ANTIBACTERIAL EFFICACY OF AQUEOUS LEAF EXTRACTS OF *Cymbopogan citratus* (POACEAE) AND *Borreria verticillata* (RUBIACEAE) ON BIOFILM PRODUCING UROPATHOGENIC BACTERIAL ISOLATES

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ABSTRACT

The antibacterial potentiality of single and combined aqueous leaf extracts of *Borreria verticillata*(ALEBV) and *Cymbopogan citratus*(ALECC) on twenty (20) biofilm producing bacterial isolates, belonging to genera *Escherichia, Staphylococcus, Streptococcus, Proteus, Pseudomonas* and *Enterococcus* obtained from mid-stream urine samples were determined using standard bacteriological and disc diffusion techniques. Assay has shown that all the twelve (100%) biofilm producers tested were sensitive to ALEBV at200 $mgml^{-1}$ with inhibitory zones (ZIDs) ranging from 9.5 ± 0.2 mm to 15.8 ± 0.2 mm, while between 66.7% (n=8) and 91.7% (n=11) biofilm producers were sensitive to ALECC at 50 and 100 ($mgml^{-1}$). More than 91.7% biofilm producers were sensitive to 50 $mgml^{-1}$ - 200 $mgml^{-1}$ of the combined ALEBV and ALECC with ZIDs and Activity Indices (A.I) ranging from 7.3 ± 0.2 mm to 17.8 ± 0.5 mm and 0.44 to 1.44, respectively. The combined ALEBV and ALECC had synergistic effect against 66.7% (n=8) biofilm producing bacterial isolates with Growth Inhibitory Indices (GIIs) ranging from 0.53 to 0.61, while the combined extracts had addictive and antagonistic effects against 16.7% (n=2) and 16.7% (n=2) biofilm producers with GIIs of 0.50 and ≤ 0.49, respectively. The regression (R^2) coefficient between different concentrations of ALEBV, ALECC and ZIDs as exhibited by the biofilm producers ranged from 0.6823 to 0.9978. The antibacterial efficacies of ALEBV and ALECC against biofilm producers (pathogenic bacteria) may be ascribed to the presence of secondary metabolites (alkaloids, flavonoids, cardiac glycosides, reducing sugar, anthraquinones, steroids, tannins, phlobatanins and saponins) in the plant leaves it and justify their usage in Nigerian ethno-medicine. Their consideration and utilization for production of potent antibiotics is strongly recommended.

INTRODUCTION

Biofilms are surface-associated multidrug resistant microbial communities embedded in a hydrated matrix composed of an extracellular polymeric substance containing proteins, polysaccharides, lipids and extracellular DNA (Costerton, 1999).Biofilms formation often leads to diverse bacterial subpopulations resulting from differential gene expression and aids bacteria in developing resistance to antibiotics, antimicrobial proteins and complement (Costerton, 1999; Donlan, 2002).

The increase in antibiotic resistant microorganisms has necessitated the continuous exploration for novel antimicrobial agents from nature to overcome life threatening infectious diseases caused by pathogenic microbial strains. Plants contain a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids and quinolones (Akinjogunla and Oluyege, 2016). *Cymbopogon citratus,* Stapf (Poaceae), also known as lemon grass, is an economically important aromatic, monocotyledonous, perennial tall grass (Omotade, 2009) that is widely cultivated worldwide, most especially in Africa, West Indies, America and tropical regions (Dama *et al.*, 2011). *C. citratus* has been exploited in the treatment of nervous condition,

hypertension, coughs, flu, leprosy, diabetes, malaria, vascular disorders and diarrhoea (Gore *et al*., 2010). The extracts of *C. citratus* has exhibited numerous biological activities including anti-inflammatory, antimicrobial and also has valuable remedy in treating ringworm (Tatiana *et al*., 2011). *Borreria verticillata* (Linn), belonging to the family Poaceae, is a perennial shrubby false-button weedy herb that is widely distributed in tropical areas in Africa, Asia and South and Central Americas (Ushie *et al*., 2013). The infusion of *B. verticillata* flower is antipyretic and analgesic. The leaves of *B. verticillata* are used for effective treatment of eczema, ringworm, dyspepsia, leprosy, diarrhoea, gonorrheal sores, paralysis, diabetes and dysmenorrhea (Ushie *et al*., 2013). This study aimed at determining the *in vitro* antibacterial activities of aqueous leaf extract of *C. citratus* and *B. verticillata* alone and in combination against biofilm producing bacterial isolates from urine samples.

MATERIALS AND METHODS

Collection of Samples

Using sterile containers, thirty midstream urine (MSU) samples were aseptically collected from the patients who had not received antibiotic treatment for the previous one week after their verbal informed consent in Uyo. The MSU samples were immediately taken to the Microbiology Laboratory, University of Uyo for bacteriological analysis

Bacteriology of Samples

The MSU samples were aseptically centrifuged and the supernatant was discarded. One (1) ml of each residue was inoculated onto each plate of Cysteine Lactose Electrolyte Deficient and the plates were incubated for 24hrs at 37^{o}C. After incubation, cultures with significant growth were further subcultured onto plates of nutrient agar and incubated for 24hrs at 37^{o}C. The isolates were characterized and identified appropriately.

Detection of Biofilm Producing Bacterial Isolates

Biofilm producing bacterial isolates were detected using Congo red agar. The Congo red stain, prepared as a concentrated aqueous solution, was autoclaved at 121 ^{o}C for 15 mins. The brain heart infusion (BHI) agar with sucrose was also prepared and autoclaved at 121 ^{o}C for 15 mins. The sterilized Congo red stain was added to the autoclaved BHI agar with sucrose at 50 ^{o}C. Each of the test isolates was streaked on each Congo red agar plate, incubated for 24 hrs at 37 ^{o}C and formation of black colonies with dry crystalline consistency indicated biofilm production.

Sources of Test Plants

The leaves of *Cymbopogan citratus* and *Borreria verticillata* (Figs 1 and 2) obtained in Uyo were authenticated by a taxonomist before taking to Pharmacology and Natural Medicine Laboratory, Faculty of Pharmacy, University of Uyo, for processing. The plant leaves were separately washed with sterile distilled water, chopped into small pieces, air-dried at room temperature and ground to fine powder using mortar and pestle. The aqueous extracts of the plants were separately prepared by soaking 2 kg of the powdered leaves in 1litre of distilled water for 72 hrs with constant shaking at room temperature. It was then filtered using Whatman No 1 filter paper, the filtrate was evaporated to dryness with steam on water bath (45°C), and the dried extract was weighed and stored in a refrigerator at 4^{o}C in a screw capped bottle until required for use. Graded concentrations (50, 100 and 200) mgml^{-1} of the extracts were aseptically prepared using 100 ml of dimethyl sulphoxide (DMSO, Sigma, USA) and shaken vigorously to obtain a homogenous mixture.

Phytochemical Screening of Aqueous Leaf Extracts of *C. citratus* and *B. verticillata*

The phytochemical constituents of the ALECC and ALEBC were determined using the methods described by Sofowora (1993) and, Trease and Evans (1996).

Antibacterial Activities of Single and Combined Extracts of *C. citratus* and *B. verticillata*

The disc diffusion technique by NCCLS (2004) was employed for the determination of the antibacterial activities of aqueous extracts of *C. citratus* (ALECC) and *B. verticillata* (ALEBC)

on the biofilm producing bacteria. Ten microlitre of each bacterial suspension, prepared directly from an overnight agar plate and adjusted to 0.5 McFarland Standard, was inoculated onto each plate of Mueller–Hinton agar (MHA). Sterile filter paper discs (6 mm) loaded with single ALECC and ALEBC (1 mL) of graded concentrations (50, 100 and 200) $mgml^{-1}$were aseptically placed onto MHA plates using a sterilized forceps. Also sterile filter paper discs of 6 mm diameter loaded with combined ALECC and ALEBC (prepared in 1:1 by volume),of graded concentrations (50, 100 and 200) $mgml^{-1}$, were aseptically placed using a sterilized forceps onto MHA plates which had previously been inoculated with the bacterial isolate. Streptomycin (10 µg) and dimethyl sulphoxide (10 %) was used as positive and negative control, respectively. All the plates in triplicates were incubated at 37°C for 24 hrs. After incubation, the diameter of a transparent circular zone corresponding to the absence of growth around each of the discs was observed, measured in millimetre (mm) and interpreted as follows: < 10 mm: mild inhibitory activity; ≥ 10 to ≤ 14 mm: moderate inhibitory activity and ≥ 15mm: strong inhibitory activity.

Evaluation of Extracts Interaction and Activity Index

The growth inhibition index (GII) was calculated by dividing the mean inhibition zone (IZD) of the combined extracts divided by the total mean inhibitory zone diameters (IZD) of the extracts in single action. The GII > 0.5 indicates synergism; GII = 0.5 indicates additive effect and GII < 0.5 indicates antagonism (Akinjogunla and Fatunla, 2017). The activity index (AI) was calculated by dividing the mean of growth inhibition zone of the extract by the mean of growth inhibition zone of standard antibiotic.

Fig 1 : *Borreria verticillata*

Fig 2 : *Cymbopogan citratus*

RESULTS

Forty six bacterial isolates belonging to the genera *Escherichia, Staphylococcus, Proteus, Streptococcus, Pseudomonas, Klebsiella* and *Enterococcus* were obtained from the thirty MSU samples analyzed. Of the 46 bacterial isolates, *E. coli* had the highest occurrence (n=13), while *K. pneumoniae* had the lowest (n=2). A total of 20 (43.5%) bacterial isolates obtained from the MSU were positive for biofilm production (Table 1). The occurrences of biofilm producing bacterial isolates were as follows: *E. coli* 8 (61.5 %), *S. pyogenes* 3 (50.0 %), *S. aureus* 4 (44.4 %), *P. aeruginosa* 2 (40.0 %), CoN *Staphylococcus* spp 1 (33.3 %), *Proteus* spp 1 (25.0 %) and *En. faecalis* 1 (25.0 %) (Table 1).

The results of the preliminary phytochemical screening of the crude aqueous leaf extracts of *B. verticillata* (ALEBV) and *C. citratus* (ALECC) are presented in Table 2, while the antibacterial activities of ALEBV on biofilm producing Gram positive and Gram negative bacterial isolates are presented in Table 3. The widest mean inhibitory zone diameter (IZD) obtained was 15.8 mm with a standard deviation of 0.2, while the narrowest mean IZD (mm ±S.D) was 8.0 ± 0.1 with the activity indices (A.I) ranging between 0.54 and 1.32. Of the 12 biofilm producing bacterial isolates tested, 8 /12 (66.7 %), 11 /12 (91.7 %) and 12/12 (100%) were sensitive to 50, 100 and 200 mg/L of ALEBV, respectively. The ALECC showed strong inhibitory activity on *E. coli* EC-B1 and *S. aureus* SA-B2; moderate inhibitory activity on *E coli* EC-B3, EC-B4, EC-B8 and *E. faecalis* EF-B1 at 200 $mgml^{-1}$. Table 4 shows the susceptibility of the biofilm producing bacterial isolates to ALECC. Of the 12 isolates tested,

disc containing the 200 $mgml^{-1}$ of ALECC exhibited strong inhibitory activity on 3 (25.0 %) with IZD ≥ 15 mm; moderate inhibitory activity on 7 (58.3 %) with IZD ranging from ≥ 10 to ≤ 14 mm and mild inhibitory activity on 2 (16.7 %) with IZD of < 10 mm. The Activity Index ranged from 0.47 to 1.34 as observed in *E coli* EC-B4 and *E coli* EC-B1, respectively (Table 4).The results showed a concentration-dependent increase in the antibacterial activities of ALECC and ALEBV decoction and concoction on the biofilm producers tested. All the 12 biofilm producing bacterial isolates showed varied degrees of sensitivity to the concoction of ALECC and ALEBV (Table 5). The concoction of ALECC and ALEBV (200 mg/ml) exerted greater antibacterial activity on *E coli* EC-B1; *S aureus* SA-B2,SA-B4 and *S. pyogenes* SP-B3 compared to the commercial antibiotic (streptomycin) with A.I ranging from ≥ 1.05 to ≤ 1.44. The *Proteus* spp PS-B1 and *En. faecalis* EF- B1 were resistant to streptomycin but showed moderate sensitivity to concoction of ALECC and ALEBV at 200 mg/ml having IZD of ≥ 11 mm (Table 5). The ALECC and ALEBV combination showed synergistic antibacterial effect against 8 (66.7%) biofilm producing bacterial isolates with GIIs ranging from ≥ 0.53 to ≥ 0.58. The combinations of ALECC and ALEBV yielded antagonistic antibacterial interactionsagainst 2 (16.7%) with GIIs ≤ 0.49 and addictive antibacterial effect against 2 (16.7%) with GIIs of 0.50 (Table 6). The regression coefficient (R^2) between different concentrations of ALECC and ALEBV and inhibitory zone diameters as exhibited by biofilm producing bacterial isolates are shown in Table 7.

Table 1: Occurrence of Biofilm Bacterial Isolates from Urine Samples

Bacterial Isolates	No. of Occurrence	Biofilm Producers No (%)	Non-Biofilm Producers No (%)
E coli	13	8 (61.5)	5 (38.5)
S aureus	9	4 (44.4)	5 (55.6)
Proteus spp	4	1 (25.0)	3 (75.0)
S. pyogenes	6	3 (50.0)	3 (50.0)
CoN *Staphylococcus* spp	3	1 (33.3)	2 (66.7)
P. aeruginosa	5	2 (40.0)	3 (60.0)
K. pneumoniae	2	0 (0.0)	2 (100)
E. faecalis	4	1 (25.0)	3 (75.0)
Total	46	20 (43.5)	26 (56.5)

Table 2: Phytochemical Constituents of Plant Extracts

Bio-Active Constituents	Occurence	
	B. verticillata	*C. citratus*
Alkaloids	++	++
Flavonoids	+	++
Saponins	+	+
Tannins	++	++
Cardiac Glycosides	+	+
Anthraquinones	+	+
Reducing Sugar	+	+
Phlobatanins	+	-
Steroids	-	+

++: Present in Moderately High Concentration, +: Present in Low Concentration,
- : Not detected

Table 3: Antibacterial Activities of *B. verticillata* on Biofilm Producing Bacterial Isolates

Bacterial Isolates	Code	mm ±S.D (50 mg/ml)	A.I	mm ±S.D (100 mg/ml)	A.I	mm ±S.D (200 mg/ml)	A.I	Streptomycin mm ±S.D	DMSO
E coli	EC-B1	13.0 ± 1.0^{b}	1.08	15.3 ± 0.5	1.28	15.8 ± 0.2^{c}	1.32	12.0 ± 1.0^{a}	NZ
	EC-B3	NZ	0.0	8.6 ± 0.1^{a}	0.52	11.1 ± 0.1^{a}	0.67	16.6 ± 0.5^{b}	NZ
	EC-B4	9.5 ± 0.1^{a}	0.55	11.0 ± 0.0^{a}	0.64	12.4 ± 0.5^{b}	0.72	17.2 ± 1.2^{b}	NZ
	EC-B8	10.2 ± 0.2^{a}	0.65	13.4 ± 1.0^{b}	0.85	14.1 ± 0.6^{b}	0.90	15.7 ± 0.2^{b}	NZ
S aureus	SA-B2	11.3 ± 0.1^{a}	0.66	14.5 ± 0.5^{b}	0.85	15.0 ± 1.0^{c}	0.88	17.0 ± 0.5^{b}	NZ
	SA-B4	10.6 ± 0.2^{a}	0.88	12.9 ± 0.1^{b}	1.08	14.0 ± 0.5^{b}	1.17	12.0 ± 1.0^{a}	NZ
Proteus spp	PS-B1	NZ	0.0	NZ	0.0	9.8 ± 0.2^{a}	0.0	NZ	NZ
S. pyogenes	SP-B2	10.5 ± 0.5^{a}	0.64	13.3 ± 0.2^{b}	0.81	13.9 ± 0.1^{b}	0.85	16.4 ± 0.4^{b}	NZ
	SP-B3	10.0 ± 0.0^{a}	0.63	11.7 ± 0.2^{a}	0.73	12.0 ± 0.1^{b}	0.75	16.0 ± 1.0^{b}	NZ
CoN *Staphylococcus* spp	CS-B1	NZ	0.0	8.0 ± 0.1^{a}	0.54	9.5 ± 0.2^{a}	0.64	14.9 ± 0.5^{a}	NZ
P. aeruginosa	PA-B1	9.6 ± 0.2^{a}	0.55	12.4 ± 0.6^{b}	0.71	13.0 ± 0.5^{b}	0.74	17.5 ± 1.0^{b}	NZ
E. faecalis	EF-B1	NZ	0.0	9.1 ± 0.1^{a}	0.0	10.8 ± 0.2^{a}	0.0	NZ	NZ

Keys: Each inhibitory zone included 6 mm diameter of the disc., SD: Standard Deviation. Each value represents the mean of three replicates and standard deviation. Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test ($P < 0.05$); NZ: No Inhibition Zone; A.I.: Activity Index.

Table 4: Antibacterial Activities of *C. citratus* on Biofilm Producing Bacterial Isolates

Bacterial Isolates	Code	mm ±S.D (50 mg/ml)	A.I	mm ±S.D (100 mg/ml)	A.I	mm ±S.D (200 mg/ml)	A.I	Streptomycinmm ±S.D	DMSO
E coli	EC-B1	12.5 ± 0.5[b]	1.04	14.7± 0.3[b]	.23	6.1 ± 1.0[c]	1.34	12.0± 1.0[a]	NZ
	EC-B3	8.6 ± 0.1[a]	0.52	9.3 ± 0.1[a]	.56	3.2 ± 0.7[b]	0.80	16.6 ± 0.5[b]	NZ
	EC-B4	8.0 ± 0.1[a]	0.47	9.9 ± 0.1[a]	.58	1.5 ± 0.5[a]	0.67	17.2 ± 1.2[b]	NZ
	EC-B8	10.0 ± 0.2[a]	0.64	12.6 ± 0.5[b]	.80	3.5 ± 0.2[b]	0.86	15.7 ± 0.2[b]	NZ
S aureus	SA-B2	12.8 ± 0.4[b]	0.75	15.7 ± 0.3	.92	6.7 ± 0.6[c]	0.98	17.0 ± 0.5[b]	NZ
	SA-B4	NZ	0.0	10.3 ± 0.1[a]	.86	2.2 ± 0.2[b]	1.02	12.0 ± 1.0[a]	NZ
Proteus spp	PS-B1	NZ	0.0	7.7 ± 0.1[a]	.0	.6 ± 0.1[a]	0.0	NZ	NZ
S. pyogenes	SP-B2	9.9 ± 0.1[a]	0.60	12.0 ± 0.2[b]	.73	2.6 ± 0.3[b]	0.77	16.4 ± 0.4[b]	NZ
	SP-B3	10.5 ± 0.2[a]	0.66	14.4 ± 0.4[b]	.9	6.8 ± 0.2[c]	1.05	16.0 ± 1.0[b]	NZ
CoN *Staphylococcus* spp	CS-B1	NZ	0.0	NZ	.0	.1 ± 0.1[a]	0.54	14.9 ± 0.5[a]	NZ
P. aeruginosa	PA-B1	NZ	0.0	10.5 ± 0.4[a]	.6	2.3 ± 0.3[b]	0.70	17.5 ± 1.0[b]	NZ
E. faecalis	EF-B1	7.4 ± 0.1[a]	0.0	8.8 ± 0.2[a]	.0	1.1 ± 0.1[a]	0.0	NZ	NZ

Table 5: Antibacterial Activities of *B. verticillata* and *C. citratus* on Biofilm Producing Bacterial Isolates

Bacterial Isolates	Code	mm ±S.D (50 mg/ml)	A.I	mm ±S.D (100 mg/ml)	A.I	mm ±S.D (200 mg/ml)	A.I	Streptomycinmm ±S.D	DMSO
E coli	EC-B1	13.9 ± 0.1[b]	1.16	15.9 ± 0.2[c]	1.33	17.4 ± 1.5[c]	.44	12.0± 1.0[a]	NZ
	EC-B3	7.3 ± 0.2[a]	0.44	11.0 ± 0.1[a]	0.66	11.6 ± 0.5[a]	.70	16.6 ± 0.5[b]	NZ
	EC-B4	9.9 ± 0.2[a]	0.58	10.6 ± 0.2[a]	0.61	12.0 ± 0.2[b]	.70	17.2 ± 1.2[b]	NZ
	EC-B8	10.5 ± 0.1[a]	0.67	14.0 ± 1.0[b]	0.89	14.5 ± 0.2[b]	.92	15.7 ± 0.2[b]	NZ
S aureus	SA-B2	11.8 ± 0.2[a]	0.69	16.3 ± 0.5[c]	0.96	17.8 ± 0.5[c]	.05	17.0 ± 0.5[b]	NZ
	SA-B4	8.9 ± 0.1[a]	0.74	14.4 ± 0.2[b]	1.2	15.1 ± 0.3[c]	.26	12.0 ± 1.0[a]	NZ
Proteus spp	PS-B1	NZ	0.0	8.1 ± 0.1[a]	0.0	11.4 ± 0.1[a]	.0	NZ	NZ
S. pyogenes	SP-B2	12.4 ± 0.3[b]	0.79	13.0 ± 0.5[b]	0.85	14.0 ± 0.2[b]	.79	16.4 ± 0.4[b]	NZ
	SP-B3	12.0 ± 0.5[b]	0.75	15.3 ± 0.2[c]	0.96	17.5 ± 0.2[c]	.09	16.0 ± 1.0[b]	NZ
CoN *Staphylococcus* spp	CS-B1	7.5 ± 0.1[a]	0.50	8.0 ± 0.1[a]	0.54	9.9 ± 0.1[a]	.66	14.9 ± 0.5[a]	NZ
P. aeruginosa	PA-B1	10.1 ± 0.1[a]	0.58	12.5 ± 0.2[b]	0.71	14.3 ± 0.3[b]	.82	17.5 ± 1.0[b]	NZ
E. faecalis	EF-B1	7.7 ± 0.1[a]	0.0	9.8 ± 0.4[a]	0.0	11.0 ± 0.2[a]	.0	NZ	NZ

Table 6: Growth Inhibitory Indices from the Combined Activities of Extracts against Biofilm Producing Bacterial Isolates

Bacterial Isolates	Codes	GIIs	Inference
E coli	EC-B1, EC-B8	≥ 0.53	Synergy
	EC-B3	≤ 0.47	Antagonism
	EC-B4	0.50	Addictive
S aureus	SA-B2, SA-B4	≥ 0.56	Synergy
Proteus spp	PS-B1	≥ 0.59	Synergy
S. pyogenes	SP-B2	≤ 0.49	Antagonism
	SP-B3	≥ 0.61	Synergy
CoN *Staphylococcus* spp	CS-B1	≥ 0.56	Synergy
P. aeruginosa	PA-B1	≥ 0.57	Synergy
E. faecalis	EF-B1	0.50	Addictive

Table 7: Regression Coefficient between different Concentrations of Extracts and Inhibitory Zone Diameters Exhibited by Bacterial Isolates

Bacterial Isolates	Code	Regression (R^2)		
		B. verticillata	*C. citratus*	*B. verticillata + C. citratus*
E coli	EC-B1	0.7308	0.9024	0.9276
	EC-B3	0.9429	0.9637	0.6955
	EC-B4	0.9565	0.9437	0.9922
	EC-B8	0.7316	0.8001	0.6823
S aureus	SA-B2	0.6915	0.7995	0.7940
	SA-B4	0.8547	0.7102	0.6714
Proteus spp	PS-B1	0.8929	0.7481	0.8273
S. pyogenes	SP-B2	0.7289	0.7697	0.9978
	SP-B3	0.7050	0.8970	0.9098
CoN *Staphylococcus* spp	CS-B1	0.7121	0.8929	0.9820
P. aeruginosa	PA-B1	0.7289	0.7017	0.9276
E. faecalis	EF-B1	0.7117	0.9974	0.8848

DISCUSSION

The occurence of biofilm producing *E. coli* and *S. aureus* in this study agrees with the study of Abdallah *et al*. (2011) who reported that *E. coli* and *S. aureus* isolated from urine samples produced .biofilm. The phytochemical screening of ALECC and ALEBV showed the presence of some secondary metabolites such as alkaloids, tannins, flavonoids, saponins and cardiac glycosides in varied concentrations. The detection of these secondary metabolites in ALEBV corroborates the reports of Aremu *et al.* (2016). These secondary metabolites are known to show antimicrobial properties as well as exhibiting physiological activity (Ushie *et al.*, 2013). The moderately high concentrations of flavonoids and tannins observed in ALECC may be responsible for its antibacterial efficacies on biofilm producers. Flavonoids are hydroxylated phenolic substances that are synthesized by plants in response to microbial infection. The activity of flavonoids is possibly due to their ability to complex with bacterial cell walls and disruption of the microbial membrane (Akinjogunla *et al*., 2012). The presence of saponins and cardiac glycosides is an indication of medicinal significance of ALECC and ALEBV. Synergism between plant extracts is a novel conception and could be synergistic, additive or antagonistic (Akinjogunla and Fatunla, 2017). The combined ALECC and ALEBV was synergistic, additive and antagonistic in their effects depending on the biofilm producing bacterial isolates used. The synergistic effects of *C. sinensis* and *J. regia* on MDR bacteria have been reported (Farooqui *et al.*, 2015).

CONCLUSION

The presence of secondary metabolites in *C. citratus* (ALECC) and *B. verticillata* (ALEBC) could provide a synergistic effect which modifies the bioavailability, effectiveness of the active components and might be responsible for their antibacterial activities against biofilm producers and also aids their efficacies for the treatment of infectious diseases.

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