

*Full Length Research Paper*

# **Antimicrobial activity of coconut water, oil and palm kernel oils extracted from coconut and palm kernel on some plasmid-mediated multi-drug resistant organisms associated with food spoilage**

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Received 25 May, 2020; Accepted 16 July, 2020

This study is aimed at evaluating the antibacterial and anticandidal activity of coconut oil, fermented and unfermented coconut water and palm kernel oil (PKO) on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Corynebacterium* sp. and *Candida albicans*. The coconut water was fermented with *Saccharomyces cerevisiae* and the pH, sugar and alcohol contents were determined before and after fermentation. The plant materials were screened for antimicrobial phytochemicals. The antimicrobial activity of the coconut water, coconut oil and palm kernel oil and the antibiotic susceptibility of the organisms were done on Muller Hinton agar using disc diffusion method. The plasmids of the organisms were cured using 10% sodium dodecyl sulphate. The pH and ethanol content of the fermented coconut water were higher than the unfermented while the sugar content decreased after fermentation. There were no phytochemicals detected in the coconut water whereas all the phytochemicals were detected in the PKO while only reducing sugar and steroids were absent in the coconut oil. All the bacteria exhibited multi-drug resistance. After plasmid curing, there were reductions in the resistance indicating that the resistance is plasmid mediated. There was a reduction in the percentage antibiotic resistance of *Corynebacterium* sp. which was 60 and 0% before and after curing respectively. The PKO was more effective in inhibiting the bacteria and *Candida* sp. than coconut oil but were all resistant to the coconut water. PKO and coconut oil may thus be used as natural substitutes to control *E. coli*, *S. aureus*, *P. aeruginosa* and *Candida* sp.

**Key words:** Bacteria, antimicrobial, resistance, coconut water, coconut oil, palm kernel oil, phytochemicals.

## **INTRODUCTION**

The presence of microorganisms in food can bring about desirable and undesirable effects. Some of the adverse

effects of their presence in food are spoilage of the food and/or food-borne diseases caused by organisms which

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include *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Pandey and Singh, 2011; Ashraf et al., 2018). Some of these diseases are life threatening and there is a growing concern of antibiotic resistance by many of these organisms (Sapkota et al., 2012; Wigmore et al., 2016; Collingnon and McEwen, 2019). Repeated applications of chemical preservatives used in the prevention of spoilage (Shan et al., 2007) bring about bioaccumulation in food and feed chain. These result in microbial resistance to the applied chemicals, consequently having detrimental side effects on the health of the consumers (Blalonska, 2010; Akinyemi et al., 2006). About 90 to 95% of *S. aureus* strains isolated worldwide are penicillin and methicillin resistant (Borges et al., 2016). *P. aeruginosa*, *S. aureus*, *Salmonella* sp., Coagulase-negative *Staphylococcus*, *Shigella*, *Enterococcus* sp. and *E. coli* are among some of the bacteria with multidrug resistance (Fisher and Phillips, 2008). The prevalence and resistance of *E. coli* isolates to the third generation cephalosporins have been reported to be very high (Uribe-Beitain et al., 2017).

The resistance of microorganisms to antimicrobials such as antibiotics can be caused by indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, organ transplants and intravenous catheters (Selvamohan et al., 2012). Due to these concerns, there are concerted efforts to develop potentially effective, safe and natural antimicrobials which can serve as food preservatives and substitutes for antibiotics in the treatment of diseases caused by multidrug-resistant organisms. The use of plants as an alternative is being studied globally since plants are considered nutritionally safe, biodegradable and possess antimicrobial phytochemicals (Berahou et al., 2007; Chika et al., 2007). Moreso, majority of the population of underdeveloped and most developing countries like Nigeria still depend mainly on traditional medicine using plants (Pallant and Steenkamp, 2008). This is because the health facilities are not well equipped, far from rural populace with expensive, or no means of transportation, and are expensive, as well as due to religious and cultural beliefs. Apart from high dependence of these plants ethnomedically in the underdeveloped and developing countries, there is also a growth trend of health and food supplements in the form of plants and their derivatives in different parts of the world.

Plants have been reported to be excellent sources of secondary metabolites which can be used in the production of modern medicines (Chandra et al., 2017). Many of the secondary metabolites such as tannins, flavonoids, alkaloids have been reported to contribute to antimicrobial activity. These secondary metabolites are produced by plants to fight against microbial attacks. The antimicrobial activity exhibited by plants and their extracts have been demonstrated in many studies (Akinpelu et al., 2015; Verma et al., 2012). Akinyemi et al. (2006) demonstrated the high antibacterial activity of three

medicinal plants (*Trema guineensis*, *Phyllanthus discoideus* and *Acalypha wilkesiana*) on *S. enteritidis*, *E. coli* and *S. aureus*. Verma et al. (2012) found the extracts of *Punica aratanum* and *Allium* sp. to be effective against some food-borne spoilage bacteria (*S. typhi*, *E. coli*, *B. cereus* and *S. aureus*). Sarid-Saklani et al. (2019) reported the presence of flavonoids, terpenoid and tannins in *Terminalia alata* which were inhibitory to *K. pneumoniae* and *S. pneumoniae*.

Essential oil which are mainly plant derivatives were reported to possess antifungal, antibacterial and antiviral properties and are potential agents of food preservation and alternatives to treat infectious diseases (Astanin et al., 2011; Safaei-Ghonu and Ahd, 2010). Efficacy of essential oils has been reported in several studies against pathogens and food contaminants (Djenane et al., 2011). Plants from which essential oils have been derived include *Cymbopogon citratus*, *Satureja montana*, *Eucalyptus* sp., *Allium sativum*, *Cinnamon*, *Cocos nucifera*, *Occimum gratissimum*, *Mentha longifolia* and *Piper nigrum*.

Coconut is cultivated for its multi-purpose values (nutritional and medicinal). The products of *C. nucifera* include coconut water, copra, coconut oil, raw kernel, coconut cake and coconut milk. It is a unique source of various natural products for the development of drugs and industrial products and is effective against fungi, bacteria, viruses, parasites and dermatophytes (Floriana et al., 2015). It is also an antioxidant (Manisha and Shyamapada, 2011). Coconut water is a refreshing beverage believed to be rich in vitamins, minerals, amino acids, carbohydrates, enzymes, hormones and phytochemicals and sometimes fermented to increase its shelf life and probiotic content (Bandalam and Galvez, 2016). Coconut water and oil is claimed to cure some diseases by rural dwellers in Nigeria (Fowoyo and Alamu, 2018). Coconut oil contains a high amount of phytochemicals in form of alkaloids, flavonoids, saponins, tannins soluble carbohydrates, hydrogen cyanide, reducing sugar, terpenoids (Ojobor et al., 2018). Coconut oil was found to inhibit *S. epidermidis*, *E. coli* and *Candida* sp. (Rajan et al., 2016; Ogbalu, 2017). The anticandidal activity of this oil was comparable to that of fluconazole (Ogbalu, 2017). Shilling et al. (2013) reported that coconut oil inhibits *Clostridium difficile*.

Palm kernel oil is extracted from the kernel endosperm of *Elaeis guineensis*. It is used traditionally in the treatment of convulsion, dandruff, skin diseases, cold and cough (Ekwenye and Ijeomah, 2004). The inhibitory effect of palm kernel oil on some bacterial and fungal isolates was reported by Ugboogu et al. (2006). Floriana et al. (2015) found palm kernel oil to be effective against *E. coli*, *S. aureus* and *Streptococcus* sp while coconut oil was active against *Listeria monocytogenes*, *S. aureus* and *Helicobacter* sp.

This study is therefore aimed at evaluating the antibacterial and anticandidal activity of coconut oil, coconut water and palm kernel oil on some microorganisms

associated with food spoilage and food-borne diseases.

## MATERIALS AND METHODS

### Collection of samples

#### Plant materials

Mature coconuts, palm kernel oil and coconut oil were bought from Abraka Market, while the tender coconuts were harvested from farms in Abraka, Delta State, Nigeria. The oils were transported to Microbiology Laboratory, Delta State University, where they were kept in sterile, screw-capped bottles and kept in the refrigerator for analyses.

### Collection of isolates

The bacterial isolates (*S. aureus*-9, *E. coli*-3, *P. aeruginosa*-1 and *Corynebacterium* sp-1) were collected from food-spoilage stock culture at Microbiology Laboratory, Delta State University, Abraka. They were cultured on Nutrient agar and Cysteine, Lactose and Electrolyte Deficient (CLED) agar, and incubated at 37°C for 24 h prior to usage.

### Confirmation of isolates

The identity of each isolate was confirmed after incubation using morphological, cultural and biochemical characteristics which included Gram staining, motility test, catalase, oxidase, indole, citrate utilization and Triple Sugar Iron (TSI) agar tests.

### Fermentation of coconut water

Coconut fruits were first washed with distilled water, then surface-sterilized using alcohol and opened at one of the eyes on the apical region using a sterile knife. The water was aseptically collected into a sterile glass measuring cylinder. The coconut water samples (mature and tender) were divided into two groups (A and B). The mature samples were labelled mA and mB while the tender ones were labelled tA and tB.

mA and tA were fermented using a modified method by Shiny et al. (2014). Dry baker's yeast (90.5 g) was added to 450 ml of each coconut water sample in a measuring cylinder. The cylinder was covered with aluminium foil and incubated at 26±2°C for 24 h.

### Determination of pH, sugar content and alcohol content

#### pH

The pH was determined using a pH meter.

#### Sugar content

A brix hydrometer was inserted into 450 mL of coconut water samples, fermented (mA and tA) and unfermented (mB and tB) and the readings were taken.

#### Alcohol content

This was done using the hydrometer to determine the specific

gravity and the alcohol content calculated using the formula:

$$\% \text{ Alcohol by volume} = (\text{FG}-\text{OG}) \times 131.25 \quad (1)$$

Where FG = Final specific gravity, OG = Original specific gravity, and 131.25 is a constant brewery factor

### Phytochemical screening of coconut water sample

Saponin, flavonoid, alkaloid, tannin, phenol, reducing sugar, terpenoid, steroid, glycoside and cardiac glycoside were screened for using the method of Hasbourn (1973) as follows:

1. Alkaloids: To 0.5 g of each extract, was added 5 ml of 2 N HCl and filtered. Dragndoff's reagent was added and formation of a red precipitate was used to indicate the presence of alkaloids.
2. Phenols: About 3 to 4 drops of ferric chloride solution was added to the extract. The presence of phenol was indicated by the formation of bluish black colouration.
3. Terpenoids: 2 ml of chloroform and 3 ml concentrated H<sub>2</sub>SO<sub>4</sub> was added to 5 ml of extract. This was done carefully to form a distinct layer. Positive result for the presence of Terpenoids was indicated by reddish brown colour formation.
4. Saponins: To a small quantity of the extract was added distilled water up to 20 ml and shaken vigorously. The formation of 1 cm layer of foam stable for 10 min indicates a positive result for saponin.
5. Flavonoids: Sodium hydroxide (10%) was added to the plant extract. The formation of yellow colouration indicated the presence of flavonoids.
6. Phenolics and Tannins: A 10% Lead acetate solution was added to little quantity of the extract already dissolved in distilled water. The presence of tannin was indicated by formation of white precipitate.

### Preparation of standard inoculum

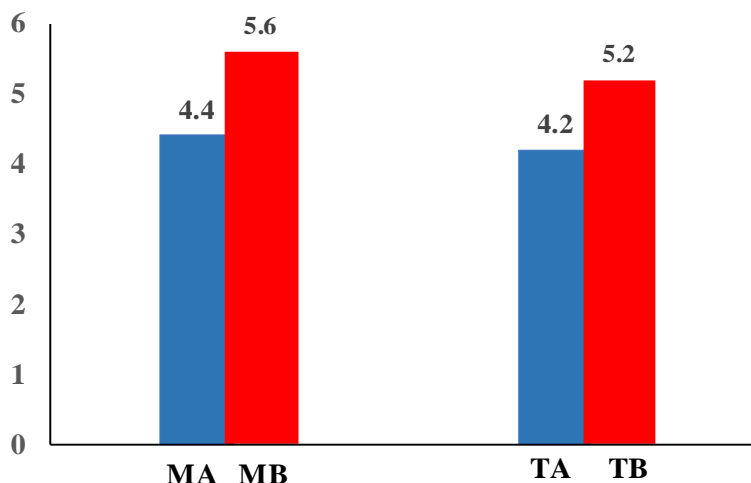
Suspension of each test organism was made by inoculating a loopful of the colony into peptone water and incubated at 37°C for 24 h. The overnight broth culture of organisms was diluted with sterile distilled water to an inoculum load of 1×10<sup>6</sup> CFU/ml. It was standardized by comparing the turbidity with 0.5 McFarland turbidity standards (Murray et al., 2016).

### Antibiotic susceptibility test

This was done using paper disc diffusion method. Sterile cotton swabs were dipped into each of the standardized inoculum suspension and used to inoculate freshly prepared Muller Hilton agar plates. The cotton swabs were pressed on the wall of the tubes to avoid flooding of the medium and the plates were completely covered for uniform growth. Appropriate antibiotic discs were aseptically placed on the inoculated agar plates which were incubated at 37°C for 24 h. They were examined for zones of inhibition (Murray et al., 2016).

### Antimicrobial susceptibility test

Antimicrobial susceptibility was done using agar Kirby-Bauer disc diffusion method as described by Abbas et al. (2017). Different concentration (20, 40, 60, 80 and 100%) of fermented and unfermented coconut water samples were made in sterile universal containers. A sterile Whatmann No. 1 filter paper disc was impregnated with appropriate concentrations of the coconut water



**Figure 1.** pH value of coconut water samples. MA = Fermented mature coconut water; MB = Unfermented mature coconut water; TA = Fermented tender coconut water, TB = Unfermented tender coconut water.

samples. The discs (equally spaced) were placed on a freshly and uniformly inoculated Muller-Hilton agar plates. The plates were incubated at 37°C for 24 h. After incubation, the plates were examined for zones.

#### Plasmid curing of bacterial isolates

Ten percent (10%) stock solution of sodium dodecyl sulphate (SDS) was prepared (Silhavy et al., 1984) by adding 10 g SDS to 90 mL of distilled water. This was heated with sterile magnetic heater for 15 min until homogeneous mixture was obtained. Bacterial isolates were inoculated into peptone water and incubated at 37°C for 24 h. Ten-fold serial dilution of the SDS solution was done and 0.5 mL of the standardized bacterial suspension were added to the dilutions in test tubes. The tubes with inoculated SDS were incubated at 37°C for 24 h. Solutions with growth were sub cultured by transferring 1 mL of the solutions and incubated at 37°C for 48 h to confirm their growth. Fresh peptone water solution was prepared and inoculated with 0.5 mL of cured tested cultures and incubated at 37°C for 24 h. Antibiotic susceptibility test was carried out on the organisms.

## RESULTS

The pH values of fermented coconut milk (mA and tA) were found to be lower than the values of unfermented samples (mB and tB) (Figure 1). The ethanol content of the fermented tender coconut samples were higher than that of fermented mature coconut water samples, while the sugar content decreased significantly after fermentation (Figure 2). The phytochemical screening of the plant extracts indicated the presence of flavonoid, tannin, alkaloid, saponin, reducing sugar, cardiac glycoside and terpenoids. This was confirmed in this study (Table 1).

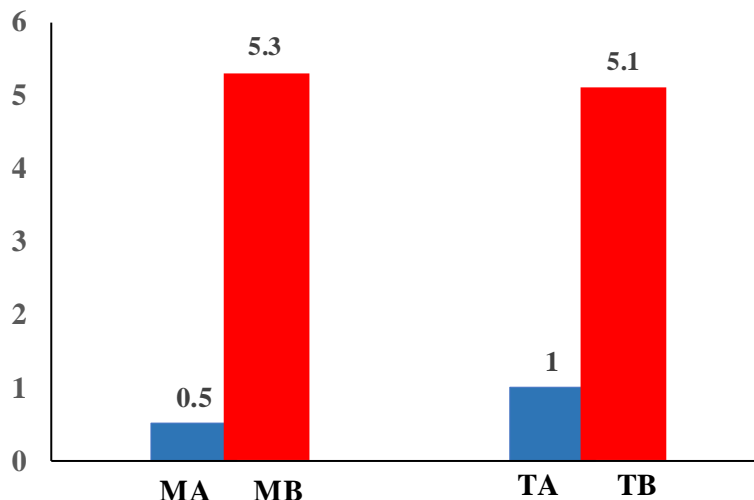
The antibiotic sensitivity profiles of the isolates showed that all the isolates exhibited multi drug resistance (Tables 2 to 3). All the isolates were resistant to tA while

81% were resistant to tB (only 2 of the 11 isolates were sensitive). There was 100 and 83% susceptibility to PKO and coconut oil respectively. The plasmid curing affected the antibiotic sensitivity profile of more than 90% of the gram negative bacteria and about 50% of the gram negative bacteria as shown in Table 4. The drug resistance of the affected isolates were reduced after the plasmid curing.

## DISCUSSION

The fourteen isolates, that is, *S. aureus* (9), *Corynebacterium* sp. (1), *E. coli* (3) and *P. aeruginosa* (1) have been reported to be common pathogens which cause urinary tract infections (Odoki et al., 2019), nosocomial infections (Khan et al., 2019) among others. They have also been implicated in the spoilage of some foods.

The absence of phytochemicals in fermented and unfermented tender and mature coconut water (sap) may be attributed to the very high content of the water which may have diluted the chemicals if present, to undetected levels. Misra (2016) reported that the composition of coconut water was mainly water but after ultrafiltration contained total sugar (14.40 g/100 mL), reducing sugar (5.58 g/100 mL) and total protein (0.23 to 0.32 g/100 mL). This study used coconut water without processes used in concentrating the chemicals. The levels of the chemicals were undetected since they might have been diluted in the high-water content. Fowoyo and Alamu (2018) reported the moisture (%) of coconut water for both mature and immature coconut to be between 95.44 to 98.00%. The increase in acidity of the water after fermentation may be attributed to the presence of organic acids, free amino acids, fatty acids and dissolved CO<sub>2</sub>



**Figure 2.** Sugar level (unit) of coconut water samples. MA = Fermented mature coconut water; MB = Unfermented mature coconut water; TA = Fermented Tender Coconut water; TB = Unfermented tender coconut water.

**Table 1.** Phytochemical screening of plant materials.

Phytochemical	Tf	tU	mF	mU	Coconut oil	PKO
Flavonoid	-	-	-	-	+	+
Tannin	-	-	-	-	+	+
Alkaloid	-	-	-	-	+	+
Saponin	-	-	-	-	+	+
Reducing sugar	-	-	-	-	-	+
Cardiac glycoside	-	-	-	-	+	+
Terpenoids	-	-	-	-	+	+
Phenols	-	-	-	-	+	+
Steroid	-	-	-	-	-	+

m = mature, t = tender, + = presents; - = absent, tF = fermented tender coconut water; tU = unfermented tender coconut water, mF = fermented mature coconut water, mU = unfermented mature coconut water, PKO = palm kernel oil.

**Table 2.** Zones of inhibition (mm) of Gram positive isolates before (and after) curing.

Isolate	Antibiotics zones of inhibition									
	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
SA1	22(22)	17(18)	12(10)	21(13)	11(8)	12(14)	20(13)	15(16)	12(14)	17(10)
SA2	10(24)	10(10)	10(10)	20(18)	10(13)	10(13)	22(18)	16(14)	10(10)	18(10)
SA3	15(20)	10(16)	10(15)	14(12)	10(10)	10(16)	18(13)	10(10)	10(16)	10(10)
SA4	18(24)	14(14)	10(10)	16(16)	12(10)	10(13)	20(14)	12(24)	10(16)	10(10)
SA5	10(22)	16(24)	10(10)	18(10)	10(12)	10(10)	14(16)	12(24)	10(16)	18(10)
SA6	20(20)	16(16)	10(10)	12(22)	14(12)	12(14)	24(15)	10(24)	12(12)	10(10)
SA7	10(19)	14(24)	10(8)	16(13)	15(18)	15(20)	10(13)	13(20)	13(12)	10(10)
SA8	10(20)	10(18)	10(22)	20(22)	15(10)	16(12)	18(13)	16(16)	15(13)	17(10)
SA9	20(22)	12(24)	10(12)	18(16)	10(14)	10(16)	22(14)	10(20)	10(12)	18(8)
<i>Corynebacterium</i>	20(26)	10(26)	10(18)	22(24)	12(26)	10(28)	24(28)	12(26)	10(18)	16(24)

0-12 = Resistant (R),  $\geq 13$  mm = sensitive (S), PEF = Pefloxacin, CN = Gentamycin, APX = Ampiclox, SA = *Staphylococcus aureus*, Z = Zinnacef, Am, = Amoxicillin, R= Rocephin, CPX = Ciprofloxacin, S = Septrin, SXT =Streptomycin, E = Erythromycin.

**Table 3.** Zones of inhibition (mm) of gram negative isolates before (and after) curing.

Isolate	Antibiotics zones of inhibition									
	PEF	CN	AU	AM	CPX	SP	CH	SXT	S	OFX
EC1	22(22)	17(16)	12(15)	21(10)	11(16)	12(18)	20(8)	15(16)	12(16)	17(13)
EC2	10(13)	10(10)	10(10)	20(10)	10(10)	10(16)	22(10)	16(13)	10(13)	18(13)
EC3	15(18)	10(16)	10(16)	14(16)	10(18)	10(10)	18(20)	10(15)	10(15)	10(18)
PA1	18(13)	14(19)	10(10)	16(15)	12(12)	10(12)	20(13)	12(10)	10(10)	10(13)

0-12 mm = Resistant (R),  $\geq 13$  mm = Sensitive (S), PEF = Pefloxacin, CN = Gentamycin, AU = Augmentin, EC = *E. coli* = *Pseudomonas aeruginosa*, CH = Chloramphenicol, AM = Amoxicillin, SP = Sparfloxacin, CPX = Ciprofloxacin, S = Septrin, SXT = Streptomycin. E = Erythromycin.

**Table 4.** Comparison of sensitivity of the isolates to the coconut water, coconut oil and palm kernel oil.

Isolate	tA	tB	PKO	CO
SA1	R	R	S*	S
SA2	R	R	S*	S
SA3	R	R	S*	S
SA4	R	R	S*	S
SA5	R	R	S*	S
SA6	R	R	S*	S
SA7	R	R	S*	R
SA8	R	R	S*	R
SA9	R	R	S	S
EC1	R	R	S**	S
EC2	R	R	S**	S
EC3	R	R	S**	S
PA1	R	R	S	S
CB	R	R	S	S
CS1	R	R	S	S

SA = *Staphylococcus aureus*, EC = *Escherichia coli*, PA = *Pseudomonas aeruginosa*; CS = *Candida* sp., CB=Corynebacterium sp., R = Resistance, S = Sensitive, S\* = Moderately sensitive, S\*\* = highly sensitive.

arising from tissue respiration (Robert and Frim, 2009). The pH of tender coconut being more acidic than that of mature coconut water in this study corresponds with that of Fowoyo and Alamu (2018).

The detection of phytochemicals may be attributed to the natural ability of plants to produce secondary metabolites to combat microbial attacks. The lower sugar content in unfermented tender coconut water (0.5) than mature (1.0) and high sugar content in the unfermented mature coconut water (5.3 and 6.1) may be due to sugar present being converted to acid during metabolic processes in the plant and that the sugar being used by the kernel during development for the synthesis of fat (Fowoyo and Alamu, 2018). The alcohol content by volume was 1.3 and 2.0% for fermented mature and tender coconut water respectively. This showed a direct relationship with the sugar content. The tender coconut had a higher sugar content resulting in a higher alcohol content. Nakuwa et al. (2012) reported that on exposure, coconut water becomes vulnerable to oxidation and

microbial contamination which brings about fermentation. Antibiotic sensitivity test showed all the gram positive organisms in the present study exhibiting multi drug resistance. Resistance may be due to prolonged use of the antibiotics, organisms putting up mechanisms to resist the antibiotics, as well as eating poultry and livestock with feeds treated with antibiotics. This is the practice in most of these countries where there are no checks and control resulting in indiscriminate use of antibiotics.

After curing, the gram positive isolates (apart from SA7) and EC1 and EC2 showed a reduction in the resistance level indicating that the resistance is plasmid mediated. This conforms with the result of Patwardhan et al. (2017) that elimination of plasmids enables effective antibiotic therapy. The gene coding for the resistance is in the plasmid hence no resistance after the removal of the plasmid. This would have constituted a strategy for removal of the gene to make the organisms susceptible but other factors contributing to the resistance still have

to be overcome, hence the need for a natural antimicrobial product.

The bacterial isolates exhibited resistance to all the tested concentrations of coconut water used (fermented and unfermented). This effect may be related to the absence of phytochemicals in coconut water. The result corresponds with the study of Rukimi et al. (2017) who stated that coconut water did not exhibit antimicrobial effect on microorganisms. However, the result disputed the report of Shiny et al. (2014) that fermented tender coconut water has antimicrobial effect on *E. coli* and the report of Fowoyo and Alamu (2018) that tender coconut water exhibited antimicrobial effect on *S. aureus*, *E. coli*, *B. cereus* and *P. mirabilis*.

All the isolates were susceptible to PKO and 86% to coconut oil. The high susceptibility of the organisms may be attributed to the many phytochemicals detected in the palm kernel and coconut oils. Coconut oil and palm kernel oil have been reported to contain lauric acid which is antibacterial, antifungal and antiviral (Manisha and Shyamapada, 2011). Contrary to the finding of this study, Abbas et al. (2017) reported a low susceptibility of *E. coli* to coconut oil extract. Ekwenye and Ijeomah (2004) also reported non-susceptibility of *E. coli* to PKO but there was susceptibility to virgin coconut oil when the PKO was mixed with coconut oil. The report of Floriana et al. (2015) that VCO and PKO were effective against *E. coli* and *S. aureus* conforms with that of this study. The difference in the effectiveness of the plant materials may be attributed to the fact that levels of the phytochemicals which invariably affect the microbial activity vary from plant to plant depending on factors such as method of extraction, solvent used in extraction and growing condition.

## Conclusion

Food spoilage and food borne diseases are attributed to the presence of diverse microorganisms in food. Many of these organisms which are pathogenic are exhibiting alarming increase of multidrug resistance, in addition to the adverse effects of the synthetic chemicals in the drugs and the food preservatives. This study showed that the coconut water was not effective against any of the organisms so cannot serve as a potential antimicrobial. However, coconut oil and palm kernel oil may be used as natural alternatives in the control of some microorganisms associated with food spoilage and food borne diseases.

They may also serve as a substitute to synthetic antibiotics which are becoming less effective and have numerous side effects. Further work can still be done on the activity of concentrates of coconut water. Other species of coconut and *E. guineensis* from other geographical locations can also be researched into for their antimicrobial activity.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors acknowledge the laboratory staff of the Department of Microbiology, Delta State University for supply of culture as well as technical support.

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