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Prevention of Bacterial Biofilms Formation on Urinary Catheter by Selected Plant Extracts

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ABSTRACT
In this study, we investigated the feasibility of using *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum* leaf extracts in preventing *Escherichia coli* biofilm formation. The plants extractions were done with methanol under cold extraction. The various concentrations 5.0, 10.0 and 20.0 mg mL^{-1} were used to coat 63 catheters under mild heat from water bath. Biofilm formation on the catheter was induced using cultures of *E. coli*. Biofilm formation was evaluated using aerobic plate count and turbidity at 600 nm. From the obtained results, *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum* delayed the onset of biofilm formation for a week. *Ocimum gratissimum* coated catheter had the highest inhibitory effect at 5.0, 10.0 and 20.0 mg mL^{-1} with bacterial count ranging from 2.2×10^5-7.0×10^4 and 5.7×10^5-3.7×10^5 for 120 and 128 h, respectively. The *Psidium guajava* coated catheter had the lowest inhibitory effect at 5.0, 10.0 and 20.0 mg mL^{-1}, with bacterial count ranging between 4.3×10^5-1.9×10^3 and 7.7×10^5-3.8×10^5 for 120 and 128 h, respectively. Despite the antimicrobial activities, the differences in the activity of these plant extracts were statistically not significant ($p<0.05$).

Key words: *Psidium guajava*, *Mangifera indica*, *Ocimum gratissimum*, *E. coli* biofilm, catheters

INTRODUCTION

Urinary catheterization has been an age-long process devised due to the emergence of medical conditions such as inability to store urine or the inability to pass out urine (Niel-Weise and van den Broek, 2005). Urinary catheters are inserted into the bladder either through the urethral (transurethral) or the anterior abdominal walls (suprapubic) (Getliffe, 2007; Tenke *et al*., 2008; Geng *et al*., 2012). The duration of catheter use differs from patient to patient, as it depends on how severe their condition occurs. Some patients may use catheters for 14 days or less (short term catheterization) while others could extend its use for about 30 days or more (long term catheterization) (Getliffe, 2007; Tenke *et al*., 2008; Geng *et al*., 2012). The use of catheter has helped immensely to keep the bladder functional in medically indisposed patients. However, catheter insertion has been a major concern because of its tendency to harbor harmful microorganisms including *Escherichia coli*, *Staphylococcus aureus*, *Proteus*, *Klebsiella*, *Enterobacter* and *Pseudomonas* species. *Candida* species are also involved in biofilm formation on catheter surfaces (Vlamakis, 2011).

Biofilms are formed when organisms adhere to catheter surfaces using flagella and other motility appendages. The organisms adapt to this new environment, grow and increase their population to become a sessile community. The community enlarges and become diversified through cooperation and quorum sensing (Trautner and Darouiche, 2004; Dwyer, 2008; Francolini and Donelli, 2010). Biofilms can colonize a whole catheter and move along the internal lumens of catheters into the bladder, kidney and sometimes the blood stream. This poses a public health problem for patients who depend on urinary catheters. Some of the nosocomial urinary tract infections that could arise due to catheters include

urethritis, cystitis, pyelonephritis, renal scarring, bacteremia and in severe cases death (Warren *et al*., 1987; Ong *et al*., 2008; Jacobsen *et al*., 2008; Niel-Weise and van den Broek, 2005; Watts *et al*., 2010; Djeribi *et al*., 2012).

About 75-80% of nosocomial catheter associated urinary tract infection and 68% recurring urinary tract infection have been associated with uropathogenic strains of *Escherichia coli* (Chibeu *et al*., 2012). *E. coli* are facultative anaerobic Gram negative bacilli that belong to the family Enterobacteriaceae (Tolg *et al*., 2011). Uropathogenic *E. coli* are serotypes of *E. coli* that have motility and adhesive structures such as fimbriae, capsule and flagella. They express virulence traits which contribute to their successful colonization and formation of biofilms on the surfaces of urinary catheter (Ranjan *et al*., 2010).

Currently, there has been great interest in the search for non-lethal antibiofilm agents. The coating of devices surfaces with either one or two antimicrobial substances or entrapping of these agents within the devices material is the approach most often used to obtain devices with different antimicrobial spectra and durations of the antimicrobial effect. Some of the different antibiotics used include: Silver oxide, kanamycin, rifampicin, gentamicin, nitrofurazone, silver oxide and silver alloy (Donlan, 2001; Getliffe, 2007). Although significant results have been achieved using these antimicrobial, however it is usually for short-term catheterization (Getliffe, 2007).

The application of medicinal plants in the treatment of human ailments has been an ancient art. *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum* have all been cited to provide medicinal properties against microbes that cause human ailments (Begum *et al*., 2002; Deo and Shastri, 2003; Adebolu and Oladimeji, 2005; Abubakar, 2009; Nwinyi *et al*., 2009; Joseph and Priya, 2011).

These plants are abundant in the tropics and have many phytochemical compositions such as essential oils, vitamins, antioxidants (flavonoids and tannins) and saponins, which contribute immensely to their antimicrobial properties (Begum *et al*., 2002; Deo and Shastri, 2003; Lima *et al*., 2006; Abubakar, 2009; Nwinyi *et al*., 2009). They have been applied in the treatment of upper respiratory and urinary tract infections (Adebolu and Oladimeji, 2005; Akinpelu and Onakoya, 2006; Nwinyi *et al*., 2008; Abubakar, 2009; Nwinyi *et al*., 2009; Rattanachaikunsopon and Phumkhachorn, 2010). In recent times, owing to the development of antibiotic resistance strains among the urinary tract disease causing microbial strains (WHO., 2002), we focused our efforts in exploring the use of medicinal plant to inhibit biofilm formation on catheter. In this study, we induced the formation of *E. coli* biofilm on the internal lumen of a sterile catheter and investigated the physiological behaviour of the *E. coli* biofilm on *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum* coated catheters. To the best of our knowledge, we report for the first time, the coating of catheters with *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum* extracts and its' delay of biofilm formation for a week.

MATERIALS AND METHODS

Media and reagents: Methanol and ethanol of analytical grade were used in this study. The nutrient agar, nutrient broth were obtained from Micro master, India. Urease base agar, starch agar, methyl red and voges proskauer medium, peptone water were obtained from the Microbiology laboratory of Covenant University. Silicone catheters were procured from Idumota, Eko, Lagos-State.

Collection of plant materials: Fresh samples of *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum* leaves were collected from Covenant University farm between the hours of 6-8 am at a prevailing temperature of about 27±2°C. All the plant collections were done in the month of February, 2013. The plants were identified and authenticated by a botanist Dr. Conrad A. Omonhinmin of the Department of Biological Sciences, Covenant University. The leaves were air dried. The dried leaves were powdered and stored in clean air tight container for further analysis.

Test organisms: Clinical isolate of *E. coli* was obtained from Lagos University Teaching Hospital (LUTH) Nigeria. The isolates were propagated on nutrient agar plates and maintained on the plate at 4°C. The isolates were sub-cultured in nutrient agar at 37°C for 24 h prior to further studies.

Preparation of extracts: Three hundred and fifty grams (350.0 g) of the dried powdered leaves of *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum* were, respectively transferred into three separate glass tanks for cold extraction using methanol as solvent. After 10 days, the extracts were strained and filtered then the filtrates were dried in vacuo at 45°C using rotary evaporator. The extracts were further dried over calcium chloride in a desiccator. The dried extracts were kept in refrigerator for further tests.

Preparation of plant extracts for catheter coating: The 700.00, 1400.00 and 2800.00 mg of the different extracts of *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum* were weighed separately into nine different sterile beakers and then dissolved each in 140 mL of ethanol to form a solution.

Coating of catheter with the plant extracts: The coating of the catheters were carried out by the selection of twenty-one catheters for each plant extract (*Mangifera indica*, *Ocimum gratissiumum* and *Psidium gujava*), respectively. These were grouped into three, with each containing seven in each group and three controls. These were arranged and labeled separately. The catheters were gently immersed in warm water bath. This caused tenderness of the catheter and allowed for easy coating of the inner surface layer of the catheters with the plant extracts. Twenty milliliters of the different plant extracts made from 700.00 mg (w/v) were injected into the first group of seven catheters, respectively. The catheters were placed such that the both end of the tubes

were out of the warm water. This allowed for quick coating of the plant extract and evaporation of the solvent (ethanol) that was used to prepare the extract. This procedure was carried out for each of the three plant extracts prepared from 1400.00 and 2800.00 mg (w/v). This procedure was repeated for each of the three plants at the remaining doses of 1400.00 and 2800.00 mg (w/v). A total of 63 catheters were used for the model.

Induction of biofilm on catheters: About 5 mL of urine sample was injected into each of the sixty-three catheters to induce biofilm formation on the internal surface of the catheter. Seven free catheters served as controls. *Escherichia coli* cultures were incubated on a freshly prepared sterile nutrient broth (1400 mL) and injected into all the 70 catheters. The catheters were stoppered with the catheter caps and incubated at 25°C for 120 h. Twenty milliters of urine obtained from several individuals (10) not on any form of drug, particularly antibiotics was injected daily into all the catheters at varying days, from 5 to 17 days. Catheters at the different concentrations of extracts were re-grouped into the following days 5, 7, 9, 11, 13, 15 and 17 for biofilm formation assay.

Estimation of biofilm prevention by the extracts: The levels of biofilm prevention assay were performed according to Ali (2012) though with some modifications. The biofilms were analyzed using the direct aerobic plate count and gradient fluxes of the cells optical density at 600 nm. In this, the catheters were surface sterilized and about 3 cm of the upper, lower and middle part of the catheters were transferred into different test tubes containing normal saline. The catheters, covered with the normal saline were left for 5 min and mixed vigorously to detach adhering organisms. Serial dilutions 10^1 to 10^5 were carried out and dilution 10^5 of each catheter were plated separately on plate count agar. The catheters that served as control followed the same procedure and were also plated out. All the plates were incubated at 37°C for 24 h. The colonies were counted using a colony counter. Further characterization based on cultural morphologies, physiological and metabolic activities were also carried out according to Fawole and Oso (2004).

Optical density count: The optical density count OD gradient fluxes were estimated by using the spectrophotometer (UNISPEC 23D) at an optical density of 0.4 at 600 nm.

Statistical analysis: Data obtained were statistically analyzed using Analysis of Variance (ANOVA) Microsoft EXCEL, 2010.

RESULTS

The physiological and metabolic activities of the organisms namely URC1-5, obtained from the catheters are as shown in Table 1. The isolates URCI 1, 3, 4 and 5 were motile, with only URCI 3 being Gram positive rod, while URCI 1, 4 and 5 were Gram negative rods. The bacterial species URCI, 1, 3, 4 and 5 were capable of fermenting glucose and catalase positive. The morphology and biochemical characterization and comparison with standard reference organism suggested the obtained organisms to be similar to the members of the genus *E. coli*, yeast, *Bacillus*, *Proteus* and *Enterobacter* species.

Figure 1 and 2 illustrates the inhibitory effects of the plant extracts at 120 and 168 h, respectively. On comparison with the controls, *Ocimum gratissimum* exhibited the highest inhibitory effect, followed by *Mangifera indica* and then *Psidium guajava*. The initial *E. coli* bacterial count for the control was 5.0×10^7 (cfu mL^{-1}). At day 5 (Fig. 1), *Ocimum gratissimum* had the *E. coli* bacterial count of 2.2×10^5 at 5.0 mg mL^{-1}, 1.2×10^5 at 10.0 mg mL^{-1} and 7.0×10^4 at 20.0 mg mL^{-1}. At day 7, *Ocimum gratissimum E. coli* bacterial count ranged from 5.7×10^5, 4.5×10^5 and 3.7×10^5 for 5.0, 10.0 and 20.0 mg mL^{-1}. The *Psidium guajava* extract showed the lowest inhibitory effects. The *E. coli* bacterial count ranged from 4.3×10^5, 3.3×10^5 and 1.9×10^3 at day 5 and the *E. coli* bacterial count ranged 7.7×10^5, 5.4×10^5 and 3.8×10^5 at day 7.

The test organism *E. coli* inhibition was not more than a week. At day 9, the population of *E. coli* had an exponential increase when compared with the control (Fig. 3). From the assessment carried out on the catheter at day 5, presence of yeast cells were also visible.

Aside from yeast cells, *Bacillus*, *Enterobacter* and *Proteus* were observed at day 9 (Fig. 3).

Escherichia coli population decreased significantly at day 17 (Fig. 4). The yeast cells had reduced population while *Proteus* species had varying growth profile on each catheter. The *Bacillus* species had a significant increase in population as shown in Fig. 4. The aerobic plate count analysis carried out between days 5-17 showed the presence of different microorganisms (Fig. 1-4). At low concentrations of the extracts (5- 10 mg mL^{-1}), there was no significant difference between *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum*. At 20 mg mL^{-1} concentrations, there

Table 1: Biochemical test of organisms isolated from coated catheters

	URCI				
Parameters	1	2	3	4	5
Shapes	Short rods	Oval yeast	Rods in chain	Rods	Rods
Gram reaction	-	NA	+	-	-
Motility	+	NA	+	+	+
Indole	+	NA	-	+	-
Citrate	-	NA	-	+	-
Methyl red	+	NA	ND	+	+
Voges-proskauer	-	NA	ND	-	-
Catalase	+	NA	+	+	-
Glucose	+	NA	+	+	+
Lactose	+	NA	-	-	+
Probable organisms	*E. coli*	Yeast	*Bacillus*	*Proteus*	*Enterobacter*

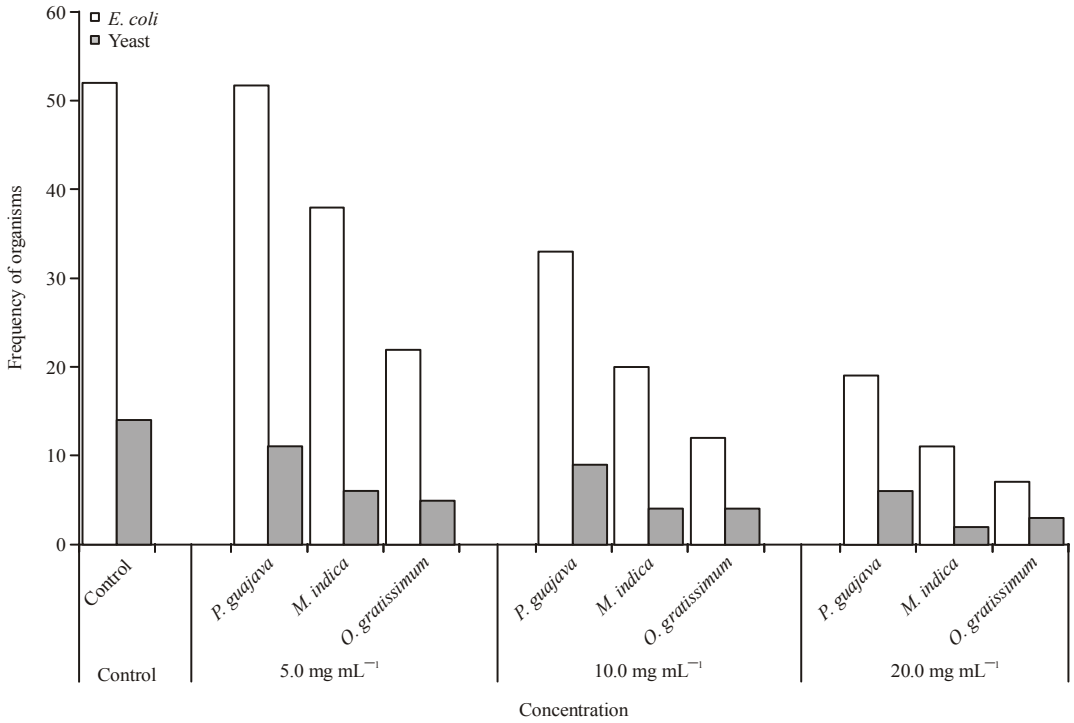

Fig. 1: Day 5 aerobic plate count analysis of organisms isolated from coated catheter surfaces

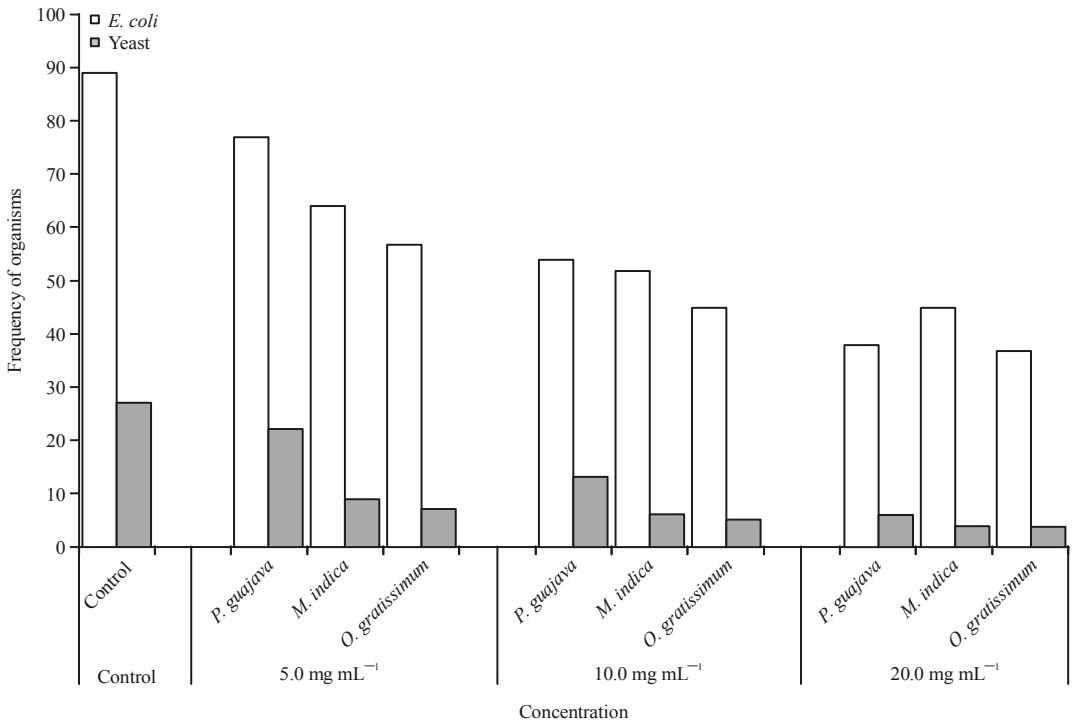

Fig. 2: Day 7 aerobic plate count analysis of organisms isolated from coated catheter surfaces

was significant difference in inhibition by *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum* extracts (Fig. 2 and 3).

The Optical Density (OD) carried at 600 nm (Fig. 5) depicted a steady increase in absorbance at day 5-17 (120-408 h). There was however, a decrease in absorbance

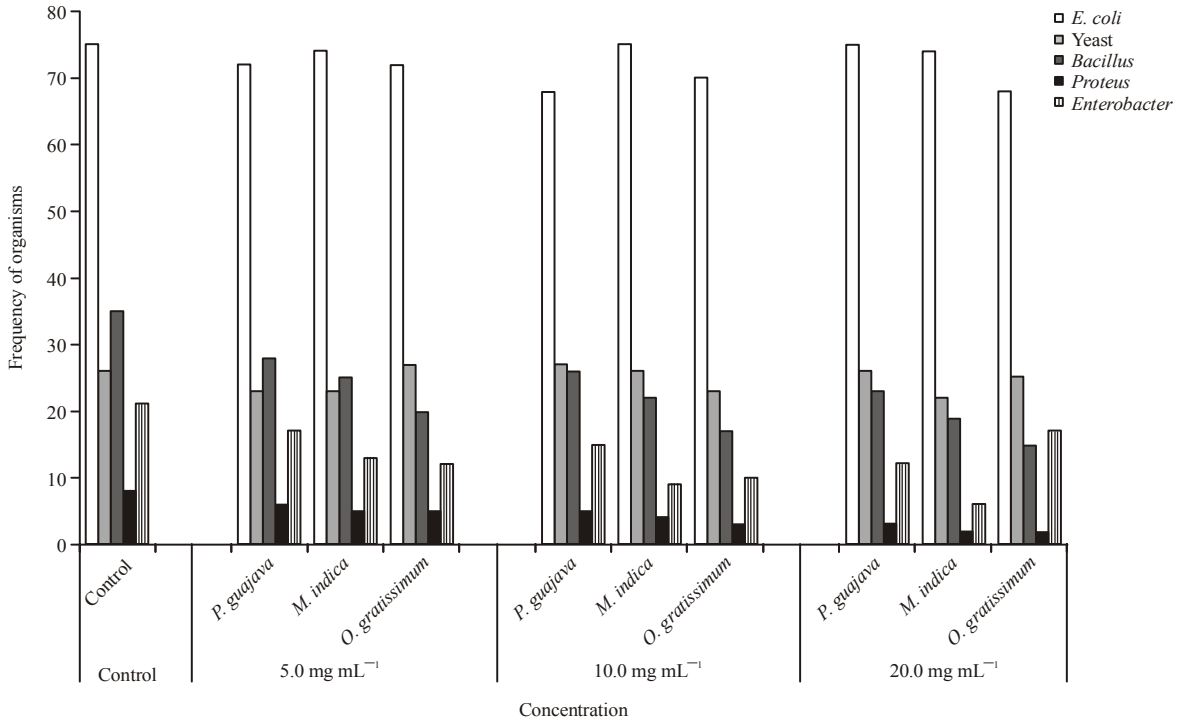

Fig. 3: Day 9 aerobic plate count analysis of organisms isolated from coated catheter surfaces

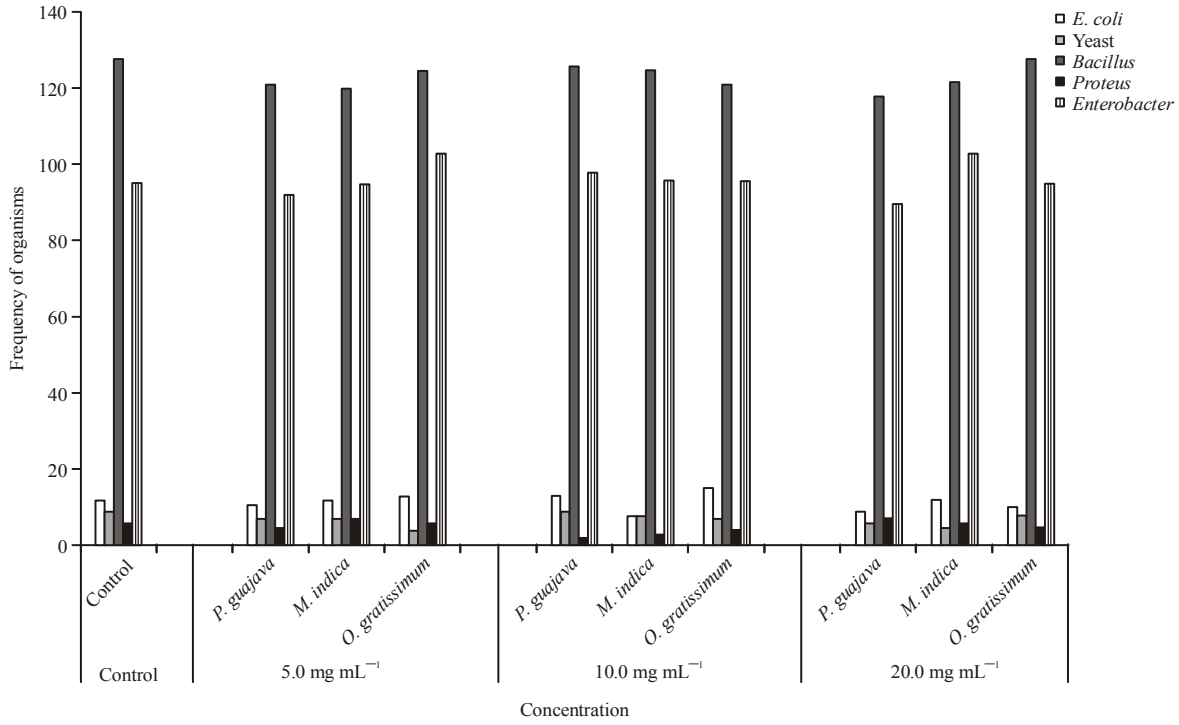

Fig. 4: Day 17 aerobic plate count analysis of organisms isolated from coated catheter surfaces

with increased concentration (turbidity). The inhibitory effect of the extracts varied among the different extracts but the difference was statistically not significant ($p<0.05$).

DISCUSSION

Biofilms are robust communities of microbes that are held by extracellular matrix. They can form on almost any surface,

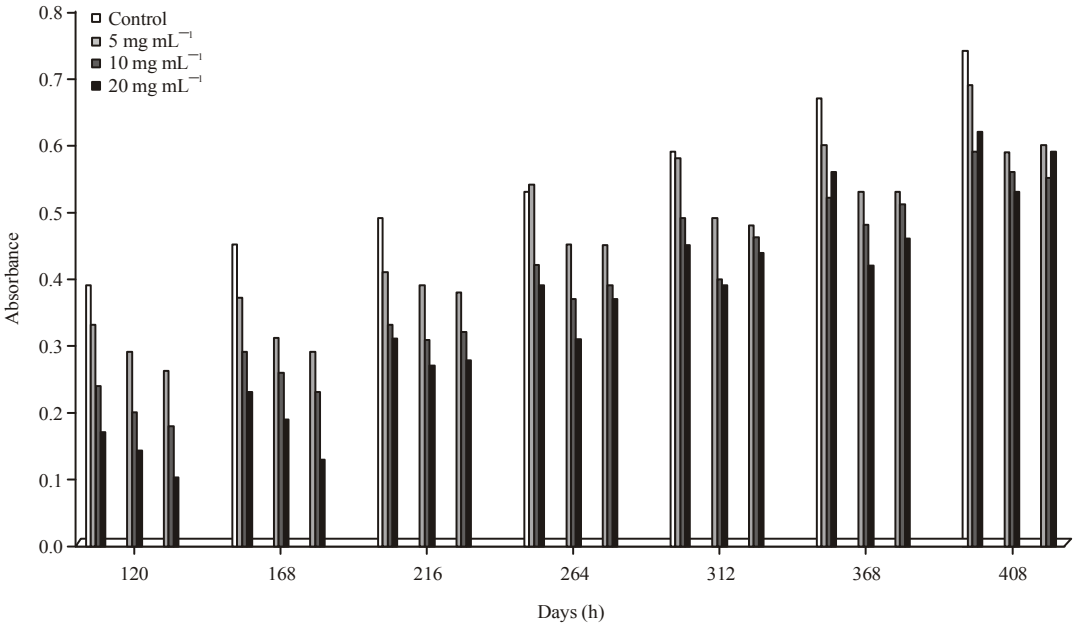

Fig. 5: Absorbance at 600 nm of the organisms isolated from coated catheter between day 5 and 17

where they can potentially cause disease or contaminate medical devices. Due to the numerous challenges of biofilms to health care, there is a continuous need to search for new antimicrobial compounds that can handle incidences and re-infection that could arise from biofilm contaminated medical devices.

In this study, *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum* leaf extracts exhibited bacteriostatic action that delayed the formation of biofilm on the catheters. It was also evident that the methanolic fractions of the leaf extracts exhibited inhibitory effects on the *E. coli* but the difference in the level of inhibition is not statistically significant among the extracts. It was observable that the reduced inhibitory activity of these plant extracts that lasted for a week was presumably due to the washing effect of urine that was introduced daily.

In the reports of Pandey and Shweta (2010), they observed low inhibitory effect of the leaf extracts of *P. guajava* when compared to other parts of the plant particularly its stem. Also, Adebolu and Oladimeji (2005) and Nwinyi *et al.* (2009) had reported of the, antimicrobial activity of *O. gratissimum*. Their reports corroborate our findings about the antimicrobial activity of *O. gratissimum*. It is also noteworthy that *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum* extracts had minimal inhibitory effect on the yeast cells when compared to their inhibitory effects on bacteria. This may be the reasons for the wide application of these plants in treating or controlling several bacterial pathogens (Adebolu and Oladimeji, 2005; Akinpelu and Onakoya, 2006; Nwinyi *et al*., 2008; Abubakar, 2009; Nwinyi *et al*., 2009; Pandey and Shweta, 2010).

The presence of other organisms apart from *E. coli* isolates that were introduced into the catheter is a reflection to other external factors that could influence biofilm formation. Some of the factors include, but not limited to the roles of endogenous flora of the genitourinary tract (Donlan, 2001; Getliffe, 2007). Among the other organisms present include: *Bacillus*, *Proteus* and *Enterobacter*. Their bacterial count ranged between 2.0×10^4 and 1.26×10^6 cfu mL^{-1}. These organisms have been introduced into the catheter due to their ubiquity and adaptation in the lumen of the catheter.

There was a continuous increase in the population of *Bacillus* species when plate count was carried out during analysis of day 9 to 17. It was also observed that *Bacillus* species inhibited the growth of other organisms on the agar plates. This might have occurred due to the presence of antibacterial producing *Bacillus* specie; most likely *Bacillus subtilis*.

We also, observed a sudden increase in absorbance during optical density count as shown in Fig. 3. In this, there were colour changes in the broth medium from day 9 to 17 which may have been caused by the growth of *Proteus*.

From this study, *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum* can only be used as coatings for short term catheterization however there are possibilities that maximum antimicrobial activity can be achieved by increasing the concentration of the extracts.

In conclusion, based on the obtained result *Psidium guajava*, *Mangifera indica* and *Ocimum gratissimum* extracts may provide alternative to short term coating for catheters.

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