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Antibacterial activities of ethyl acetate and methanol leaf extracts of *Psidium guajava* and *Carica papaya* on bacterial pathogens isolated from manual toothbrushes

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Toothbrush has become a potential source of infection, owing to contamination by various pathogens as a result of poor oral hygiene awareness and practices. This study investigated the antibacterial activities of ethyl acetate and methanol leaf-extracts of *Psidium guajava* (guava) and *Carica papaya* (paw-paw) on bacteria pathogens isolated from toothbrushes. A total of 100 manual used toothbrushes of different brands were collected from patients attending Federal School of Dental Therapy, Enugu Dental Clinic and analyzed for bacterial growth. *In vitro* antibacterial study of *Carica papaya* and *P. guajava* leaf extracts was conducted using Kirby-Bauer agar well diffusion technique. *Staphylococcus aureus* 76 (69.1%), *Escherichia coli* 23 (20.9%), and *Pseudomonas aeruginosa* 11(10%) were isolated from toothbrushes using standard microbiological techniques. Results showed that ethyl acetate extract of *P. guajava* had inhibition zone diameter (IZD) ranging from 9 - 29 mm against bacterial isolates, while its methanol extract had IZD of 8- 26 mm. Ethyl acetate extract of *C. papaya* had IZD of 5 - 21 mm, while its methanol extract had IZD of 5- 10 mm. The Minimum inhibitory concentration of the extracts was 50 mg/ml, while minimum bactericidal concentration was 100 mg/ml for all the isolates. The bacterial contamination frequency of manual toothbrushes recorded in this study was high and this calls for urgent public health attention.

Key words: *Psidium guajava*, *Carica papaya*, antibacterial activity, extracts, toothbrush.

INTRODUCTION

Toothbrushes are the most common oral hygiene aid used to promote oral health and prevent dental diseases (Glass, 1992). Toothbrushes have been characterized as a means of microbial retention, transport and growth

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(Wetzel et al., 2005). According to American Society of Microbiologists general meeting (ASM GM, 2015) report, toothbrushes have been shown to harbour bacteria; including faecal coliform bacteria that can be released into the air when the toilet is flushed or can be spread to the toothbrush when the owner touches a contaminated surface before handling his or her brush. Retention and survival of microorganisms on toothbrushes after brushing represents a possible cause of re-contamination of the mouth (Wetzel et al*.,* 2005).

Numerous studies have shown that prolonged use of one toothbrush facilitates its contamination by various microorganisms such as species of *Streptococcus*, *Staphylococcus*, *Lactobacilli*, *Klebsiella*, *Candida, Pseudomonas* spp, *Escherichia coli*, and Herpes simplex virus (Wetzel et al., 2005; Karibasappa et al., 2011; Sogi et al., 2002; Glass, 1992). These microorganisms have been implicated to cause dental caries, gingivitis, stomatitis, infective endocarditis (Karibasappa et al., 2011), arthritis, bacteremia and stroke (Warren et al., 2011; Sammons et al., 2004), affecting both oral and general health. Cockroaches, wall gecko, flies, and other household rodents are attracted to our toothbrushes by the toothpaste remnants which often times are not properly washed off after brushing (El-Sherbini, 2011). Microorganisms grow and flourish in warm and moist conditions as could be provided by the brush especially when kept in a container (Taji and Rogers, 1998). There seems to be lack of awareness among the public regarding what happens to toothbrushes when not in use and less attention is often paid to their care and maintenance. A vast majority of the people do not know the implication of covering their toothbrush after use and even the civilized population still considers the toilet a convenient place to store their toothbrushes oblivious of the health implication and so does not consider its decontamination using any antimicrobial agent. Although rinsing with tap water is the common disinfecting protocol, it results in continued high levels of contamination (Vignesh et al*.,* 2017). Highly contaminated toothbrushes may cause a possible constant “re-infection”, which is a risk factor for periodontal disease (Goldschmidt et al., 2004). While there are various ways of toothbrush sanitization, one study indicates that soaking a toothbrush in diluted hydrogen peroxide or Listerine mouthwash greatly reduces (85%) bacterial load, (Beneduce et al., 2010). Once a week, a toothbrush (or electric-toothbrush head) should be soaked in a solution of hydrogen peroxide (3% in 97% water). The use of plant extracts with known antimicrobial properties can be of great significance in microbial control.

Many plants have been used in microbial control because of their active antimicrobial properties which are usually synthesized as secondary metabolites (Saxena et al., 1993). *Psidium guajava Linn*. (Guava), a fruit plant of the family Myrtaceae is found globally. Numerous studies have shown significant antibacterial activity of guava against common food borne diarrhoea-causing bacteria including *Staphylococcus* spp*., Shigella* spp., *Salmonella* spp., *Bacillus* spp., *E. coli, Clostridium* spp, and food spoilage bacteria such as *Pseudomonas* spp (Alnieida et al., 1995). Many parts of the Guava plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothaches, cough, sore throats, inflamed gums, and a number of other conditions (Abdelrahim et al., 2002). Pawpaw (*Carica papaya*) of the family caricaceae is a small, bushy tree having a hollow trunk. *C. papaya* is cultivated for its edible ripe fruit; its juice is a popular beverage, and its young leaves, shoots, and fruits are cooked as a vegetable. Paw-paw has assorted medicinal properties for treatment of diabetes, as birth control, as an antiseptic, antimicrobial, or diuretic, to control parasites, reduce inflammation, lower blood pressure, and lower cholesterol. *C. papaya* is known to exert a proteolytic effect on bacteria resulting from the production of a coagulum that immobilizes microorganisms and protects the host against bacterial infections. In addition, *C. papaya* may improve the efficiency of phagocytic cells that destroy bacteria; also it contains the alkaloid, carpaine, which has antibacterial properties (Vij and Prashar, 2015). Assertions have been made that toothbrushes which play a pivotal role in the fight against tooth decay can itself lead to dental diseases as well as many other systemic diseases; including septicaemia, gastrointestinal, cardiovascular, respiratory, and renal diseases, if not properly stored and maintained (Basman et al., 2016). This study therefore investigates the antibacterial activities of ethyl acetate and methanol leaf extracts of *P. guajava* (guava) and *C. papaya* (paw-paw) on bacteria isolated from toothbrushes.

MATERIALS AND METHODS

Sample collection

A total of 100 used toothbrushes were randomly collected from members of the public visiting dental clinic in Trans-Ekulu area in Enugu-East local government council, Enugu State in exchange for new toothbrush (Oral-B, Nigeria) between February, 2017 and January, 2019. One new unused toothbrush was used as control sample. Each toothbrush collected was wrapped with tin foil to avoid cross contamination and transported in ice-pack to the laboratory for microbial analysis within 1 h. New unused toothbrush was used as control.

Collection of plant materials

C. papaya and *P. guajava* leaves identified by a taxonomist, Dr. (Mrs) N. C. Nnamani in Biological Sciences Department of Ebonyi State University, were collected from domestic gardens in Trans-Ekulu, Enugu East local government, Enugu state.

Methanolic and ethyl acetate extraction of *Psidium guajava* and *Carica papaya* leaves

P. guajava and *Carica papaya* leaves were collected, washed with clean water, air-dried under room temperature into crispy form, and

grounded into powdery form using a blender. The methanol and ethyl acetate extraction of *P. guajava* and *C. papaya* leaves were made by soaking separately, 20 g each of the powdered *P. guajava* and *C. papaya* leaves into 100 ml of both 70% methanol and 70% ethyl acetate in conical flasks and placed in a shaker for 24 h. The extracts were filtered through Whatman No 1filter paper to obtain the fresh extracts of the products and poured into round bottom sterile stainless plates and allow to gradually evaporate. After drying, 2 g dried yield of each extract was dissolved in 10 ml of 95% dimethyl sulphoxide (DMSO) using properly labeled sterile sample bottles, and stored in refrigerator for subsequent use (Kamrul et al., 2014).

Isolation, characterization, and identification of the bacterial isolates

Each of the used toothbrushes was decapitated using a sterile end cutting nipper on a disinfected surface and the brush head aseptically transferred into a sterile sample bottle containing 10 ml of sterilized nutrient broth (A new unused toothbrush was included in the experiment as a control). This was incubated at 37°C for 18-24 h and observed for turbidity. Turbid tubes were selected and a loopful of the content was streaked on the surface of sterile nutrient agar, mannitol salt agar, and MacConkey agar plates. Inoculated plates were then incubated at 37°C for 18-24 h. After 18-24 h incubation, all streaked plates were examined for microbial growth and the morphological characteristics of all colonies were recorded. Bacterial colonies observed were further subjected to the following tests for proper identification and characterization (Cheesbrough, 2010); Gram Stain, motility test, catalase test, citrate utilization test, oxidase test, coagulase test, indole test, Voges-Proskauer (VP) test, and sugar fermentation test (lactose, maltose, and *sucrose*).

Inoculation of test plates

Using a sterile wire loop, pure bacterial colony was picked and emulsified in 3 ml of sterile water and adjusted to 0.5 McFarland standard turbidity equivalents. Sterile swab was then inserted into the standardized test organism; excess fluid was removed by pressing and rotating the swab against the side of the tube above level of the suspension, and inoculated onto Mueller-Hinton agar plate. Suspension was streaked evenly over the surface of the medium in three directions, rotating the plate to ensure even distribution. With the Petri dish in place, the surface of the agar was allowed to dry for 5 min (Cheesbrough, 2006).

Antibiotics susceptibility testing (AST)

This was done using Kirby-Bauer disc diffusion technique. Using a sterile wire loop, pure bacterial colony was picked and emulsified in 3 ml of sterile water and adjusted to 0.5 McFarland standard turbidity equivalents. Sterile swab was then inserted into the standardized test organism; excess fluid was removed by pressing and rotating the swab against the side of the tube above level of the suspension, and inoculated onto Mueller-Hinton agar plate. Suspension was streaked evenly over the surface of the medium in three directions, rotating the plate to ensure even distribution. With the Petri dish in place, the surface of the agar was allowed to dry for 5 min (CLSI, 2015). The following standard antibiotic discs were used against the isolates: Amikacin (AK, 30 µg), amoxycillin-Clavulanic acid (AMC, 20 µg), cefepime (FEP, 30 µg), cefotaxime (CTX, 30 µg), clindamycin (DA, 2 µg), erythromycin (E, 15 µg), gentamicin (CN, 10 µg), ofloxacin (OFX 5 µg), oxacillin (OX, 1 µg), piperacillin (PRL, 100 µg), tetracycline (TE, 30 µg), trimethoprim/sulfamethoxazole (SXT, 125 µg), amoxicillin (AML, 30 µg) cefotetan (CTT, 30 µg /OB) (Oxoid Ltd, UK). Sterilized forceps were used to place the antibiotic discs evenly on the inoculated Mueller-Hinton agar so that the disc should be about 15mm from the edge of the plate and not closer than 25 mm from disc to disc. After 30 min, the plates were inverted and incubated for 24 h. A ruler was used to measure the diameter of each zone of inhibition in mm on the underside of the plate. The inhibitory zone diameter was interpreted as susceptible or resistant according to the criteria of Clinical Laboratory Standards Institute (CLSI, 2015).

Antibacterial activity of ethyl aceteate and methanolic leaf extracts of *C. papaya* (pawpaw) and *P. guajava* leaves against bacteria isolates from used toothbrush

Antibacterial activity study was done using the Kirby-Bauer agar well-diffusion technique (CLSI, 2015). Plant extract antibacterial activity was tested on Mueller-Hinton agar plates by streaking standardized (0.5 McFarland standard) test organism on already prepared Mueller-Hinton agar plate using a sterile swab stick. Wells were bored into the Mueller Hinton agar medium with a flame-sterilized 6 mm diameter cork borer. An aliquot of each test extract was then dispensed into each well after the inoculation of the plates with bacterial isolate. Each plate contained three wells for each of the two plants (*C. papaya* and *P. guajava*) investigated (i) Methanolic extract each of *C. papaya* and *P. guajava* (ii) Ethyl acetate extracts each of *C. papaya* and *P. guajava* (iii) 0.2% of chlorhexidine gluconate (Colgate) mouthwash as a positive control. After the extract's introduction, each plate was allowed to stand undisturbed for 18 h, after which the remaining extracts were tipped off the wells. Zones of inhibition were read and measured in mm with a transparent meter rule.

Determination of the minimum inhibitory concentration (MIC) of ethyl acetate and methanolic leaf extract of *C. papaya* and *P. guajava*

The MIC of methanolic leaf extract of *C. papaya* and *P. guajava* was determined using Mueller-Hinton agar plates. Mueller-Hinton agar was prepared according to manufacturer's instruction and the 0.5 McFarland equivalent of the standardized test organism was streaked on the surface of the Mueller-Hinton agar plates. Holes were bored in the plates using a flamed 6 mm diameter cock borer. Different concentrations of the plant extracts were prepared by serially diluting 4 ml of the plant extracts with 4 ml of sterile water in sterile test tubes; to give the following concentrations of the extracts; 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml. These diluted extract concentrations were then introduced into the bored holes using calibrated rubber pipettes (one for each concentration). The plates were left undisturbed for 24 h, after which the remaining extracts were tipped off the plates. Clear zones of inhibition were measured to the nearest millimeter using a transparent meter rule under a bright light. The minimum inhibitory concentration was taken as the lowest concentration that inhibited bacterial growth.

Determination of the minimum bactericidal concentration (MBC) of ethyl acetate and methanolic leaf extract of *C. papaya* and *P. guajava*

The determination of MBC follows the determination of MIC by plating technique. The MBC is the lowest concentration of the antibacterial agent that kills at least 99.9% of the test organism (Jasmin et al., 2017). To determine the MBC, the plates of the MIC with clear zones of inhibitions were selected and samples were taken from it and inoculated on a fresh agar media and incubated at 37°C for 18-24 h. After incubation, extract concentration without

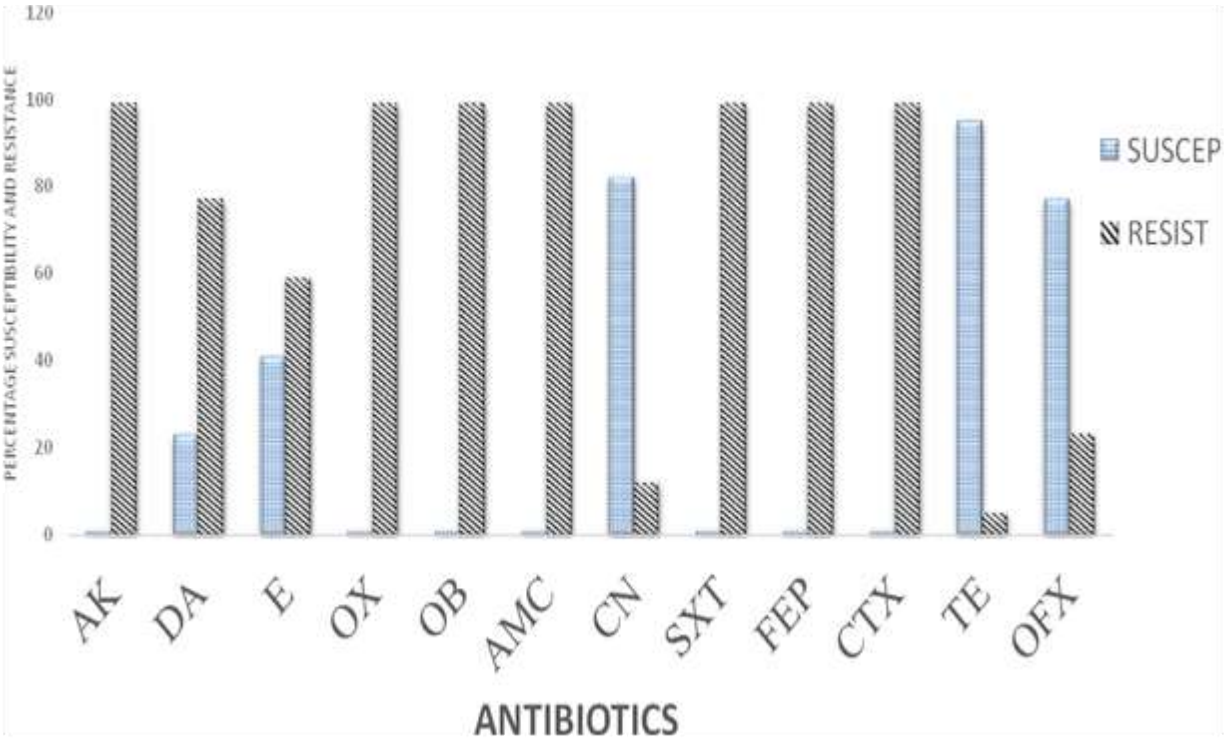

Figure 1. Graphic representation of the antibiotic susceptibility profile of *Staphylococcus aureus* isolated from used manual toothbrush.
Key: Amikacin (AK), Clindamycin (DA), Erythromycin (E), Oxacillin (OX), Tobramycin (OB), Amoxycillin-Clavulanic acid (AMC), Gentamicin (CN), Sulfamethoxazole/trimethoprim (SXT), Cefepime (FEP), Cefotaxime (CTX), Tetracycline (TE), Ofloxacin (OFX).

microbial growth was taken as the MBC (Jasmin et al., 2017).

Statistical analysis

Statistical analysis was performed using SPSS 17.0 version statistical software package. Comparison between categorical variables was calculated using Independent samples T-test. Results were considered statistically significant if the p value is less than 0.05 ($p < 0.05$).

RESULTS

Out of the 100 used toothbrushes analyzed 28 bacterial isolates: *Staphylococcus aureus* (78.6%), *Escherichia coli* (17.9%), and *Pseudomonas aeruginosa* (3.6%) were isolated. No microbial growth was recorded in the new unused manual toothbrush used as control sample. Antibiotic susceptibility test results indicated that the *Staphylococcus aureus* isolates were completely resistant (100%) to oxacillin, amikacin, tobramycin, amoxycillin-clavulanic acid, sulfamethoxazole/ trimethoprim, cefepime, and cefotaxime (Figure 1). Isolates were highly susceptible to tetracycline (97%), gentamicin (86.3%), and ofloxacin (77%) (Figure 1). Antibiotic sensitivity test showed that *E. coli* isolates were susceptible (33.3%) to tobramycin while high resistance frequency of 90% were each exhibited to tetracycline, ofloxacin, ticarcillin, amikacin, cefotaxime, and gentamicin (Figure 2).

All the *P. aeruginosa* isolates exhibited complete resistance (100%) to tobramycin, amikacin, ofloxacin, tetracycline, and gentamicin but highly susceptible (100%) to ticarcillin and cefotaxime (Figure 3).

Table 1 shows the antibacterial activity of the plant substances on *S. aureus* isolated from used manual toothbrushes. Ethyl acetate guava extract recorded the highest inhibitory effect with inhibition zone diameter (IZD) range of 4-28 mm. This was closely followed by ethyl acetate *C. papaya* extract with IZD range of 4- 26 mm. Methanolic *P. gaujava* extract exhibited IZD range of 5– 16 mm while the methanolic *C. papaya* extract had IZD range of 2– 14 mm (Table 1). The highest IZD exhibited by the mouthwash solution tested in this study was 11 mm while its lowest IZD was 5 mm (Table 1). Results showed that the methanol extract of paw-paw (*C. papaya*) which exhibited the least IZD was more effective than the conventional mouthwash solution normally used for mouth rinse and toothbrush decontamination.

There was no statistically significant difference in the inhibition zone diameter (IZD) of ethyl acetate extract and

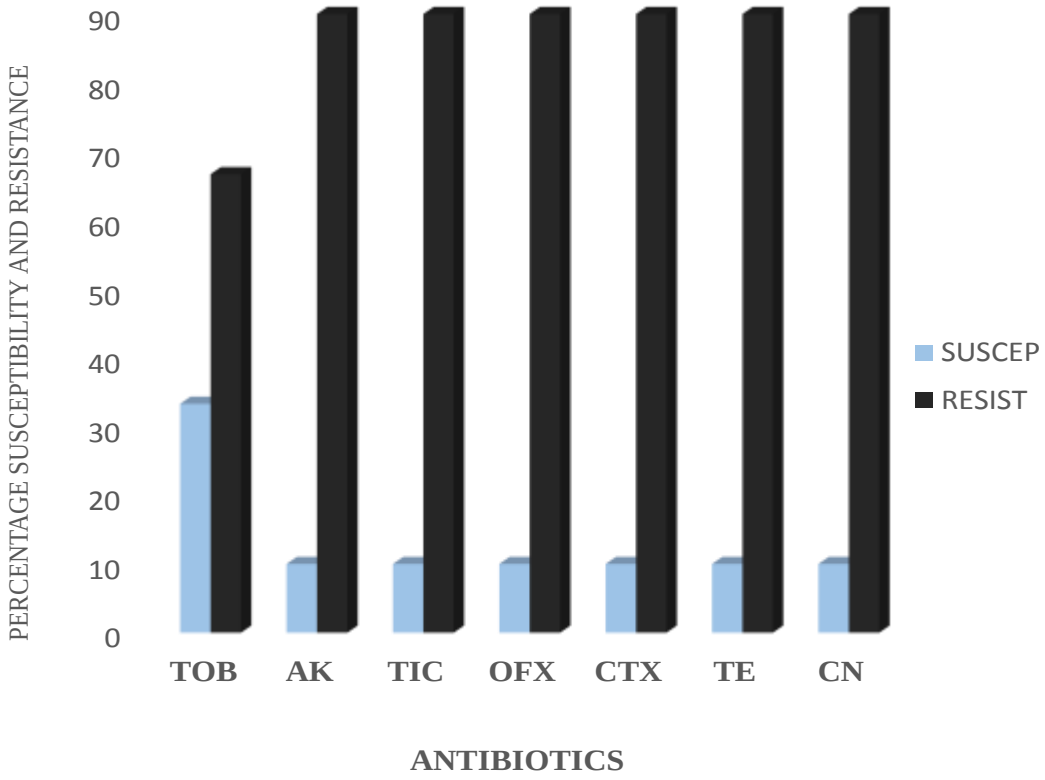

Figure 2. Antibiotic sensitivity pattern of *Escherichia coli* isolated from used manual toothbrush wash. Key: Amikacin (AK), Cefotaxime (CTX), Gentamicin (CN), Tobramycin (TOB), Tetracycline (TE), Ticarcillin (TIC), and Ofloxacin (OFX).

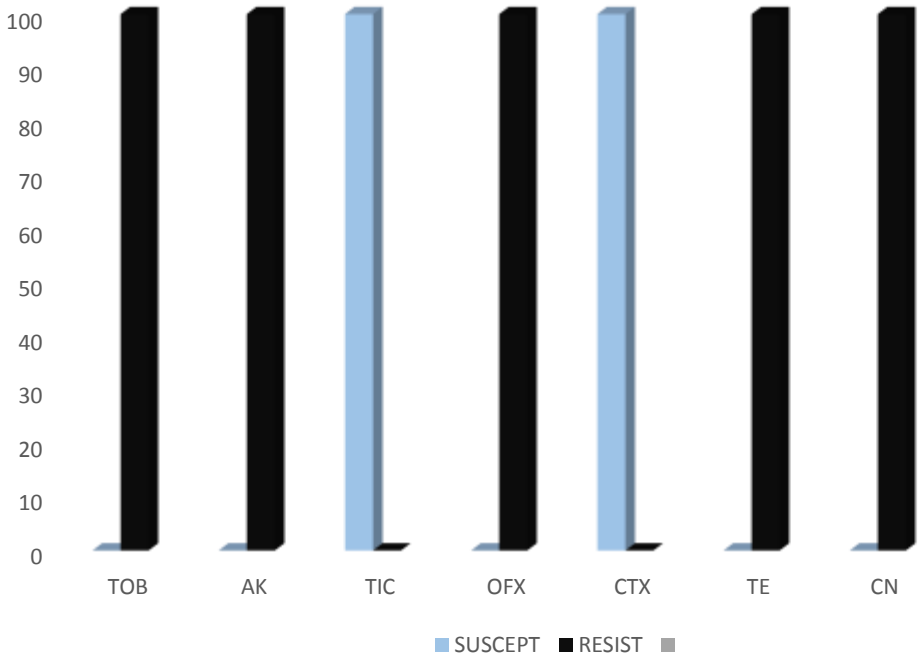

Figure 3. Graphic representation of antibiotic sensitivity pattern of *P. aeruginosa* isolated from used manual toothbrushes.
Key: Amikacin (AK), Cefotaxime (CTX), Gentamicin (CN), Tobramycin (TOB), Tetracycline (TE), Ticarcillin (TIC) and Ofloxacin (OFX).

Table 1. Antibacterial activity of ethyl acetate and methanol leaf extracts of *P. guajava* and *C. papaya* on *S. aureus* isolated from used manual toothbrush.

Inhibition Zone Diameter (mm)				
MW (mm)	**EG (mm)**	**MG (mm)**	**EP (mm)**	**MP (mm)**
8	14	9	9	9
9	11	4	6	12
7	11	6	7	7
7	10	14	7	6
9	14	18	11	6
6	9	9	11	14
6	11	8	5	6
9	14	8	9	7
10	11	10	6	6
6	9	12	6	9
10	11	12	6	7
6	6	9	12	7
14	28	12	9	9
10	8	12	11	6
5	9	15	9	6
10	11	12	7	8
11	12	12	9	11
7	16	18	12	NIL
11	6	26	7	9
NIL	14	7	6	2
11	19	17	16	2
NIL	4	6	13	NIL
Mean	11.7 (S.D. = 4.99)	11.6 (S.D. = 5.00)	8.8 (S.D. = 2.86)	6.8 (S.D. = 5.01)

Mouth wash-MW, Ethyl acetate guava extract-EG, Methanol Guava extract-MG, Ethyl acetate paw-paw extract-EP, Methanol paw-paw extract-MP, mm = millimeter.

methanol extract of *P. gaujava* ($p < 0.05$); while there was a statistically significant difference in the inhibition zone diameter (IZD) of ethyl acetate extract and methanol extract of *C. papaya* ($p < 0.05$). Results showed that the methanol extract of *C. papaya* was significantly more effective against bacterial isolates than ethyl acetate extracts.

There was a statistically significant difference in the inhibition zone diameter (IZD) of *P. gaujava* ethyl acetate extract and *C. papaya* ethyl acetate extract ($p < 0.05$). The ethyl acetate extract of *P. gaujava* was significantly more effective against bacterial isolates than the ethyl acetate extract of *C. papaya*. There was also a statistically significant difference in the inhibition zone diameter (IZD) of *P. gaujava* methanol extract and *C. papaya* ethyl acetate extract. The methanol extract of *P. gaujava* was significantly more effective against bacterial isolates than the methanol extract of *C. papaya* ($p < 0.05$).

Results showed that all the plant extracts had inhibitory effect against the *E. coli* isolated from used manual toothbrush (Table 2). The highest mean inhibition zone diameter (IZD) was recorded for ethyl acetate extract of *P. gaujava* (EG) with mean IZD of 12.2 mm, thus making it the most outstanding extract investigated. This was followed by methanol extract of *P. gaujava* (MG) with a mean IZD of 11.8 mm. There was no statistically significant difference in the mean inhibition zone diameter (IZD) of ethyl acetate extract and methanol extract of *P. gaujava* ($p < 0.05$).The mean IZD of Ethyl acetate extract of *C. papaya* was 10.6 mm while that of methanol extract was 6.6 mm, being the least effective (Table 2). There was a statistically significant difference in the mean inhibition zone diameter (IZD) of ethyl acetate extract and methanol extract of *C. papaya* ($p < 0.05$). The mouthwash solution recorded a mean IZD of 7.4 mm against *E. coli* isolates. Results showed that the mouthwash solution (Oral B) and the least effective plant (*C. papaya*) extract recorded the same inhibitory effect range.

The methanol extract of *C. papaya* (MP) exhibited no antibacterial activity against multidrug-resistant *P. aeruginosa* isolated from used toothbrush while the ethyl acetate extract of guava extract (EG) had IZD of 16 mm.

Table 2. Antibacterial activity of ethyl acetate and methanol leaf extracts of *P. guajava* and *C.papaya* on *E. coli* isolated from used manual toothbrush.

Inhibition Zone Diameter (mm)				
MW (mm)	**EG (mm)**	**MG (mm)**	**EP (mm)**	**MP(mm)**
5	16	14	9	11
9	12	10	10	4
11	10	11	11	4
7	8	14	13	9
5	15	10	10	5
Mean	12.2 (S.D. = 4.99)	11.8 (S.D. = 5.01)	10.6 (S.D. = 3.52)	6.6 (S.D. = 3.52)

Key: MW = Mouth wash, EG = Ethyl acetate guava extract, MG = Methanol guava extract, EP = Ethyl acetate paw-paw extract, MP = Methanol paw-paw extract, mm = millimeter.

Table 3. Antibacterial activity of ethyl acetate and methanol leaf extracts of *P. guajava* and *C. papaya* on *P. aeruginosa* isolated from used manual toothbrush.

Inhibition zone diameter (mm)				
MW (mm)	**EG (mm)**	**MG (mm)**	**EP (mm)**	**MP (mm) IZD**
7	16	10	12	NIL

Key: MW = Mouth wash, EG = Ethyl acetate guava extract, MG = Methanol guava extract, EP = Ethyl acetate paw-paw extract, MP = Methanol paw-paw extract, mm = millimeter.

Ethyl acetate extract of *C. papaya* (EP) had IZD of 12 mm, while methanol extract of guava extract and the control (mouth wash) exhibited IZD values of 10 mm and 7 mm respectively (Table 3). The MIC is the lowest concentration of the agent that completely inhibits visible growth as judged by the naked eye, disregarding a single colony or a thin haze within the area of the inoculated spot (Wiegand et al., 2008). All the plant extracts exhibited their minimum inhibitory effects at 50% concentration of the agents but at varying degrees of inhibition zone diameter (Tables 4 to 6). The colony forming unit as observed after sub-plating of the clear zones of inhibition recorded by MICs of plant extracts is shown in Tables 7 and 8. The minimum bactericidal concentration (MBC) exhibited by extracts tested was recorded only at 100% concentration for all the plant extracts used (Tables 7 and 8).

DISCUSSION

Our toothbrushes can be a friend, a good tool for oral healthcare or a foe when neglected, serving as a vehicle for potential pathogenic agents spread with its attendant health implications. Contrary to the prevailing perception, pathogenic organisms are well spread through instruments of oral hygiene care, even as the notorious pathogens implicated in toilet infections could as well be spread through contaminated toothbrushes as shown by the literatures reviewed. This study investigated the antibacterial activities of ethyl acetate and methanol leaf-extracts of *P. gaujava* (guava) and *C. papaya* (paw-paw) on bacteria isolated from toothbrushes. A total of 28 bacterial isolates including *S. aureus* (78.6%), *E. coli* (17.9%), and *P. aeruginosa* (3.6%) were isolated from 100 used toothbrushes. The isolated bacterial pathogens from the used toothbrushes were multidrug-resistant as they exhibited resistance to at least two different classes of antibiotics. Methanol extract of paw-paw (*C. papaya*) and guava (*P. gaujava*) exhibited higher antibacterial activities than the conventional mouthwash solution which is normally used as mouth rinse and toothbrush decontamination. Extracts of guava (*P. gaujava*) were significantly more effective against bacterial isolates than *Carica papaya* extracts. Interestingly, the methanol extracts from paw-paw (*C.papaya*) and guava (*P. gaujava*) were generally better in antibacterial activities than ethyl acetate extracts. Obika and Onuorah (2015) posited that manual used toothbrushes were contaminated with microorganisms. This is in line with this study which recorded high frequency (10 – 69.1%) of bacteria in manual toothbrush. This report however corroborated the positions of Caudry et al. (1995) and Glass and Jensen (1994) who asserted that toothbrushes were not sold sterile but were contaminated prior to its use. This could possibly be as a result of contamination from packaging and distribution of these manual tootbrushes. In line with the same observation, this study

Table 4. Minimum inhibitory concentration of ethyl acetate and methanol leaf extracts of P. guajava and C.papaya on S. aureus isolated from used manual toothbrush.

S/N	EG (mg/ml)	MG (mg/ml)	EP (mg/ml)	MP (mg/ml)
1	50	50	50	50
2	50	50	50	50
3	50	50	50	50
4	50	50	50	50
5	50	50	50	50
6	50	50	50	50
7	50	50	50	50
8	50	50	50	50
9	50	50	50	50
10	50	50	50	50
11	50	50	50	50
12	50	50	50	50
13	50	50	50	50
14	50	50	50	50
15	50	50	50	50
16	50	50	50	50
17	50	50	50	50
18	50	50	50	50
19	50	50	50	50
20	50	50	50	50
21	50	50	50	50
22	50	50	50	50

Key: MW = Mouth wash, EG = Ethyl acetate guava extract, MG = Methanol guava extract, EP = Ethyl acetate paw-paw extract, MP = Methanol paw-paw extract, milligram per milliliter = mg/ml.

Table 5. Minimum inhibitory concentration of ethyl acetate and methanol leaf extracts of P. guajava and C. papaya on P. aeruginosa isolated from used manual toothbrushes.

S/N	EG (mg/ml)	MG(mg/ml)	EP(mg/ml)	MP (mg/ml)
1	50	50	50	50

Key: MW = Mouth wash, EG = Ethyl acetate guava extract, MG = Methanol guava extract, EP = Ethyl acetate paw-paw extract, MP = Methanol paw-paw extract, milligram per milliliter = mg/ml

Table 6. Minimum inhibitory concentration of ethyl acetate and methanol leaf extracts of P. guajava and C. papaya on E. coli isolated from used manual toothbrush.

S/N	EG (mg/ml)	MG(mg/ml)	EP(mg/ml)	MP(mg/ml)
1	50	50	50	50
2	50	50	50	50
3	50	50	50	50
4	50	50	50	50
5	50	50	50	50

Keys: MW = Mouth wash, EG = Ethyl acetate guava extract, MG = Methanol guava extract, EP = Ethyl acetate paw-paw extract, MP = Methanol paw-paw extract, milligram per milliliter = mg/ml.

Table 7. Minimum bactericidal concentration of ethyl acetate and methanol leaf extracts of *P. guajava* and *C. papaya* on *S. aureus* isolated from used manual toothbrushes.

Isolate S/N	EG (mg/ml)	MG(mg/ml)	EP(mg/ml)	MP(mg/ml)
1	100	100	100	100
2	100	100	100	100
3	100	100	100	100
4	100	100	100	100
5	100	100	100	100
6	100	100	100	100
7	100	100	100	100
8	100	100	100	100
9	100	100	100	100
10	100	100	100	100
11	100	100	100	100
12	100	100	100	100
13	100	100	100	100
14	100	100	100	100
15	100	100	100	100
16	100	100	100	100
17	100	100	100	100
18	100	100	100	100
19	100	100	100	100
20	100	100	100	100
21	100	100	100	100
22	100	100	100	100
23	100	100	100	100
24	100	100	100	100
25	100	100	100	100
26	100	100	100	100

Key: MW = Mouth wash, EG = Ethyl acetate guava extract, MG = Methanol guava extract, EP = Ethyl acetate paw-paw extract, MP = Methanol paw-paw extract, milligram per milliliter = mg/ml.

Table 8. The minimum bactericidal concentration of plant extracts against *E. coli* isolated from used manual toothbrushes.

Isolate S/N	EG (mg/ml)	MG (mg/ml)	EP (mg/ml)	MP (mg/ml)
1	100	100	100	100
2	100	100	100	100

Key: MW = Mouth wash, EG = Ethyl acetate guava extract, MG = Methanol guava extract, EP = Ethyl acetate paw-paw extract, MP = Methanol paw-paw extract, milligram per milliliter = mg/ml.

confirmed that manual toothbrushes are contaminated by oral and non-oral flora as *S. aureus, E. coli*, and *P. aeruginosa* were all isolated from the manual toothbrush samples used in this study. Also, ADA (2009) agrees that microorganisms can grow on toothbrushes after use as toothbrushes become contaminated with bacteria, blood, saliva, oral debris, and toothpaste during brushing. Even after being rinsed visibly clean with tap water, toothbrushes can remain contaminated with potentially harmful bacteria. Contrary to the submission of Osungunna and Oyajoju (2016) that *Bacillus* species were predominantly isolated, *S. aureus* was the most predominantly isolated bacteria in this study, hence supporting the observation made by Obika and Onuorah (2015).

In a study conducted by Osungunna and Oyajoju (2016), the bacteria isolated from manual toothbrushes recorded multidrug resistance against conventional antibiotics tested. This is in accord with this study, but however disagrees with the submission that gentamicin

was not effective against isolated bacteria. All the bacterial isolates were multidrug-resistant as they exhibited resistance to at least two different classes of antibiotics. Antibacterial activities of the ethyl acetate and methanol extracts of *P.gaujava* and *C. papaya* had antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa* isolates from manual toothbrushes at varying degrees. Sogi et al. (2002) reported that chlorhexidine mouthwash was effective in the control of oral microbiota, and for the daily decontamination of toothbrushes. The extracts from guava plants were generally more effective against bacterial isolates than the extracts from *C. papaya*. This report is also in agreement with this study but interestingly, this study reported greater antibacterial efficacy for ethyl acetate and methanol leaf extracts of *P. guajava* (guava) and *C. papaya* (paw-paw) in decontamination of toothbrushes. This antibacterial efficacy of plant extracts in controlling the microbial contamination of toothbrushes and pathogenic organisms in this study has also been reported by Vignesh et al. (2017) and Bipul et al. (2013).

Conclusion

It is rather disturbing to say the least, that toothbrushes analyzed in this study were contaminated by multidrug-resistant bacteria; an indication of poor oral hygiene awareness and practice among the sampled population. This study showed that extracts from paw-paw (*C. papaya*) and guava (*P. gaujava*) exhibited higher antibacterial activities than the conventional “Oral-B” mouthwash solution. Of note, extracts from *P. gaujava* had higher antibacterial activity than extracts from *Carica papaya*. Interestingly, this study also showed that methanol extracts from the selected plants (*P. gaujava* and *Carica papaya*) were generally better in antibacterial activities than ethyl acetate extracts. The antibacterial activity of the plants extracts (*P. gaujava* and *C. papaya*) in this study is encouraging; an indication that natural medicinal plants can actually come to our rescue in the face of current increasing antimicrobial resistance menace. Thus, efforts should be geared towards exploring natural plants for their potential antimicrobial activities in order to develop alternative therapeutics. The isolation of bacterial pathogens in used toothbrushes in this present study calls for public health enlightenment on good oral hygienic practices so as to improve oral health and curtail the spread of diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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