2015; Ohyama et al., 1998; Padmanabhan and Jangle, 2012). In ethnomedicine, extracts from plants are useful in treatment of an array of infections and diseases as plants contain several phytochemicals which can concurrently act on different targets (Tonthubthimthong et al., 2001; Gupta et al., 2012). While modern medicine has succeeded in controlling and even eradicating some infectious diseases and disorders, antimicrobial resistance poses a significant challenge for treatment of bacterial and fungal infections (Willey et al. 2020). Several last resort antibiotics including carbapenems (Mills and Lee, 2019; Nowak and Paluchowska, 2016), polymycins (Poirel et al., 2017; Caniaux et al., 2016), tigecycline, linezolid, daptomycin and vancomycin (Bender et al., 2018; Ahmed and Baptiste, 2017) are becoming increasingly ineffective for therapy due to antimicrobial resistance. The upsurge of antimicrobial resistance amongst other factors necessitates the continuing search for novel antimicrobial treatment options.

Euphorbia lateriflora (*E. lateriflora*) (Schum. and Thonn.) is locally used to treat urinary tract infections (UTIs), blood disorders,

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Table 1
Qualitati ve and Quantitative Phytochemical Screening of Powdered Leaves of *E. lateriflora.*

Test	Observation	Inferences	Qualitative	Quantitative (%)
Terpenoids	Reddish-brown colour at interphase	Presence of terpenoids	+	ND
Steroids	Greenish colouration at the upper part	Presence of steroids	+	ND
Saponins	Persistent frothing/foaming	Presence of saponins	++	2.84
Tannins	Brownish-green colouration	Presence of tannins	++	0.44
Cardiac glycosides	No colour change	Absence of cardiac glycosides	–	ND
Flavonoids	Persistent yellow colouration	Presence of flavonoids	++	8.64
Anthraquinones	Colour change	Presence of anthraquinones	+	ND
Alkaloids	Formation of precipitate	Presence of alkaloids	+	10.80

Key: '+' = presence of metabolite, '++' = high concentration of metabolite inferred from instantaneous change or persistency of observation, '-' = absence of metabolite, 'ND' = Not determined.

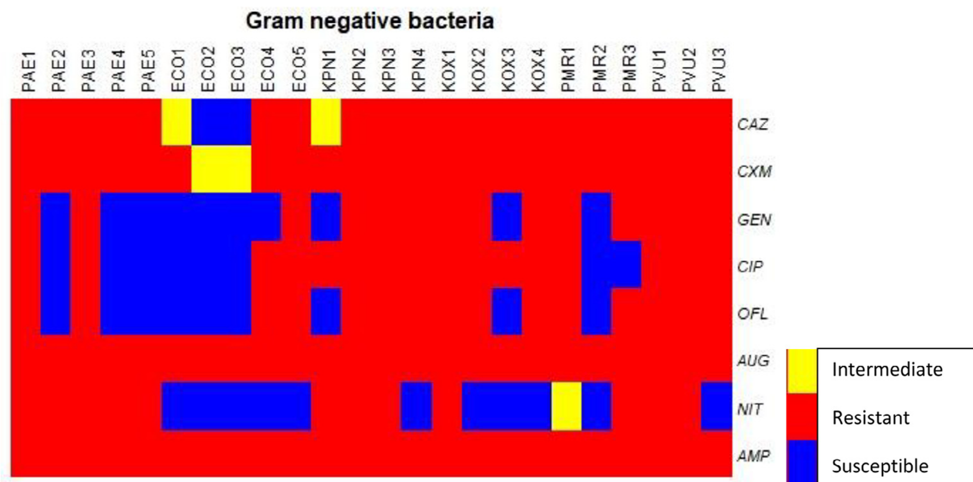

Fig. 1. Antibiogram of Gram Negative Isolates. Key: 'ECO' = *Escherichia coli*, 'PAE' = *Pseudomonas aeruginosa*, 'KPN' = *Klebsiella pneumonia*, 'KOX'= *Klebsiella oxytoca* 'PMR' = *Proteus mirabilis*, 'PVU' = *Proteus vulgaris*, 'CAZ'=Ceftazidime, 'CXM'=Cefuroxime, 'GEN'=Gentamicin, 'CIP'=Ciprofloxacin, 'OFL'=Ofloxacin, 'AUG'=Augmentin, 'NIT'=Nitrofurantoin, 'AMP'=Ampicillin.

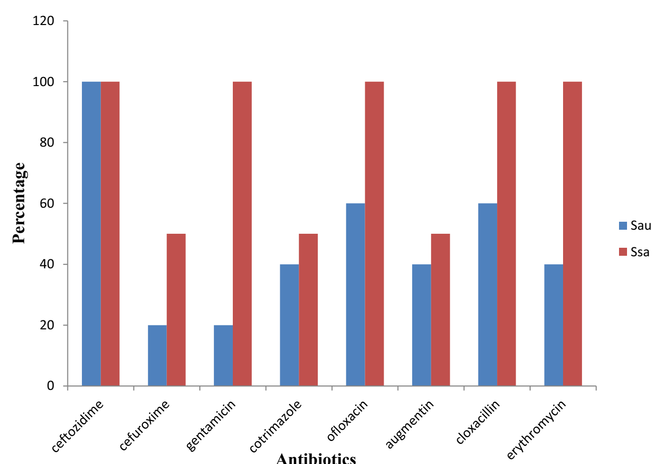

Fig. 2. Percentage resistance of Gram Positive Isolates to Antibiotics. Key: 'Sau' = *Staphylococcus aureus*, 'Ssa' = *Staphylococcus saprophyticus*,

veneral diseases, sub-cutaneous parasitic infections and other diseases (Burkill, 1994; Abubakar et al., 2007; Emmanuel, 2010) notably due to its broad spectrum of activity.

The burden of Sexually Transmitted Infections (STIs) and UTIs world over is of particular interest, as well as the harmful effects of conventional antibiotics on protective normal vaginal flora. Urinary Tract Infections are the most common bacterial infections occurring in women; with 10% of women becoming infected yearly and 60% at one time in their lives (Nicolle, 2008; Colgan and Williams, 2011; Salvatore et al., 2011). Sexually Transmitted Infections on the other hand are important from a public health point of view partly because symptoms do not surface all the time and hence can be transferred without the person(s) knowing. In 2008, an estimated 500 million people were infected with STI (WHO, 2013). Also of interest is the unwanted depletion of the normal flora of the genito-urinary tract by conventional antibiotics.

We investigated the *in-vitro* antimicrobial activity of *E. lateriflora* on clinical bacteria and yeasts isolated from urine and vagina respectively

2. Methods

2.1. Test microorganisms

Clinical isolates of *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), *Staphylococcus saprophyticus* (*S. saprophyticus*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Klebsiella oxytoca* (*K. oxytoca*), *Proteus vulgaris* (*P. vulgaris*), *Proteus mirabilis* (*P. mirabilis*) and *Candida albicans* (*C. albicans*) were obtained from Department of Medical Microbiology, University College Hospital, Ibadan and Lancet Clinical Laboratories, Ibadan. These isolates had been previously identified with Microbact and API by the respective clinical laboratories. Information about the isolates obtained from the clinical laboratories revealed that they were isolated from symptomatic urinary tract infections and vaginal swabs.

Table 2
Antimicrobial screening of extracts on test isolates.

	Hexane extract			Ethyl acetate extract			Methanol extract			Ciprofloxacin	Fluconazole
Concentration	100 mg/mL	50 mg/mL	25 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	100 mg/mL	50 mg/mL	25 mg/mL	10 µg/mL	50 µg/mL
Isolate	Zones of Inhibition (mm)										
P. aeruginosa 1	12 ± 0.82	10 ± 0.82	11 ± 0.82	23 ± 0.82	18.33 ± 0.94	11.67 ± 0.47	14 ± 0.82	NZI	NZI	NZI	NT
P. aeruginosa 2	NZI	NZI	NZI	26.33 ± 0.94	16 ± 0.82	12.33 ± 0.47	11.33 ± 0.47	11.33 ± 0.47	10 ± 0.82	29 ± 0.82	NT
P. aeruginosa 3	NZI	NZI	NZI	20.67 ± 2.49	15.33 ± 0.47	13 ± 1.41	12 ± 0.82	11 ± 0.82	NZI	NZI	NT
P. aeruginosa 4	12.33 ± 0.47	13 ± 1.63	16 ± 0.82	14.33 ± 1.25	12 ± 0	10 ± 0	11.33 ± 0.94	NZI	NZI	23.67 ± 1.25	NT
P. aeruginosa 5	NZI	NZI	NZI	22.33 ± 0.47	15 ± 0.82	12.33 ± 0.47	NZI	NZI	NZI	30.33 ± 1.25	NT
E. coli 1	13 ± 0.82	10 ± 0	10 ± 0	16.67 ± 0.47	12.33 ± 1.25	NZI	12.67 ± 1.25	10.33 ± 0.47	13.33 ± 1.25	27.67 ± 0.94	NT
E. coli 2	10 ± 0	NZI	9.33 ± 1.25	26 ± 0.82	15 ± 0.82	10.33 ± 0.47	NZI	NZI	NZI	30 ± 0.82	NT
E. coli 3	11.67 ± 0.47	10.33 ± 1.7	14 ± 0.82	19.33 ± 0.47	16.33 ± 0.47	12.33 ± 1.25	NZI	NZI	NZI	27.33 ± 0.47	NT
E. coli 4	12 ± 1.41	NZI	11.67 ± 0.47	20.33 ± 0.47	14 ± 0	10.33 ± 0.47	11.33 ± 0.47	NZI	NZI	NZI	NT
E. coli 5	NZI	NZI	NZI	16 ± 0.82	15 ± 1.63	12.33 ± 1.25	12 ± 1.63	11 ± 0.82	10.33 ± 1.25	NZI	NT
K. pneumoniae 1	NZI	NZI	NZI	24 ± 1.63	18.33 ± 0.47	13 ± 0.82	14 ± 0	10.67 ± 0.47	NZI	NZI	NT
K. pneumoniae 2	NZI	NZI	NZI	24 ± 1.41	20.33 ± 0.47	12.33 ± 1.25	14.33 ± 2.49	14 ± 1.63	12 ± 2.16	NZI	NT
K. pneumoniae 3	11.67 ± 1.25	12.33 ± 0.47	15.33 ± 0.47	17 ± 0	12 ± 0	10.33 ± 0.47	NZI	10.33 ± 0.47	12.33 ± 0.47	NZI	NT
K. pneumoniae 4	24.67 ± 0.47	18.33 ± 0.47	23.33 ± 0.47	16 ± 0.82	13 ± 0.82	NZI	NZI	NZI	NZI	NZI	NT
K. oxytoca 1	NZI	NZI	NZI	NZI	NZI	NZI	NZI	NZI	NZI	NZI	NT
K. oxytoca 2	NZI	NZI	NZI	20.33 ± 1.25	19.33 ± 1.25	11 ± 0	10 ± 0.82	11 ± 0	NZI	NZI	NT
K. oxytoca 3	NZI	NZI	NZI	21.33 ± 0.47	NZI	NZI	NZI	NZI	NZI	20.33 ± 2.05	NT
K. oxytoca 4	11 ± 0	10 ± 1.41	NZI	20 ± 1.41	11 ± 0.82	10 ± 0.82	NZI	10.33 ± 1.25	10 ± 0	NZI	NT
P. mirabilis 1	NZI	NZI	10.33 ± 0.47	16 ± 0.82	12.33 ± 0.47	NZI	10.67 ± 0.94	NZI	NZI	NZI	NT
P. mirabilis 2	NZI	10.33 ± 0.47	12 ± 0.82	12.67 ± 0.47	11 ± 0.82	10.33 ± 1.25	10 ± 0	10.33 ± 0.94	NZI	20.33 ± 1.25	NT
P. mirabilis 3	13 ± 0.82	10.33 ± 2.05	9.33 ± 0.47	20.33 ± 1.7	15.33 ± 1.25	11.33 ± 0.47	15.33 ± 1.89	10.67 ± 0.47	11.33 ± 0.47	NZI	NT
P. vulgaris 1	10.33 ± 0.47	10.33 ± 0.47	12.33 ± 0.47	11.33 ± 1.25	10 ± 0	NZI	9.67 ± 1.25	10 ± 1.63	9 ± 0	NZI	NT
P. vulgaris 2	12.67 ± 0.94	15 ± 0.82	22 ± 1.63	13 ± 0.82	10.33 ± 0.47	NZI	13.33 ± 2.05	10.67 ± 0.47	NZI	NZI	NT
P. vulgaris 3	10.33 ± 1.25	12.33 ± 0.47	NZI	NZI	NZI	NZI	13 ± 0	10.33 ± 0.47	NZI	NZI	NT
S. aureus 1	NZI	NZI	NZI	12 ± 0	11 ± 0	9.67 ± 0.47	11.33 ± 0.47	9.33 ± 0.47	NZI	NZI	NT
S. aureus 2	NZI	NZI	NZI	19 ± 0.82	16.33 ± 1.25	15 ± 0.82	14.67 ± 0.47	11 ± 0.82	10.33 ± 0.94	22 ± 1.63	NT
S. aureus 3	NZI	NZI	NZI	16.33 ± 0.47	11.33 ± 0.47	10.33 ± 0.94	13 ± 0.82	12 ± 0	10.33 ± 1.25	29 ± 0.82	NT
S. aureus 4	NZI	NZI	10 ± 0	16.33 ± 1.25	15 ± 0.82	11.33 ± 0.47	11 ± 0	10.33 ± 1.25	NZI	NZI	NT
S. aureus 5	NZI	NZI	NZI	17.33 ± 0.47	19.33 ± 1.25	13.33 ± 0.47	15.33 ± 2.05	14.33 ± 0.47	14 ± 0.82	NZI	NT
S. saprophyticus 1	NZI	NZI	NZI	19.33 ± 0.47	15 ± 0	13 ± 1.41	12.33 ± 0.47	9.67 ± 0.47	NZI	NZI	NT
S. saprophyticus 2	NZI	NZI	NZI	21.67 ± 1.25	18 ± 0.82	11.33 ± 1.25	14 ± 0.82	11.33 ± 0.47	10 ± 0	NZI	NT
C. albicans 1	NZI	NZI	NZI	11.33 ± 0.94	10 ± 0	NZI	NZI	12.33 ± 0.47	21.33 ± 2.05	NT	NZI
C. albicans 2	NZI	NZI	NZI	23.33 ± 1.25	14 ± 0.82	12.33 ± 1.25	NZI	NZI	9 ± 0	NT	NZI
C. albicans 3	NZI	NZI	NZI	NZI	NZI	NZI	10 ± 0.82	NZI	12 ± 1.63	NT	NZI
C. albicans 4	NZI	NZI	NZI	20.33 ± 0.47	16.33 ± 0.47	NZI	15.33 ± 0.47	13 ± 0.82	NZI	NT	16.33 ± 1.25

Key: '*E. coli*' = *Escherichia coli*; '*P. aeruginosa*' = *Pseudomonas aeruginosa*; '*K. pneumoniae*' = *Klebsiella pneumoniae*; '*K. oxytoca*' = *Klebsiella oxytoca*; '*P. mirabilis*' = *Proteus mirabilis*; '*P. vulgaris*' = *Proteus vulgaris*; '*S. aureus*' = *Staphylococcus aureus*; '*S. saprophyticus*' = *Staphylococcus saprophyticus* 'NZI' = no zone of inhibition 'NT' = not tested.

2.2. Plant collection and preparation

Fresh leaves and stems of *E. lateriflora* were collected from Ikire in Osun state, Nigeria. The plant was identified and authenticated by Mr Adeyemo at Forestry Research Institute of Nigeria (FRIN) and assigned a voucher specimen number FHI 110801. Plant materials were air-dried at ambient temperatures, away from direct sunlight for four months to constant weight and pulverized with an industrial grinder.

2.3. Phytochemical screening

Phytochemical screening was determined following simple qualitative tests previously described by Vinoth et al., (2012) to confirm presence or absence of secondary metabolites.

2.4. Plant extraction

Dried, pulverized leaves of both plants were extracted successively with the soxhlet apparatus using organic solvents of increasing polarities from n-hexane, through ethyl acetate and methanol. The extracts were concentrated to dryness with the aid of a shaker water bath (Lab Tech Shaker, model; LSI-3016R, Korea) set at 60 °C. The weight of the extracts was taken after which they were stored in the refrigerator at 4 °C for subsequent use.

2.5. Identification and characterization of test isolates

All clinical isolates used were resuscitated by sub-culturing into appropriate differential and/or selective media and thereafter incubated at 37 °C for a period of 18 to 24 h. They were then subjected to appropriate biochemical tests in order to verify their identity (Cheesbrough 2006). The biochemical tests performed include: Gram staining, catalase, citrate utilization, coagulase, indole, oxidase, carbohydrate fermentation, urease, methyl-red/Vogues-Proskauer, motility-hydrogen sulphide production, novobiocin, DNAse and germ tube fermentation tests.

2.6. Antibiogram of isolates

The antibiotic discs employed in this study include; ceftazidime (30 µg), cefuroxime (30 µg), gentamycin (10 µg), erythromycin (5 µg), cloxacillin (5 µg), ofloxacin (5 µg), amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), cotrimazole (25 µg), novobiocin (5 µg), cefoxitin (30 µg), nitrofurantoin (300 µg) and ciprofloxacillin

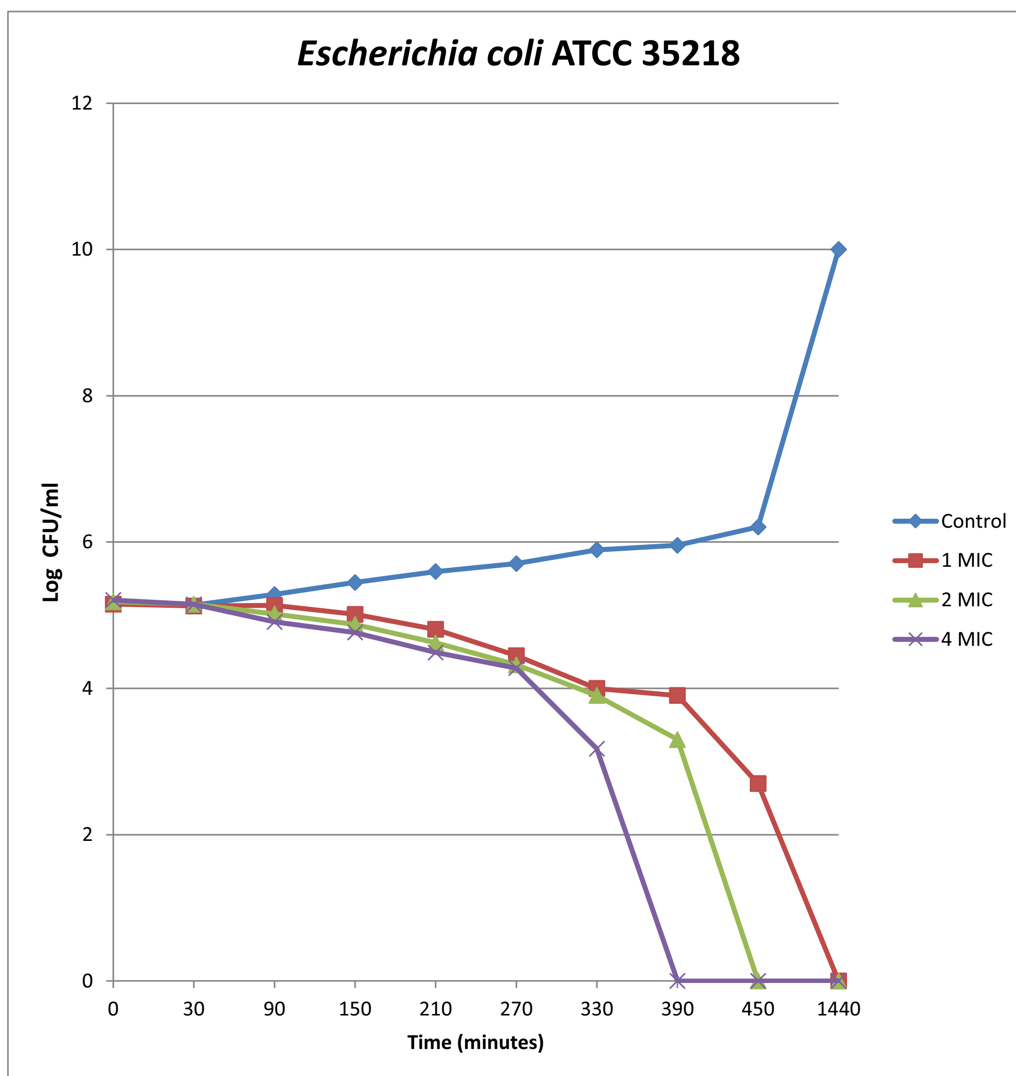

Fig. 3. Kill Kinetics of the Ethyl Acetate Extract of *E. lateriflora* on *Escherichia coli* ATCC 35218.

Table 3
Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Ethyl Acetate Extract.

Isolate	MIC	MBC
P. aeruginosa 1	6.25 mg/mL	25 mg/mL
P. aeruginosa 2	6.25 mg/mL	25 mg/mL
P. aeruginosa 3	6.25 mg/mL	12.5 mg/mL
P. aeruginosa 4	6.25 mg/mL	25 mg/mL
P. aeruginosa 5	6.25 mg/mL	12.5 mg/mL
E. coli 1	6.25 mg/mL	50 mg/mL
E. coli 2	6.25 mg/mL	25 mg/ml
E. coli 3	6.25 mg/mL	25 mg/mL
E. coli 4	6.25 mg/mL	25 mg/mL
E. coli 5	6.25 mg/mL	25 mg/mL
K. pneumoniae 1	6.25 mg/mL	25 mg/mL
K. pneumoniae 2	6.25 mg/mL	12.5 mg/mL
K. pneumoniae 3	6.25 mg/mL	12.5 mg/mL
K. pneumoniae 4	6.25 mg/mL	25 mg/mL
K. oxytoca 1	6.25 mg/mL	≥50 mg/mL
K. oxytoca 2	6.25 mg/mL	6.25 mg/mL
K. oxytoca 3	6.25 mg/mL	12.5 mg/mL
K. oxytoca 4	6.25 mg/mL	12.5 mg/mL
P. mirabilis 1	6.25 mg/mL	12.5 mg/mL
P. mirabilis 2	6.25 mg/mL	6.25 mg/mL
P. mirabilis 3	6.25 mg/mL	12.5 mg/mL
P. vulgaris 1	6.25 mg/mL	50 mg/mL
P. vulgaris 2	6.25 mg/mL	25 mg/mL
P. vulgaris 3	6.25 mg/mL	50 mg/mL
S. aureus 1	6.25 mg/mL	6.25 mg/mL
S. aureus 2	6.25 mg/mL	6.25 mg/mL
S. aureus 3	6.25 mg/mL	12.5 mg/mL
S. aureus 4	6.25 mg/mL	12.5 mg/mL
S. aureus 5	6.25 mg/mL	6.25 mg/mL
S. saprophyticus 1	6.25 mg/mL	12.5 mg/mL
S. saprophyticus 2	6.25 mg/mL	25 mg/mL

Key: '*E. coli*' = *Escherichia coli*, '*P. aeruginosa*' = *Pseudomonas aeruginosa*, '*K. pneumoniae*' = *Klebsiella pneumoniae*, '*K. oxytoca*' = *Klebsiella oxytoca*, '*P. mirabilis*' = *Proteus mirabilis*, '*P. vulgaris*' = *Proteus vulgaris*, '*S. aureus*' = *Staphylococcus aureus*, '*S. saprophyticus*' = *Staphylococcus saprophyticus*.

(5 μg). Ciprofloxacin (10 μg/ml) was used as the drug control for antimicrobial screening of extracts.

An overnight broth culture of the clinical isolates on Tryptone Soy Agar (TSB) was diluted in 0.85% saline. Dilutions corresponding to a 0.5 McFarland equivalent were employed in the test. Petri dishes of Mueller Hinton Agar containing 20 mL each were prepared and with the aid of sterile cotton tipped applicators, an inoculum from each isolate was spread evenly on the surface of corresponding plate. With the aid of sterile forceps, appropriate multiple-antibiotic disc was placed firmly on the surface of inoculated plates. The plates were thereafter left on bench for about 10 mins to dry after which they were incubated at 37 °C for 24 h. Zones of inhibition were measured in millimeters and interpreted following the standards of CLSI (CLSI, 2018). Data were further analyzed using WHONET.

2.7. Antimicrobial screening of extracts

Antimicrobial screening of the n-hexane, ethyl acetate and methanol extracts of both plants on test isolates was conducted using the agar well diffusion method. A 0.5 McFarland equivalent of each test isolate was prepared and 100 μL of this was used to inoculate sterile agar plates of Mueller Hinton Agar and Saboraud's Dextrose Agar for bacteria and yeast respectively. The plates were labeled accordingly with isolate's and dilutions codes. Equidistant wells were bored on the agar plates with the aid of a cork borer (8 mm). With the aid of a sterile glass pipette, 200 μL of prepared extracts was dispensed into corresponding wells. Ciprofloxacin (10 μg/mL) and fluconazole (50 μg/mL) were used as chemotherapeutic controls while methanol was used as the negative control. Plates were then left on bench for about an hour to allow optimum diffusion of extracts and controls before incubating at 37 °C for 24 h and 25 °C for 48 h for bacteria and fungi respectively. Tests were performed in triplicates and the mean and standard deviation recorded in each case.

2.8. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts

MICs of the ethyl acetate extract was conducted following standard protocols (Klancnik et al., 2010; Wiegand et al., 2008). Double-fold dilutions of the extract were made in Mueller Hinton Agar, yielding final concentration range of 0.39–50 mg/mL. An overnight broth culture of each isolate on TSB was diluted. 200 μL of dilutions equivalent to a 0.5 McFarland standard was aseptically transferred into a sterile 96 well microtitre plate. The scheme on the 96-well plate was noted and with the aid of a sterile 48-points multipoint inoculator, inoculums from the broths were transferred into all MIC plates, one at a time. Plates were incubated at 37 °C for 24 h. The lowest concentration of antimicrobials which inhibited the growth of each isolate was recorded as its MIC.

MBC of the ethyl acetate extract was also determined using multiples of MICs up to 10^3. Overnight broth cultures of isolates were diluted and 500 μL of the 10^{-2} dilution was inoculated in 3.5 mL of sterile TSB. One milliliter of appropriate extract dilution was dispensed into the tubes before incubation at 37 °C for 24 h and 25 °C for 48 h for bacteria and fungi respectively. A loop-full of cultures was streaked onto sterile TSA plates after appropriate incubation. The least concentration inhibiting growth of organism was taken as the MBC.

2.9. Determination of bactericidal kinetics of extracts.

The bactericidal kinetics was determined following already described procedures (Lajubutu et al. 1995). One milliliter of overnight broth cultures of *E. coli*, *S. aureus* and *C. albicans* in 5 mL TSB

Table 4
Antimicrobial Screening of Vacuum Layer Chromatography pooled fractions of *E. lateriflora.*

	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5		Fraction 6	
Concentration	50 mg/mL	25 mg/mL	50 mg/mL	25 mg/mL	50 mg/mL	25 mg/mL	50 mg/mL	25 mg/mL	50 mg/mL	25 mg/mL	50 mg/mL	25 mg/mL
Isolate	Zones of Inhibition (mm)											
P. aeruginosa 2	10	NZI	15	NZI	12	NZI	12	NZI	13	NZI	13	NZI
E. coli 2	24	18	22	18	30	24	35	31	22	19	21	14
K. pneumoniae 1	10	NZI	20	17	18	13	25	23	26	22	NZI	NZI
K. oxytoca 2	12	NZI	19	12	18	12	30	26	12	10	13	10
S. aureus 2	NZI	NZI	10	NZI	13	NZI	NZI	NZI	21	14	19	10
S. saprophyticus 2	12	NZI	17	10	10	NZI	12	NZI	20	15	10	NZI
C. albicans 2	NZI	NZI	15	NZI	13	NZI	12	NZI	19	11	12	NZI

Key: '*E. coli*' = *Escherichia coli*; '*P. aeruginosa*' = *Pseudomonas aeruginosa*; '*K. pneumoniae*' = *Klebsiella pneumoniae*; '*K. oxytoca*' = *Klebsiella oxytoca*; '*P. mirabilis*' = *Proteus mirabilis*; '*P. vulgaris*' = *Proteus vulgaris*; '*S. aureus*' = *Staphylococcus aureus*; '*S. saprophyticus*' = *Staphylococcus saprophyticus* 'NZI' = no zone of inhibition.

was used to inoculate 4 mL of TSB which was subsequently incubated for 18 h in order to obtain actively growing cultures. The resultant cultures were diluted and 100 µL of the 10^{-2} dilution was used to inoculate 3.9 mL TSB containing 1 mL of the extract at a final concentration equivalent to the MIC of each isolate. The resultant mixture, containing extract, culture and broth, was serially diluted and 100 µL of the 1 in 4 and 1 in 8 dilutions were used to inoculate plates of TSA at different time intervals beginning at 0 min, 1 h, 1 h 30 mins, 2 h, 3 h, 4 h, 5 h, 6 h and 24 h. The inoculum was evenly spread with the aid of a sterile glass spreader after which the plates were left for a few minutes to dry. The plates were then incubated at 37 °C for 24 h and 25 °C for 48 h for bacteria and fungi respectively. The entire procedure was repeated for extract concentrations containing 2 times the MIC and 4 times the MIC for each test isolate as well as control plates containing no extract. After incubation period, the colony forming unit (CFU) was counted and a graph of the log of CFU per mL was plotted against time.

2.10. Vacuum liquid chromatography

Fifteen grams of the ethyl acetate fraction adsorbed on silica gel (60–200 mesh size) was subjected to Vacuum liquid chromatography (VLC) using 100 mL mixtures of solvents in increasing polarities (n-hexane, ethyl acetate and methanol). Fractions were collected into clean tubes, spotted on a thin layer chromatographic plate (TLC) and developed in a mobile phase mixture of chloroform:methanol (9:1). Fractions with similar retention factors were pooled together and concentrated to dryness.

2.11. Antimicrobial screening of VLC fractions

Serial dilutions of each pooled fraction were tested against the isolates using agar well diffusion method as described under section 2.7 above.

3. Results

3.1. Summary

Phytochemical screening revealed the presence of saponins, tannins and flavonoids in abundance as shown in (Table 1). The percentage yield of hexane, ethyl acetate and methanol extracts were 5.02%, 4.03% and 4.58% respectively.

Bacterial isolates employed in this study were resistant to most antibiotics in the panel consisting of commonly employed antibiotics (Figs. 1 and 2).

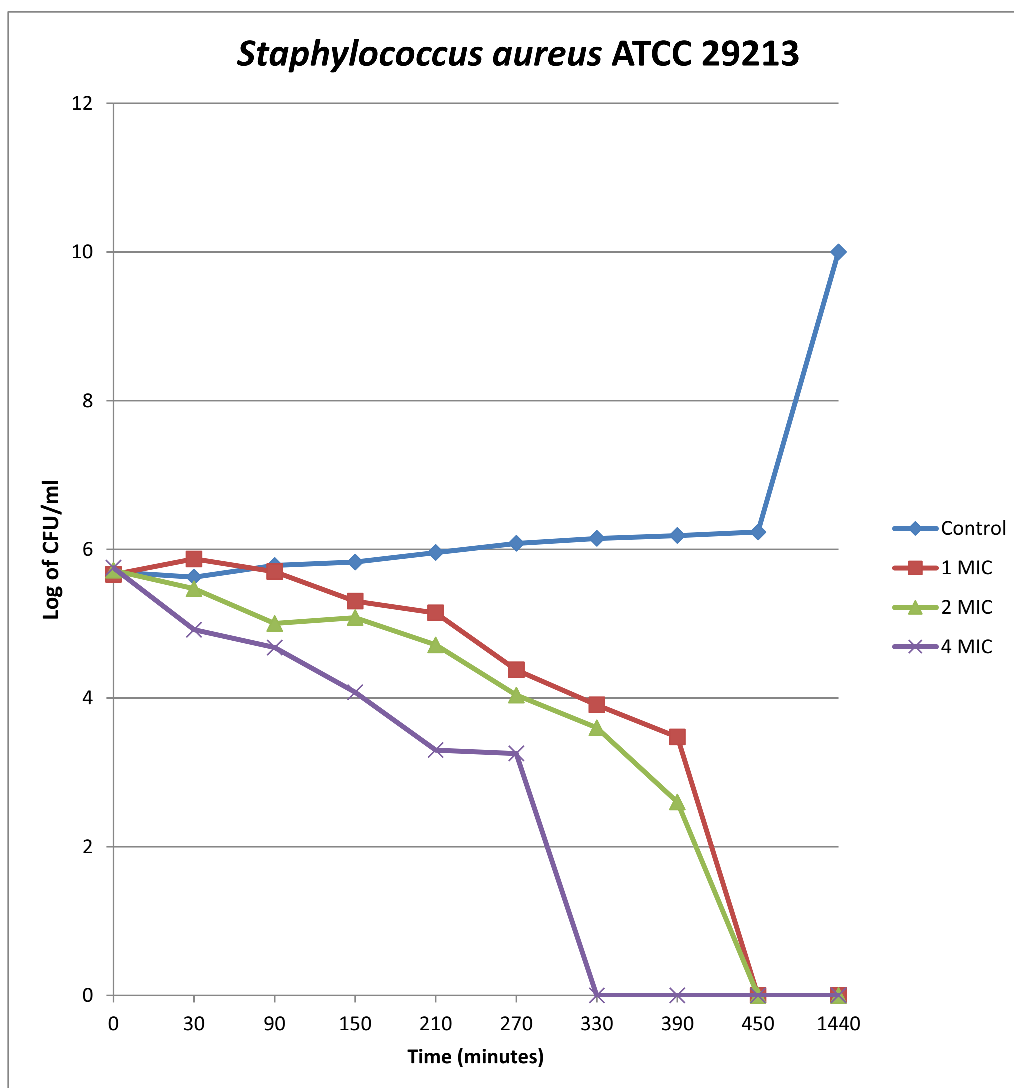

Fig. 4. Kill Kinetics of the Ethyl Acetate Extract of *E. lateriflora* on *Staphylococcus aureus* ATCC 29213.

Extracts (Table 2) and fractions (Table 4) of *E. lateriflora* produced appreciable zones of inhibition on both bacterial and fungal isolates. In some cases, zones of inhibition produced by the extracts and fractions were greater than those of the drug control. The MIC of the ethyl acetate extract was 6.25 mg/mL on all test isolates while MBCs ranged from 6.25 mg/mL to 50 mg/mL (see Table 3).

Bactericidal kinetics revealed that the ethyl acetate extract produced a total kill on *E. coli*, *S. aureus* and *C. albicans* within 24 h (Figs. 3–5).

4. Discussion

E. lateriflora is a popular medicinal plant used widely in ethnomedicinal practice in all parts of Nigeria. This study investigated the activity of extracts and fractions of *E. lateriflora* on genitourinary tract pathogens.

Phytochemical screening revealed presence of terpenoids, saponins, tannins, flavonoids, anthraquinones, steroids, and alkaloids while cardiac glycosides were absent. Pure terpenoids as well as alkaloids have been previously reported to be present in *E. lateriflora* (Lavib and Jain, 1968; Sule et al., 2001). Literature confirms that presence of secondary metabolites confer potent antimicrobial activity on medicinal plants. The overall activity seen is thought to be produced mainly by different phytochemicals simultaneously targeting different sites in susceptible bacteria, making evolution of antimicrobial resistance difficult (Padmanabhan and Jangle 2012). Flavonoids for example produce broad spectrum antibacterial activity by binding irreversibly to cell walls and other extracellular soluble proteins, resulting in bactericidal activity (Cowan, 1999; Ohyama et al., 1998). Alkaloids are also remarkable for producing potent antimicrobial activity by essential enzyme inhibition as well as tampering with cell membrane integrity. They may also interfere with cell anabolism (Ogunshe et al. 2013). Terpenoids kill bacterial cells by altering cell membrane structure, leading to cell lysis (Cowan 1999). These and other phytochemicals present in *E. lateriflora* may be responsible for observed antimicrobial activity of the plant.

The percentage yield of extracts was higher for the hexane extract followed by methanol and ethylacetate extracts. This could be partly because the plant has large amounts of non-polar constituents, inferred by the waxy nature of the plant. Conversely, the relatively lower yield recorded for the ethylacetate extract

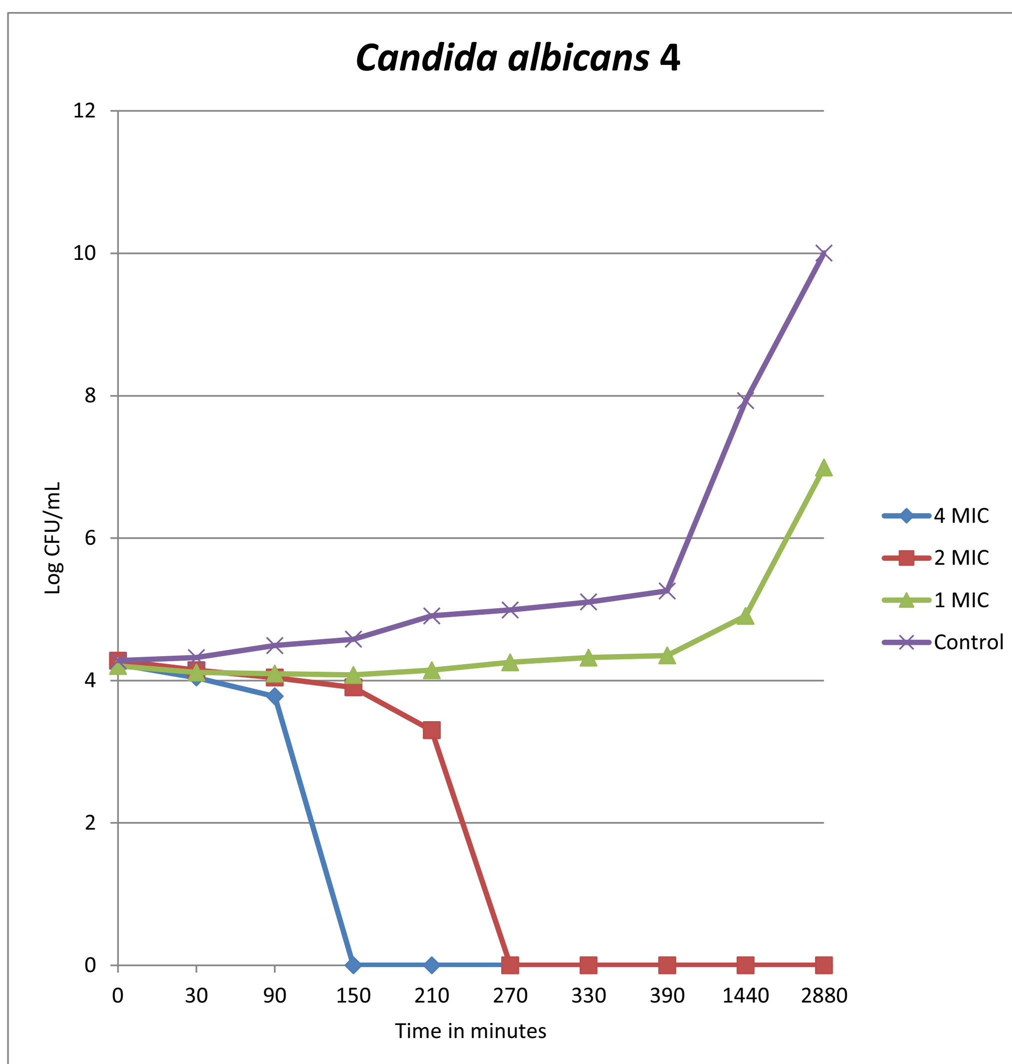

Fig. 5. Kill Kinetics of the Ethyl Acetate Extract of *E. lateriflora* on *Candida albicans* 4.

could be an indication that the plant's components are mostly on the ends of polarity.

The majority of test isolates were Multi Drug-Resistant (MDR) (CLSI, 2018; Magiorakos et al., 2011). Resistance rate was particularly alarming for *P. aeruginosa* as all isolates were resistant to 3rd generation cephalosporins and other beta-lactam antibiotics as well as nitrofurantoin, making them possibly Extended Drug-Resistant. Similar high resistance rates were observed for all the other isolates, with a few isolates resistant to the panel of antibiotics used. Reports of high resistance rates of Gram negative bacteria abound (Neuhauser et al., 2003; Salem et al., 2013; Okon et al., 2014). The antibacterial to which the lowest resistance was observed was nitrofurantoin, followed by ofloxacin, ciprofloxacin and gentamicin indicating that these antibacterial agents may still be useful in treating infections caused by such organisms, albeit on the basis of results from susceptibility testing. The alarming rate of resistance is probably due to the widespread and inappropriate use of antibiotics both in humans and animals which selects for resistant strains of bacteria. Also, these isolates were collected from secondary tertiary institutions which are usually the last resort facilities for untreatable infections, probably accounting for selection of drug resistant bacteria.

The hexane extract of *E. lateriflora* was only active on a few clinical isolates, showing small zones of inhibition at the highest concentrations employed. However the zones of inhibition increased as concentration reduced and again reduced on further decrease in concentration for a good number of isolates. This could be as a result of difficulty in diffusion of the extract at high concentrations as the hexane extract was waxy and as such diffusion through the agar may have been impeded. The hexane extract had no zone of inhibition whatsoever on the fungal isolates employed in this study. The majority of fungal isolates were also resistant to fluconazole at the concentration employed. In contrast, the ethyl acetate extract of *E. lateriflora* had potent antimicrobial activity on all bacterial isolates except *Proteus vulgaris* 3. In some cases, the zones were comparable to those produced by the drug control. It had consistently wider zones of inhibition than both hexane and methanol extracts. It could also be seen from the results that the activity was concentration dependent, reducing with reduction in concentration. Similar to the observation with the hexane extract on some isolates, the ethyl acetate extract was active on *K. pneumoniae* 4 only at the middle concentrations employed. The activity of the ethylacetate extract on fungi was somewhat moderate and this was also concentration dependent. The methanol extract, while more active than hexane, was less active than ethyl acetate as more isolates showed no zones of inhibition. Those which were susceptible showed smaller zones in comparison to the ethyl acetate extract. The methanol extract had moderate activity on fungi which was not consistent with concentration. A study conducted by Sule et al., (2001) however reported moderate activity of *E. lateriflora* extract on only three strains of bacteria.

Ciprofloxacin, the drug control was ineffective on most of the isolates. While plants extracts cannot be compared to standard antibiotics due to their complex nature, it is remarkable to observe that the extracts produced large zones of inhibition on multiply resistant isolates. It is therefore probable that the plant extracts and fractions contain particles that can be used in the treatment of infections caused by MDR bacteria.

MICs and MBCs of the ethyl acetate extracts were a bit high for substances intended for treatment of infections. This is most likely as a result of the complexity of plants' extracts, containing several inert compounds which ultimately account for the total weight.

Antimicrobial screening of pooled fractions against representative isolates produced relatively larger zones of inhibition when compared to crude extracts, further supporting our thoughts on high MIC and MBC values. Fractions 4 and 5 produced larger zones of inhibitions, suggesting that the active components of *E. lateriflora* are concentrated middle of the polarity spectrum.

The ethyl acetate extract of *E. lateriflora,* resulted in death of *E. coli, S. aureus* and *C. albicans* in a concentration dependent manner at varying time intervals. The rate of kill on *C. albicans* at the 4 MIC and 2 MIC of the ethyl acetate extract of *E. lateriflora* was faster, resulting in complete clearance at 2 h 30 min and 4 h 30 min respectively. However, the MIC reduced colony counts for the first three hours after which counts increased consistently, indicating that that concentration was not suitable for killing the organism. In all cases, the controls, containing no extract increased substantially.

5. Conclusion

We have shown in this study that *E. lateriflora* extracts and fractions produced potent antimicrobial activities on pathogens of the urinary tracts, including multidrug resistant organisms thus validating its use in ethnomedicine. This therefore suggests that *E. lateriflora* could be an important source of antimicrobial compounds with broad spectrum activity against drug resistant bacteria.

6. Authors' contributions

MEC partook in research concept and design, collection and assembly of data, data analysis and interpretation, writing the article, critical review of the article and final approval of the article.

AOO took part in collection and assembly of data, data analysis and interpretation, writing the article, critical revision of the article and final approval of the article.

TOA partook in data analysis and interpretation, critical revision of the article and final approval of the article.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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