Original Research Article

Antibacterial Activity of *Zea Mays* Silks and Husks Crude Extract on Biofilm Producing Multi-Drug Resistant Bacteria from Urinary Catheters

ABSTRACT

Aims: This study was carried out to investigate the *in-vitro* antibacterial effect of the crude extracts of Corn (*Zea mays*) silks and husks on selected biofilm producing multi-drug resistant bacteria isolated from urinary catheter tips.
Study design: Experimental design
Place and Duration of Study: This study was conducted at the Federal University of Technology, Akure (FUTA) Ondo State, Nigeria.
Methodology: Bacterial isolates from urinary catheter tips were screened for biofilm production. The biofilm producing isolates were subjected to commercial antibiotics and isolates resistant to more than three classes of antibiotics were used for the study. Methanol and distilled water were used as extracting solvents for the corn silks and husks. The antibacterial activity and phytochemical analysis of the extracts were carried out using standard procedures.
Results: The phytochemical analysis of the methanol and aqueous extracts of corn husks and silks revealed the presence of saponins, tannins, flavonoids, steroids, terpenoids and cardiac glycosides. The aqueous extract of corn husk showed highest inhibitory effects on *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli* and Coagulase-Negative *Staphylococc*i with zones of inhibition ranging from 14.80 ± 1.89 mm to 24.40 ± 2.51 mm at 200 mg/mL. *Proteus mirabilis* exhibited resistance against all the extracts.
Conclusion: Findings from this study revealed the antibacterial potential of corn silks and husks extracts at varying concentrations. The potency of aqueous extract of husk at lower concentration (200 mg/mL) suggests its potential use in the treatment of urinary tract bacteria especially *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and Coagulase-Negative *Staphylococc*i.

Keywords: Urinary catheter; Biofilm; antibacterial; phytochemicals; corn silks; corn husks.

1. INTRODUCTION

One of the most important medical interventions of the last century was the introduction of antibiotics which reduced human morbidity and mortality [1]. However, the frequency of resistance among human pathogens has increased strikingly due to intensive use of antibiotics and the adaptability of the organisms. Therefore, this reduces therapeutic drug options, making the effective treatment of infections difficult or posing more risk of complications and fatal outcomes [2]. Thus, the evolution of antibiotic-resistant bacteria and in addition, the emergence of new pathogens has raised a need for novel antimicrobial drugs [3].
Bacteria live a complex lifestyle by growing on surfaces either abiotic or biotic to form biofilms, which represent an adaptation that provides protection from environmental stresses. It has been estimated that biofilm cells are up to 1,000 times more resistant to most antimicrobial agents than planktonic cells and it is also estimated that

80% of all causes of bacterial infections are biofilm related [4]. Biofilms are simply surface-associated communities of microorganisms (bacteria and fungi) that are prevalent in environmental and clinical settings [5]. Microorganisms involved in biofilm formation have been implicated in many infectious diseases including urinary tract infections, native valve endocarditis, chronic otitis media, gastrointestinal ulcers, chronic lung infections in cystic fibrosis (CF) patients and colonization of several medical devices [6].

Medicinal plants have been widely used for restorative rationale right through generations [7]. Many plants possess certain compounds that present important therapeutics properties for the care and treatment of human and other animal diseases but most of these plants are yet to be explored for their medicinal properties [8]. Nigerian traditional medical practitioners have adopted several means of treating infectious diseases with varieties of herbal preparations [9].

Corn, scientifically known as *Zea mays* belonging to the family Poaceae is believed to be first cultivated in North America (Mexico) and now widely cultivated all over the world most especially in Africa and Asia. The corn silks have been reported to be effective in the treatment of renal diseases such as chronic nephritis and urinary tract infections including cystitis [10, 11]. Corn silk is used by people from different countries to control blood pressure and manage a string of maladies comprising fever, gout and urinary tract infections with other bacterial diseases [11]. Sani [12] stated that in China, corn silks have been widely used as antidiabetic agent for many decades.

The corn husk is the multiple of leaf-like structure covering corn and part of the silk. Decoctions of corn husk have been reported to be used as traditional treatment of malaria in Nigeria and also for the treatment of arthritis and pains [13]. However, the bioactivity of the husk is underexplored.

Methanol and aqueous extracts of corn silk have been reported to be rich in alkaloids, flavonoids, tannins and saponins [10, 14]. Feng *et al*. [15] also demonstrated that ethanol extracts of corn silk were more active against Gram-positive bacteria. The incidence of urine bacterial consortia is affected by diverse dynamics comprising age, gender, weakened immunity, and use of catheter [16]. The bacterial continuum of urine bacterial consortia largely consists of *Escherichia coli*, and other uropathogens comprising *Staphylococcus saprophyticus*, *Enterococcus* spp., *Proteus* spp., *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are seldom isolated from urine tracts of patients [17]. The present study investigated the antibacterial activity of *Zea mays* silks and husks extracts on the biofilm producing bacteria isolated from urinary catheter tips.

2. MATERIALS AND METHODS

2.1 Collection of urinary catheter tips

A total of Hundred and eighty (180) urine catheter tips were aseptically collected in sterile bottles from patients in eight (8) wards of Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) Ile-Ife, Nigeria following ethical approval. They were transported immediately after collection to the Microbiology laboratory of the Federal University of Technology, Akure (FUTA) for culturing.

2.2 Isolation of bacteria from urinary catheter tips

Already prepared MacConkey agar (Hi-Media, India) and Cysteine Lactose Electrolyte Deficient Agar (CLED) (Hi-Media, India) media were inoculated by making a tiny smear with the urinary catheter tips. A sterile wire loop was used to spread the smear on the entire surface of the media by streak-plating method. The media plates were incubated aerobically at 37 ^{o}C for 24 h, then a single pure isolated colony was maintained on nutrient agar medium and each experiment was carried out using a fresh overnight culture.

2.3 Identification of bacterial isolates

The bacterial isolates were identified by morphological and biochemical characterization using standard techniques as described by Hemraj *et al.* [18]. Gram staining procedure was carried out followed by various biochemical tests.

2.4 Biofilm Formation Assay

The bacterial isolates were subjected to biofilm formation assay. This assay was based on the ability of the test organisms to form biofilms on plastics surfaces. This was carried using 1 mL plastic cuvettes. The culture of the different isolate that was tested was grown overnight in Nutrient broth. The overnight culture was diluted 1:100 into a fresh Nutrient broth and 0.6 mL of the diluted culture was added to sterile cuvettes in triplicate using sterile broth as control. The cuvettes were secured with sterile thick aluminum corks to prevent desiccation. The aforementioned procedure was setup respectively for 6, 12, 18 and 24 h aerobic incubation at 37 ^{o}C. After each incubation time, the cuvettes were washed with distilled water 2-3 times to remove unattached cells and media in the cuvettes. 0.8 mL of 10 % Crystal Violet was added to each cuvette and were incubated at room temperature for 10-15 min and rinsed thoroughly 3-4 times with distilled water, shaken vigorously and blotted

thoroughly on a stack of clean paper towels to remove excess dyes and cells. The cuvettes were left to dry for few hours. 0.8 mL of 30 % acetic acid was added to each stained cuvette to solubilize the Crystal Violet and incubated at room temperature for about 10-15 min. The biofilm was read at absorbance 600 nm with acetic acid as the blank.

2.5 Preparation of standard inoculums

Nutrient broth tube was inoculated with a loop full of bacterial isolate growth and incubated at 37 °C for 18 h. The bacterial suspension was then diluted with sterile distilled water, adjusted the turbidity to match the 0.5 McFarland standard using the mass spectrophotometer at 625 nm to a value of 0.08 – 0.1 to obtain bacterial suspension with cell density of 1.5×10^8 CFU/mL.

2.6 Antibiotic sensitivity testing

Antibiotic sensitivity tests were performed on the biofilm producing isolates using standard agar diffusion protocols as described by Clinical Laboratory Standard Institute (CLSI) [19], with commercially available antibiotics (ABTEK, UK). All sensitivity tests were carried out using fresh overnight cultures. About 0.2 mL suspension of each bacterial isolates, equivalent to 0.5 McFarland standards was aseptically spread on already prepared Mueller-Hinton agar (MHA) (Hi-Media, India) plates respectively. This was allowed to stand for few minutes. Commercially produced antibiotic discs containing different classes of antibiotics such as Cephalosporins (Cefuroxime (CRX) (30 µg), Ceftazidime (CAZ) (30 µg), Cefixime (CXM) (30 µg) and Ceftriaxone (CTR) (30 µg)), Penicillins (Augmentin (AUG) (30 µg) and Cloxacillin (CXC) (5 µg)), Aminoglycoside (Gentamycin (GEN) (10 µg)), Fluoroquinolones (Ofloxacin (OFL) (5 µg) and Ciprofloxacin (CPR) (5 µg)), Macrolide (Erythromycin (ERY) (5 µg)) and Nitrofurantoin (NIT) (300 µg) were aseptically placed on the surface of the media using a sterile forcep. These were incubated for 18-24 h at 37 ^{o}C, after which the diameter of zone of inhibition was measured using a ruler and the results were interpreted using standard interpretative charts as recommended by CLSI, [19]. Multidrug resistance was indicated by resistance to a minimum of three different classes of antibiotics.

2.7 Collection of test plant

Fresh corn husks and silks were collected from a self-cultivated farm located at Bolorunduro area of Ilesa, Osun State, Nigeria, with geographic coordinates 7.4905° N, 4.7096° E. The silks were carefully separated from the husks, washed with water to remove dust and any other unwanted particles. They were then allowed to air dry very well on a clean surface and then grinded to powder using a grinding machine.

2.8 Methods of corn silk and husk extraction

The cold extraction method was adopted, whereby the ground silks and husks were soaked in distilled water and methanol respectively from which the extraction procedure was carried out as described by Ogundare, [8].

2.8.1 Aqueous Extract

Exactly 150 g of silks and 250 g of husks were soaked in 1 L and 2 L of distilled water respectively following the procedure for N-hexane extraction. The extracts obtained were labelled as SA (aqueous silks extract) and HA (aqueous husks extracts).

2.8.2 Methanol Extract

Exactly 150 g of silks and 250 g of husks were soaked in 1 L and 2 L of Methanol respectively for 72 h. Following the extraction procedure above, the subsequent extracts obtained were labelled as SM (methanol silk extract) and HM (methanol husk extract).

2.9 *In-vitro* antimicrobial activity of *Zea mays* silk and husk extracts

The agar well diffusion method was adopted. Sterile swab was dipped into standardized bacterial suspensions and streaked on the entire Mueller-Hinton agar plates. The plates were left for 5-15 min to allow the medium absorb the inoculums. With the aid of a cork borer (6 mm in diameter), holes were bore in the inoculated agar and 50 µL of the extracts were dispensed into the holes. The plates were incubated at 37 ^{o}C for 18-24 h. The plates were observed for zone of inhibition.

2.10 Minimum Inhibitory Concentration (MIC)

This was carried out using the agar well diffusion method as described by Luitel & Dahal [20]. Extracts were reconstituted to different concentrations of 500 mg/mL, 400 mg/mL, 300 mg/mL, 200 mg/mL, 100 mg/mL, 50

mg/mL, 25 mg/mL and 12.5 mg/mL. The MIC was regarded as the lowest concentration which completely inhibited the bacterial growth.

2.11 Statistical analysis of data

Experiments were performed in triplicates and data derived were expressed as mean ± standard deviation and were subjected to Analysis of Variance (ANOVA) with level of significance documented at $P \leq 0.05$. Separation of means were performed using Duncan's new multiple range test (DNMRT) at 95 % confidence level.

3. RESULTS

3.1 Catheters used for this study

A total of 180 urinary indwelling catheter tips were collected from patients in 8 wards of Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State Nigeria. The catheter tips differed in colour and texture depending on the length of stay in the patient before removal. The catheters that stayed one month and above in the patients had brown to black colouration on the tips, those that stayed for about three weeks had a slight colour change while those that stayed for less than two weeks had no remarkable visible change when compared to new ones as shown on Plate 1.

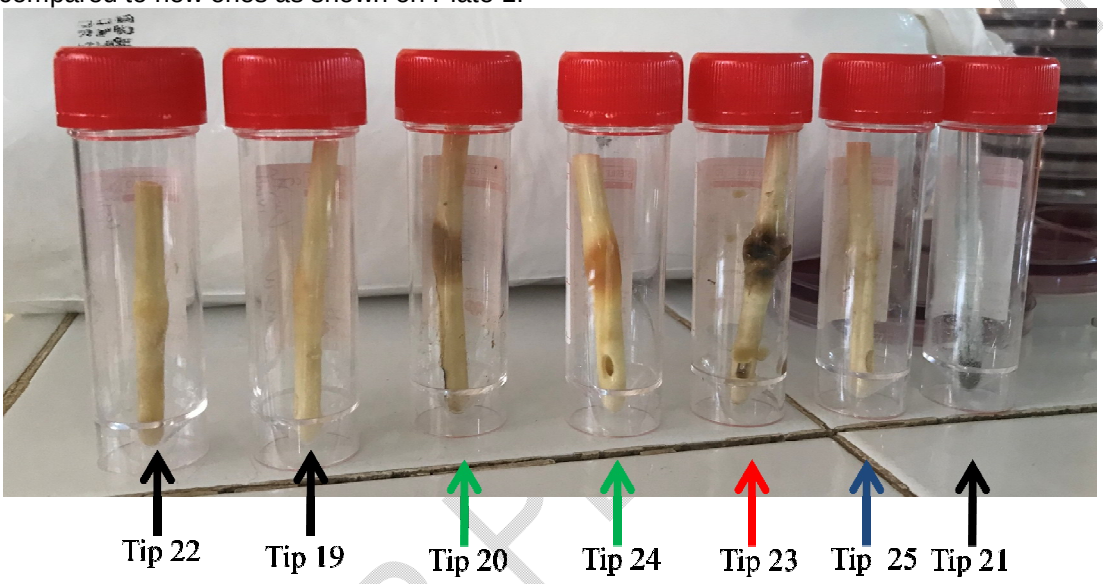

Plate 1: Some of the catheter tips collected for the study.

Key

Tip 25 stayed for 2 weeks
Tips 22, 19 and 21 stayed for 3 weeks
Tips 20 and 24 stayed for about 1 month
Tip 23 stayed for more than 1 month

3.2 Prevalence of microorganisms associated with the urinary catheter tips

A total of one hundred and twenty six (126) organisms were isolated from one hundred and eighty (180) catheter tips sampled on CLED and MacConkey agars. The catheters that stayed in the patient up to a month had at least one organism isolated from them while those that stayed for less than two weeks had no visible growth from them. The organisms were subjected to Gram staining procedure. Thirty-four (34) of the isolates were identified as yeast, twenty (20) were suspected to be *Lactobacillus* spp. (Gram Positive Rods) while the remaining seventy-two (72) isolates were subjected to further biochemical tests for identification. The most prevalent bacteria was *Staphylococcus aureus* (23.6 %), followed by *Escherichia coli* (19.4 %), *Klebsiella pneumoniae* (16.7 %), *Pseudomonas aeruginosa* (15.3 %), *Proteus mirabilis* (13.9 %) while Coagulase-Negative *Staphylococci* had the least prevalence at 11.1 % as shown in Table 1.

Table 1: Prevalence of bacteria isolated from cultured catheter tips

Bacterial Isolates	Number Isolated	Percentage (%)
Staphylococcus aureus	17	23.6
Escherichia coli	14	19.4
Klebsiella pneumoniae	12	16.7
Pseudomonas aeruginosa	11	15.3
Proteus mirabilis	10	13.9
Coagulase-Negative *Staphylococci*	8	11.1

3.3 Biochemical identification of bacteria associated with the urinary catheter tips

Gram-negative bacteria isolated include *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus mirabilis* while *Staphylococcus aureus* and Coagulase-Negative *Staphylococci* were the Gram-positive bacteria implicated in urinary catheter tips in this study as illustrated in Table 2.

Table 2: Morphological and biochemical characteristics of the bacterial isolates

Cultural characteristics	**Organisms**					
	A	**B**	**C**	**D**	**E**	**F**
Colour	Pale yellow	Cream	Greenish blue	Cream	Cream	Cream/White
Shape	Circular	Circular	Irregular	Circular	Irregular	Circular
Edge	Entire	Entire	Entire	Entire	Lobate	Entire
Elevation	Raised	Flat	Flat	Flat	Flat	Raised
Surface	Smooth	Smooth	Smooth	Smooth	Rough	Smooth
Morphological characteristics						
Shape	Cocci	Rod	Rod	Rod	Rod	Cocci
Biochemical tests						
Gram Reaction	+	-	-	-	-	+
Catalase	+	+	+	+	+	+
Coagulase	+	-	±	-	±	-
Oxidase	-	-	+	-	-	-
Citrate	+	-	+	+	+	-
Urease	±	-	-	+	±	±
Indole	-	+	-	-	-	-
Motility	-	+	+	-	+	-
H_2S	-	-	-	-	+	±
Sugar fermentation						
Glucose	A	A	A	AG	A	AG
Sucrose	A	-	-	-	-	A
Lactose	A	-	-	-	-	A
Galactose	-	A	-	A	A	-
Maltose	A	-	-	-	-	A
Mannitol	AG	-	-	-	-	±

Key: A = *Staphylococcus aureus*, B = *Escherichia coli*, C = *Pseudomonas aeruginosa*, D = *Klebsiella pneumoniae*, E = *Proteus mirabilis*, F = Coagulase-Negative *Staphylococci* (a = Acid, a/g = Acid and Gas, (-) = Negative, (+) = Positive).

3.4 Biofilm Formation Assay

The biofilm result showed that 64 (88.9 %) out of 72 bacterial isolates produced biofilm significantly ($P \leq .05$) with different quantities at 6, 12, 18 and 24 h while 8 (11.1 %) isolates did not form any remarkable biofilm on the cuvettes . Figures 1 to 6 shows biofilm formation by different bacteria.

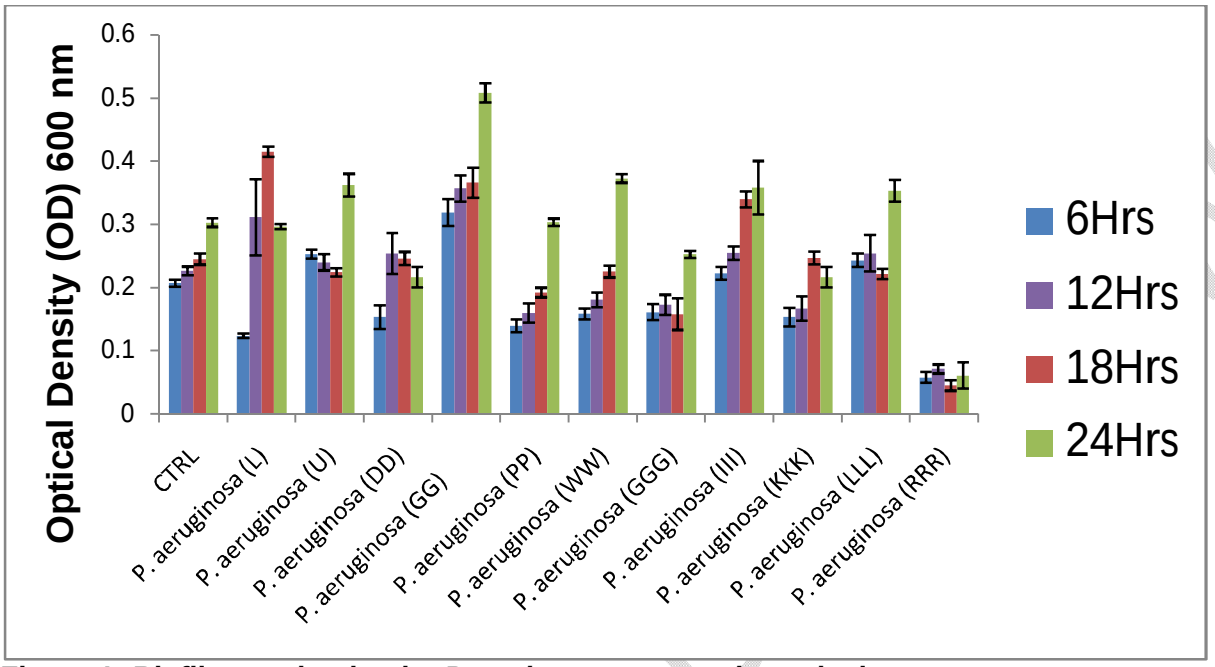

Figure 1: Biofilm production by *Pseudomonas aeruginosa* isolates

(**Key:** L, U, DD, GG, PP, WW, GGG, III, KKK, LLL & RRR = isolate codes)

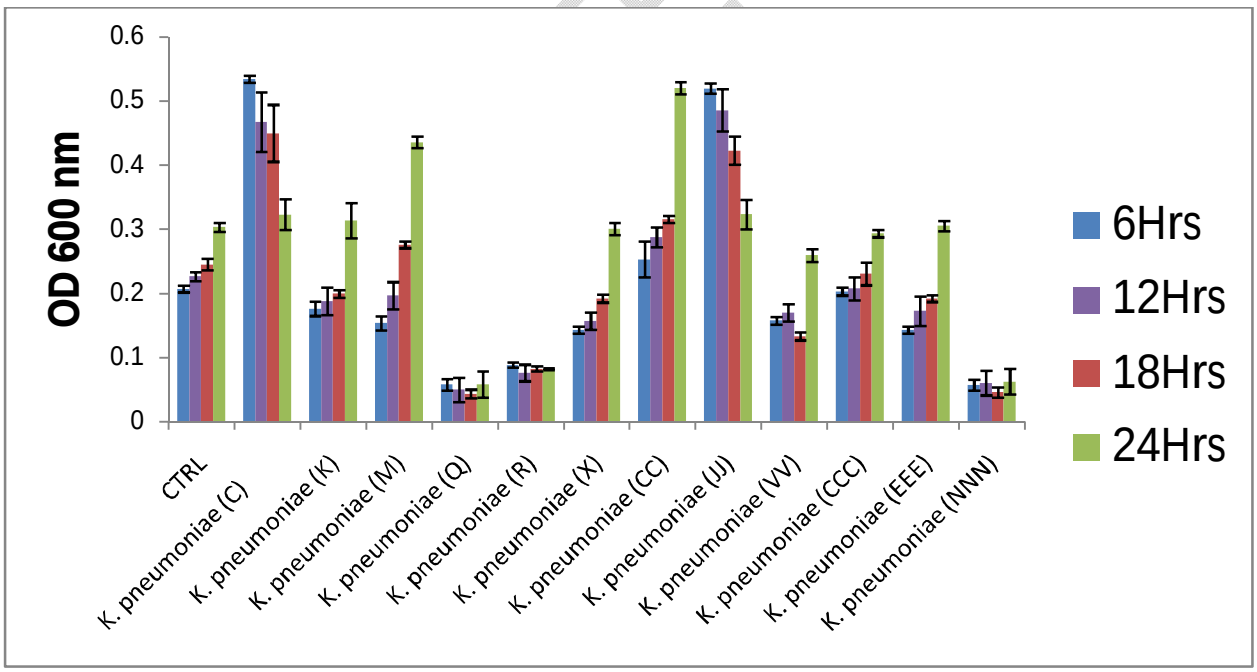

Figure 2: Biofilm production by *Klebsiella pneumoniae* isolates

(Key: C, K, M, Q, R, X, CC, JJ, VV, CCC, EEE & NNN = Isolate codes)

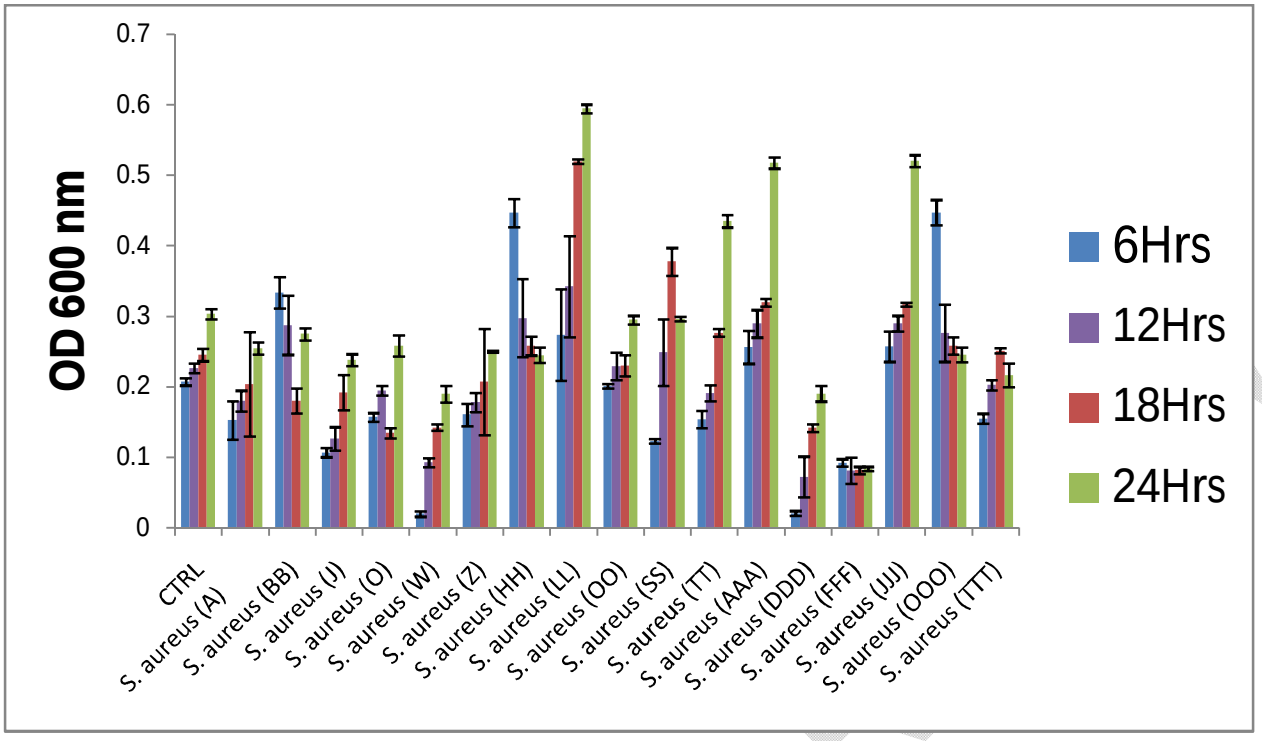

Figure 3: Biofilm production by *Staphylococcus aureus* isolates

(**Key:** A, BB, J, O, W, Z, HH, LL, OO, SS, TT, AAA, DDD, FFF, JJJ, OOO & TTT = Isolate codes

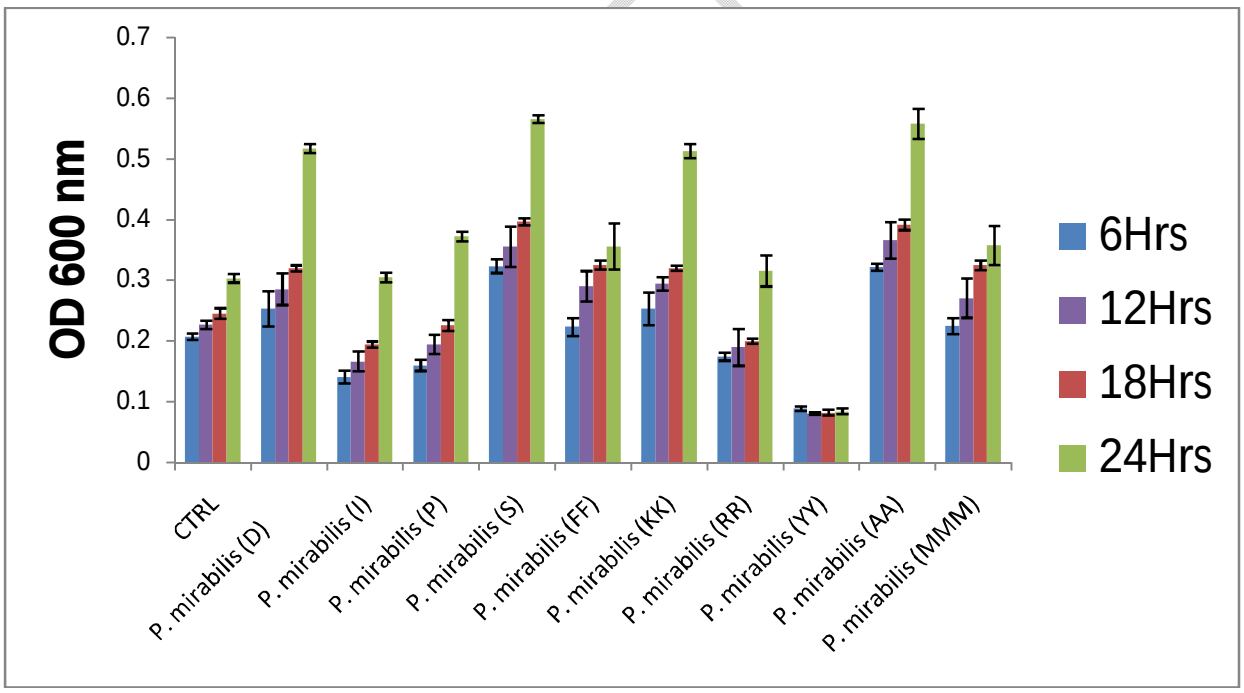

Figure 4: Biofilm production by *Proteus mirabilis* isolates

(Key: D, I, P, S, FF, KK, RR, YY, AA & MMM = Isolate codes)

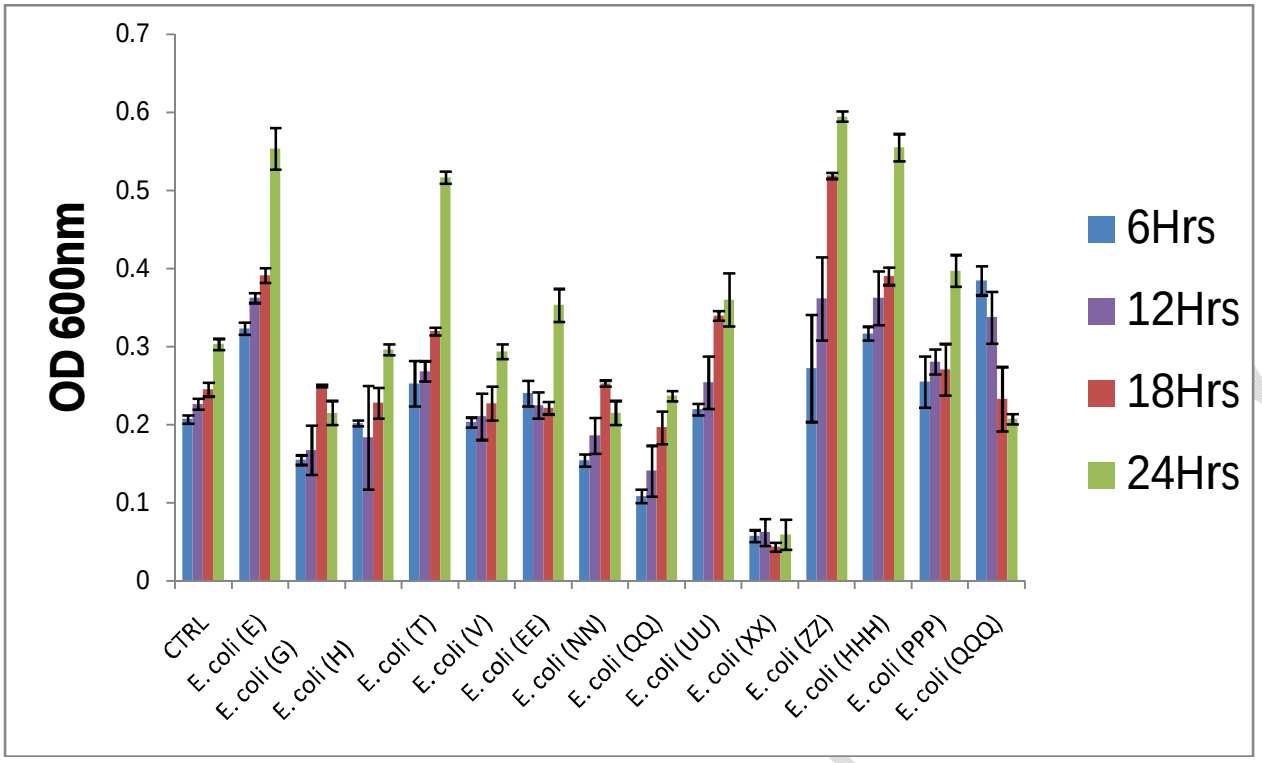

Figure 5: Biofilm production by *Escherichia coli* isolates

(Key: E, G, H, T, V, EE, NN, QQ, UU, XX, ZZ, HHH, PPP & QQQ = Isolate codes)

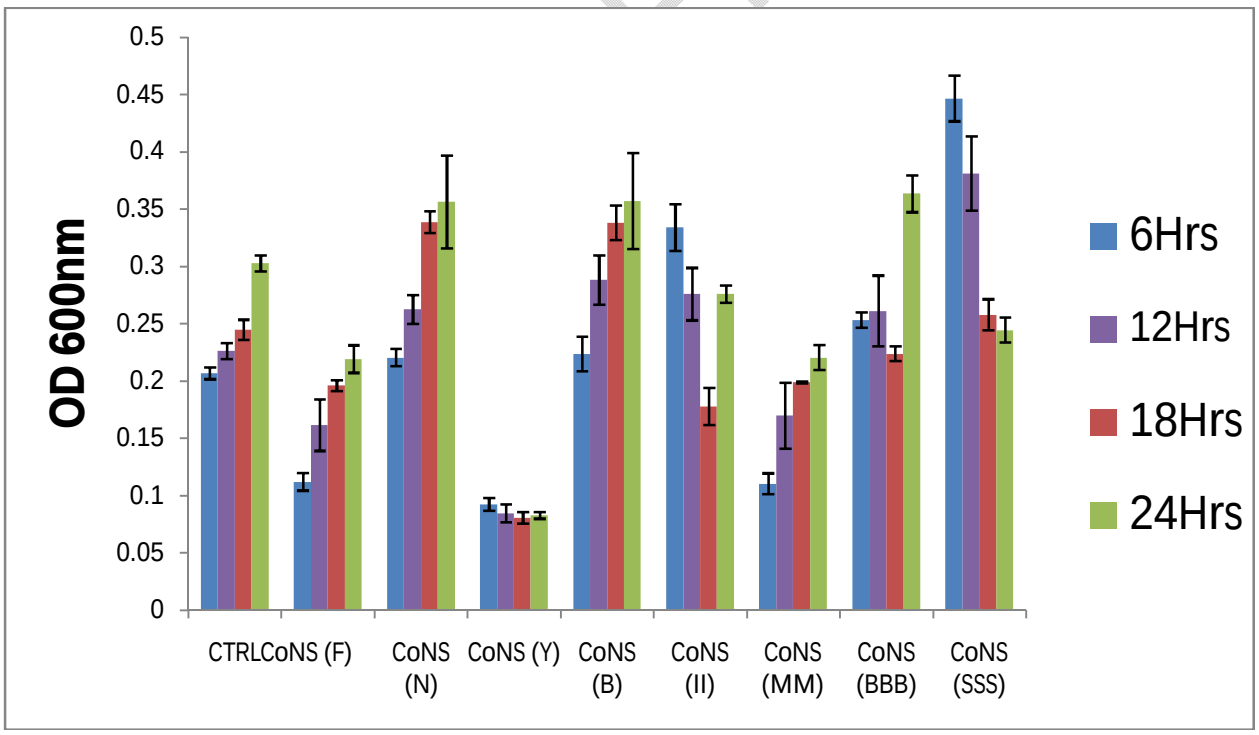

Figure 6: Biofilm production by Coagulase-Negative *Staphylococci* isolates

(Key: F, N, Y, B, II, MM, BBB & SSS = Isolate codes)

3.5 Antibiotic sensitivity patterns of bacterial isolates from urinary catheter tips

Klebsiella pneumoniae had the highest level of resistance (100 %) to Cefuroxime, Ofloxacin, Nitrofurantoin, Ceftazidime and Ceftriaxone while the resistance rate to other antibiotics was Augmentin (33.3 %), Ciprofloxacin (22.2 %) and Gentamicin (22.2 %). *Staphylococcus aureus* and Coagulase negative *Staphylococci* isolates were highly resistant (100 %) to Augmentin, Ceftazidime, Cefuroxime, Erythromycin and Cloxacillin. *Pseudomonas aeruginosa* isolates were all resistant (100 %) to Cefixime, Ofloxacin and Ceftazidime. The resistant pattern to other antibiotics include Augmentin (90 %), Ciprofloxacin (50 %), as well as Nitrofurantoin, Cefuroxime and Gentamicin (80 %). *Proteus mirabilis* isolates had 100 % resistance to Augmentin, Nitrofurantoin and Cefuroxime. *Escherichia coli* was highly sensitive (100 %) to Augmentin and Gentamycin. Both *Staphylococcus aureus* and Coagulase-negative *Staphylococci* also had a sensitivity pattern of (62.5 %) and (100 %) to gentamycin respectively. *Klebsiella pneumoniae* had the highest sensitivity pattern of (66.7 %) to Augmentin, (77.8 %) to Ciprofloxacin and (77.8 %) to Gentamycin as illustrated in Table 3.

Table 3: Antibiogram of bacteria isolated from urinary catheters

			Antibiotics									
Bacterial Isolate	**Total No. (%)**	**S, I, R**	**CXM 5µg**	**OFL 5µg**	**AUG 30µg**	**NIT 300µg**	**CPR 5µg**	**CAZ 30µg**	**CRX 30µg**	**GEN 10µg**	**ERY 5µg**	**CXC 5µg**
Pseudomonas aeruginosa	10(15.6)	S (%)	0(0)	0(0)	1(10)	2(20)	1(10)	0(0)	2(20)	0(0)	-	-
		I (%)	0(0)	0(0)	0(0)	0(0)	4(40)	0(0)	0(0)	2(20)	-	-
		R (%)	10(100)	10(100)	9(90)	8(80)	5(50)	10(100)	8(80)	8(80)	-	-
Klebsiella pneumoniae	9(14.1)	S (%)	0(0)	0(0)	6(66.7)	0(0)	7(77.8)	0(0)	0(0)	7(77.8)	-	-
		I (%)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	-	-
		R (%)	9(100)	9(100)	3(33.3)	9(100)	2(22.2)	9(100)	9(100)	2(22.2)	-	-
Escherichia coli	13(20.3)	S (%)	6(46.2)	8(61.5)	13(100)	7(53.8)	2(15.4)	4(30.8)	11(84.6)	13(100)	-	-
		I (%)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	-	-
		R (%)	7(53.8)	5(38.5)	0(0)	6(46.2)	11(84.6)	9(69.2)	2(15.4)	0(0)	-	-
Proteus mirabilis	9(14.1)	S (%)	7(77.8)	4(44.4)	0(0)	0(0)	7(77.8)	3(33.3)	0(0)	6(66.7)	-	-
		I (%)	1(11.1)	2(22.2)	0(0)	0(0)	0(0)	1(11.1)	0(0)	3(33.3)	-	-
		R (%)	1(11.1)	3(33.3)	9(100)	9(100)	2(22.2)	5(55.6)	9(100)	0(0)	-	-
Staphylococcus aureus	16(25)	S (%)	-	5(31.3)	0	-	-	0	0	10(62.5)	0	0
		I (%)	-	2(12.5)	0	-	-	0	0	6(37.5)	0	0
		R (%)	-	9(56.3)	16(100)	-	-	16(100)	16(100)	0(0)	16(100)	16(100)
Coagulase-Negative *Staphylococci*	7(10.9)	S (%)	-	5(71.4)	0(0)	-	-	0(0)	0(0)	7(100)	0(0)	0(0)
		I (%)	-	0(0)	0(0)	-	-	0(0)	0(0)	0(0)	0(0)	0(0)
		R (%)	-	2(28.6)	7(100)	-	-	7(100)	7(100)	0(0)	7(100)	7(100)

Keys: S = Sensitive, I = Intermediate, R = Resistant, CXM = Cefixime, OFL = Ofloxacin, AUG = Augmentin, NIT = Nitrofurantoin, CPR = Ciprofloxacin, CAZ = Ceftazidime, CRX = Cefuroxime, GEN = Gentamicin, ERY = Erythromycin, CTR = Ceftriaxone, CXC = Cloxacillin

3.6 Percentage recovery profile of corn silk and corn husk crude extracts

The methanol extract of corn husk had the highest recovery of 9.8 % (24.5 g) while aqueous extract had 24.11 g (6.94 %) recovery. The aqueous extract of corn silk had the lowest extract recovered (9.77 g) and the lowest percentage recovery of 6.51 % as shown in Table 4.

Table 4: Percentage yield of Corn Silks and Husks crude extracts

Plant	Solvents	Powdered Sample (g)	Extract Recovered (g)	Percentage Yield (%)
Corn Silks	Methanol	150	13.04	8.69 %
	Aqueous	150	9.77	6.51 %
Corn Husks	Methanol	250	24.50	9.8 %
	Aqueous	250	24.11	9.64 %

3.7 Qualitative and quantitative phytochemical analysis of corn silks and husks crude extracts

Saponin, tannin, flavonoids, steroids, and terpenoids were present in both aqueous and methanolic crude extracts of corn silks while phlobotannin, anthraquinone and alkaloids were absent. Saponin had the highest quantitative phytochemical content of 58.36 mg/g while flavonoid had the least at 0.34 mg/g for methanolic extract. Saponin was the highest phytochemical at 51.64 mg/g while flavonoid was the least at 0.30 mg/g for aqueous crude extract (Table 5). Saponin, tannin, flavonoids, steroids, and terpenoids were also present in both aqueous and methanolic crude extracts of corn husk while phlobotannin, alkaloids and anthraquinone were absent. Saponin had the highest quantitative phytochemical content of 37.45 mg/g while flavonoid had the least at 0.24 mg/g for methanolic extract. Saponin was the highest phytochemical at 17.45 mg/g while flavonoid was the least at 0.09 mg/g for aqueous crude extract as demonstrated in Table 6.

Table 5: Qualitative and Quantitative Phytochemical Constituents of Corn Silks

PARAMETERS	METHANOL		AQUEOUS	
	Qualitative	Quantitative (mg/g)	Qualitative	Quantitative (mg/g)
Saponin	+	58.36	+	51.64
Tannin	+	5.81	+	5.15
Phlobatannin	-	-	-	-
Flavonoid	+	0.34	+	0.30
Steroid	+	4.99	+	3.29
Terpenoid	+	20.03	+	15.45
Alkaloid	-		-	-
Anthraquinone	-	-	-	-
CARDIAC GLYCOSIDES		26.75		19.29
Legal test	+		+	
Keller kiliani test	+		+	
Salkwoski test	+		+	
Lieberman test	+		+	

Key: + = Positive; - = Negative

Table 6: Qualitative and Quantitative Phytochemical Constituents of Corn Husks

PARAMETERS	METHANOL		AQUEOUS	
	Qualitative	Quantitative (mg/g)	Qualitative	Quantitative (mg/g)
Saponin	+	37.45	+	17.45
Tannin	+	4.16	+	4.00
Phlobatannin	-	-	-	-
Flavonoid	+	0.24	+	0.09
Steroid	+	2.40	+	1.26
Terpenoid	+	12.90	+	8.11
Alkaloid	-	-	-	-
Anthraquinone	-	-	-	-
CARDIAC GLYCOSIDES		19.16		13.18
Legal test	+		+	
Keller kiliani test	+		+	
Salkwoski test	+		+	
Lieberman test	+		+	

Key: + = Positive; - = Negative

3.8 Antibacterial activity of corn silks and husks extracts on bacteria from urinary catheter tips

The aqueous extract of corn husks (HA) showed highest antibacterial activity on all the bacterial isolates except *Proteus mirabilis* isolates which showed no sensitivity to all the extracts. At the highest concentration of 500 mg/mL, *Staphylococcus aureus* isolates had the highest average zone of inhibition (24.40 ± 2.51 mm) while *Klebsiella pneumoniae* isolates had the least at 14.80 ± 1.89 mm for aqueous corn husk extract. *Pseudomonas aeruginosa* isolate had the highest zone of inhibition at 16.00 ± 0.00 mm for corn silk aqueous extract while Coagulase-Negative *Staphylococci* isolate had the least at 12.00 ± 0.00 mm. *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis* showed no zone of inhibition to methanolic extracts of corn husks and silks. *Staphylococcus aureus* had the highest ZOI at 20.00 ± 4.24 mm and 15.00 ± 0.00 mm while Coagulase-Negative *Staphylococci* had the least at 12.80 ± 3.74 mm and 13.30 ± 2.08 mm for methanolic extracts of corn silks and husks respectively as shown in Table 7.

Table 7: Zones of inhibition (mm) induced by corn husks and silks extracts at 500 mg/mL against biofilm producing bacteria isolated from catheter tips

Bacterial isolates	HM	HA	SM	SA
Pseudomonas aeruginosa	0.00 ± 0.00^{a}	18.60 ± 2.61^{b}	0.00 ± 0.00^{a}	16.00 ± 0.00^{b}
Escherichia coli	0.00 ± 0.00^{a}	17.80 ± 3.87^{b}	0.00 ± 0.00^{a}	14.50 ± 0.71^{b}
Staphylococcus aureus	15.00 ± 0.00^{b}	24.40 ± 2.51^{c}	20.00 ± 4.24^{b}	14.00 ± 0.00^{b}
Klebsiella pneumoniae	0.00 ± 0.00^{a}	14.80 ± 1.89^{b}	0.00 ± 0.00^{a}	13.00 ± 1.41^{b}
Proteus mirabilis	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Coagulase-Negative *Staphylococci*	13.30 ± 2.08^{b}	18.80 ± 3.27^{b}	12.8 ± 3.74^{b}	12.00 ± 0.00^{b}

Key: HM = Husk Methanol, HA = Husk Aqueous, SM = Silk Methanol, SA = Silk Aqueous. Values are mean ± standard deviation of replicates. Values with the same superscript letter along the same column are not significantly different ($P \leq 0.05$).

3.9 Minimum Inhibitory Concentration (MIC) of methanolic and aqueous extracts of corn husks and silks on the test bacteria

The Minimum Inhibitory Concentration (MIC) of aqueous extract of corn husks was 200 mg/mL for all the test isolates. Aqueous extract of corn silks had higher MIC at 500 mg/mL for all the test isolates while methanolic extract of silks had MIC of 500 mg/mL for only Coagulase-Negative *Staphylococci* species. The MIC for methanolic extract of husks was 500 mg/mL for *Staphylococcus aureus* and Coagulase-Negative *Staphylococci.*

Table 8: Minimum Inhibitory Concentration (MIC) of corn silks and husks extracts on test bacteria

	MIC (mg/mL)			
	Corn Silks		Corn Husks	
Bacterial Isolates	**Aqueous**	**Methanolic**	**Aqueous**	**Methanolic**
Escherichia coli	500	N.D	200	N.D
Klebsiella pneumoniae	500	N.D	200	N.D
Staphylococcus aureus	500	N.D	200	500
Pseudomonas aeruginosa	500	N.D	200	N.D
Coagulase-Negative *Staphylococci*	500	500	200	500

Key: N.D = Not Detected

4. DISCUSSION

Urethral catheterization has been reported to be a prominent risk factor associated with urinary tract infections [21]. This study revealed that 30 % of the catheters tips collected did not produce any visible growth on CLED and MacConkey agars. The percentage of bacteria isolated from these catheter tips was dependent on the length of stay of the catheters on the patients. Morphological and biochemical identification of the bacterial isolates revealed *Staphylococcus aureus* as the most prevalent in this study. The high occurrence of *Staphylococcus aureus* in this study could be attributed to contamination of the catheter tips with skin bacterial flora at the point of insertion or removal as supported by Bayode *et al*. [22]. This is similar to another study by Pondei *et al.* [23] where *Staphylococcus aureus* was reported as the most predominant urinary tract pathogen isolated, followed closely by *Escherichia coli, Proteus mirabilis and Klebsiella pneumoniae*. However, the high occurrence of *Staphylococcus aureus* negates the report from some other previous studies [24, 25] where *Escherichia coli* was found to be the most prevalent uropathogen.

Biofilms Production by the isolates varied in quantities and patterns per time when compared with the control organism and this could be attributed to the length of stay of the catheters on the patients which influenced the bacterial adhesion and the different surface adaptability nature of the bacterial isolates [26]. Regarding the antimicrobial susceptibility testing, *Escherichia coli* isolates showed more susceptibility to the available antibiotics than other bacterial isolates with 100 % sensitivity to Augmentin and Gentamicin as shown in table 3. However, overall susceptibility rates of the isolates to the antibiotics were generally low and highly variable in this study. Similar rates have been reported from tertiary hospitals in South west Nigeria by Ochada *et al*. [25], while another study by Oluwafemi *et al*. [21] reported higher susceptibility rates. This infers that antibiogram pattern of uropathogens varies from place to place depending on the samples collected, methods of isolation as well as the adaptation of the isolates.

Corn silks and husks extracts showed antibacterial activity with varying degrees as suggested by the diameters of inhibition zones. Minimum inhibitory concentration test revealed that methanolic extract of silks had no inhibitory effect on *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* at the highest concentration (500 mg/mL) tested. Similarly, methanolic extract of husks showed no inhibitory effect on *E. coli, K. pneumoniae* and *P. aeruginosa* which suggests that the extracts may have higher MIC against the isolates. The antibacterial activities of corn silk extracts can be attributed to the presence of various organic compounds such as tannins, alkaloids, flavonoids, terpenoids, glycosides and steroids [27, 28]. Similarly, the presence of saponins, tannins, flavonoids and cardiac glycosides observed in corn husk extracts in this present study bears credence with the report of Okokon *et al*. [13] who divulged these compounds as the contributing factor to the antimicrobial nature of the husks. Notable phytochemicals in corn husks and silks crude extracts in this study also bears similarities with the studies of Hasanudin *et al*. [29] and Nawaz *et al*. [30] who all revealed the antioxidant potential and biological activities of corn with emphasis on the phytochemical composition. Therefore, non-nutritive bioactive molecules found in various plants parts referred to as

phytochemicals are important plants components conferring antioxidant, antibacterial and other biological qualities to the plants [30].

5. CONCLUSION

Findings of this study revealed that aqueous extract of corn husks possesses better antibacterial potential than the methanolic extract of husks as well as the aqueous and methanolic extracts of corn silks. The extracts of silks and husks have shown antibacterial activity against one or more urinary tract isolates. This provides a rationale for the use of this plant part in traditional medicine in Nigeria. The antimicrobial activities of these plant parts have been earlier reported and this can be further explored to provide more insight as to how they can be used as alternative treatment against synthetic drugs. This calls for the adoption of the corn silk and husk as primary raw materials in the production of future natural products for the management of urinary tract bacteria.

ETHICAL APPROVAL

Ethical approval was obtained from the Ethics and Research Committee of Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria. The clearance document carries the International Registration number: IRB/IEC/0004553, National Number: NHREC/27/02/2009a and Protocol Number: ERC/2019/05/17

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

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