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Antibacterial activity of *Garcinia kola* and *Hunteria umbellata* extracts on bacterial isolates from consumed sachet water in Edo State, Nigeria

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Abstract. Water is an essential resource and its purity should not be negotiated when it comes to human consumption. This study investigated the antibacterial activity of *Garcinia kola* and *Hunteria umbellata* seed and epicarp on some bacterial isolates from sachet water. Duplicate samples of ten brands of sachet water were purchased from sales outlets around Ugbowo community, Benin City. Plate count techniques, minimum inhibitory and minimum bactericidal concentrations of the extracts were adopted in this investigation. Heterotrophic bacterial counts revealed highest range of $3.72\pm0.50x10^2$ cfu/mL in EJ water and lowest in IB water ($0.00\pm0.0x10^2$ cfu/mL) while total coliform counts revealed its highest value in EJ water ($3.62\pm0.30x10^2$ cfu/mL) and lowest value (0.00 ± 0.0 $x10^2$ cfu/mL), in OL, IB, NOS and UNI water. *Aeromonas* sp., *Bacillus* sp., *Pseudomonas* sp., *Staphylococcus aereus* and *Enterobacter* sp. were isolated. The antimicrobial susceptibility profile revealed varying zones of inhibition of 4 mm for *Aeromonas* sp., for *Garcinia Kola* and 20 mm recorded against *Staphylococcus aureus* and *Bacillus* sp. for *Hunteria umbellata* epicarp extract. The Minimum Inhibitory Concentration of susceptible bacteria to *Hunteria umbellata* and *Garcinia kola* extract were 6.25mg/mL and 12.5mg/mL,

respectively, except *Aeromonas* sp. which had 50 mg/mL. *Hunteria umbellata* epicarp had a greater bactericidal effect of 6.25mg/mL against *Staphylococcus aureus,* while *Garcinia kola* had its greatest bactericidal effect on *Enterobacte*r sp. with a minimum bactericidal concentration of 12.5mg/mL. This study has revealed the potentials of *Hunteria umbellata* epicarp and *Garcinia kola* as effective natural therapeutic agents against some harmful bacteria, preventing their pathogenic effect.

Keywords: Herbal extracts, Hygiene education, Minimum Bactericidal Concentration, Minimum Inhibitory Concentration, Sachet water.

Introduction

Water is indispensable to life so that it has a substantial effect on public health, living standard and has an uneven distribution the world over (Kılıç, 2020). It's of immense importance and an essential substance needed to maintain vital actions of humans such as respiration, nutrition, circulation, excretion and reproduction (Kılıç, 2020). The adult human body consists of 70 percent water, while 95 to 98 percent of the bodies of lower animals/aquatic animals are made up of water (Gordalla *et al.,* 2007). Most times water ascertained as clean is often non available to countless human populations globally, which remains relatively distressing with the harmful aspects linked with contaminated water frequently used (Khalifa and Bidaisee, 2018). Generally, people prefer to drink and use unhygienic water than using nothing at all. The amount of people that consume unhygienic water across the globe are about two billion people, which is alarming (Khalifa and Bidaisee, 2018).

Water is prone to contamination by microorganisms and organic matter, among other contaminants, irrespective of source (Gangil *et al.,* 2013; Anyamene and Ojiagu, 2014; Oludairo and Aiyedun, 2016). Potable water encourages economic growth as well as helps to improve public health. Contaminated water is often the reason for economic and social costs via water-related ailments like dysentery, typhoid fever, hepatitis A, poliomyelitis, Vibrio illness, *E. coli* infection and increases in therapeutic treatment expenses (Rossi *et al.,* 2012; Mohsin *et al.,* 2013). The presence of *Escherichia coli*, *Klebsiella* and *Enterobacter* spp. in water possibly connotes the existence of infective organisms such as *Clostridium pafringens*, *Salmonella* and Protozoa (Anyamene and Ojiagu 2014).

Antimicrobials refer to a constituent that kills or impedes the development of microorganisms like bacteria, fungi and viruses (Ajayi and Ojelere, 2014). Antimicrobial drugs either kill microorganisms (micro-biocidal) or impede the development of microorganisms (micro-biostatic) (Ajayi and Ojelere, 2014). Antibiotics either occur naturally or are synthetic organic compounds known to impede or terminate choosy bacteria, normally at low concentrations (Brooks *et al.,* 2007).

Hunteria umbellata (K. Schum) is a tropical rainforest plant which belongs to the Apocynaceae family. It is referred to locally as 'abeere' amongst the Yoruba (South-West Nigeria), 'nkpokiri' by Ibos and 'osu' by Edos (Adeneye and Adeyemi, 2009b). It is a therapeutic plant having a history of usage in treatment of infections, illnesses and diseases in Nigeria and Ghana (Adeneye and Adeyemi, 2009b). It has been reported that numerous extracts prepared from its various parts have been used for the treatment of several human diseases like sexually transmitted infections, yaws, stomach ulcers, pains and swellings, diabetes mellitus, dysmenorrhea and to induce or augment birth labor by African folklore medicine (Falodun *et al.,* 2006; Adeneye and Adeyemi 2009). Studies have shown that various medicinal plant extracts have several biological properties such as antimicrobial, antioxidant, anti-inflammatory, anticancer and anti-diabetic activities (Wang *et al.,* 2018; Ahmed *et al.,* 2019; Cai *et al.,* 2019; Tuama and Mohammed 2019; Olaokun *et al.,* 2020). Also, the analgesic and antipyretic effects the aqueous extract of its fruit pulp has been investigated and proven to be effective in the regulation of pain and fever and these effects were independent of its antibacterial activities (Igbe *et al.,* 2009).

Garcinia kola commonly known as bitter kola, belongs to the family Guittiferal, it is valued in Nigeria for its medicinal nut within many Nigerian communities. Bitter kola is chewed extensively by Nigerian locals as a masticator to enhance nervous alertness and has been proven to exhibit pharmacological uses in the treatment of coughs and throat infections (Farombi *et al*., 2005). *Garcinia kola* stem bark has been shown to contain a complex mixture of phenolic compounds such as tannins, guttiferin, biflavonoids, xanthenes, benzophenone, kola flavanone and garcinia flavanone Adamu *et al.,* 2020 and Niemenak *et al*., 2008, all of which are reported as having antimicrobial activity. Besides, *G. kola* has been reported as exhibiting purgative, anti-parasitic, anti-inflammatory, anti-bacterial and antiviral properties (Akoachere *et al*., 2002).

It is against this background that this study attempts to assess the antimicrobial effect of *Garcinia kola* (bitter kola) and *Hunteria umbellata* on bacterial isolates from selected sachet water brands sold around Ugbowo community in Benin Metropolis to provide data for future reference purposes.

Materials and methods

Study Area

The study was carried out around Ugbowo community in Egor Local Government area, Benin City, the capital of Edo state, in the southern part of Nigeria. Its geographic location is at latitude 6°11′ and 6°29'N, and longitude 5°33′ and 5°47'E.

Sample collection

Ten different brands of sachet water were purchased from a total of five shops within Ugbowo environs in Benin City. Duplicate samples were purchased for each brand. The sachet water samples were purchased at the following locations: Faculty of Life Sciences Shopping Complex, University of Benin (6°23'46" N, 5°37'8" E); Faculty of Physical Sciences Shopping Complex, University of Benin (6°23'48" N, 5°36'59" E); Ekosodin (6°24'19" N, 5°37'35" E); and two locations in BDPA area (6°23'44" N, 5°36'13" E and 623'41" N, 5°36'9" E).

The purchased sachet water samples were stored under ice and transported to the laboratory for analysis (Figs. 1, 2).

Figure 1. Sachet water displayed for sales (Photo credit: Blessing Offeh)

Figure 2. Sampling locations

Sterilization and preparation of media

The glare wares used (conical flask, round bottom flask) were washed, drained and dried. They were wrapped with aluminum foil and sterilized using autoclave at 121°C for 15 minutes. An aseptic working environment was achieved with the use of Bunsen burner flame and disinfection of work surfaces with alcohol. The media were prepared according to manufacturers' manufacturer's instructions. Pour plate technique was employed (Holt *et al*., 1994).

Enumeration and isolation of bacterial species

The isolation, enumeration and characterization of the bacterial species were carried out using standard procedures and characterization of the isolates were done using Bergey's manual (Holt *et al*., 1994).

Preparation of plant organic extracts

The *Garcinia kola* (bitter kola) and *Hunteria umbellata* (osu) were grinded to fine powder to increase the surface area. 100 g of each extract was soaked in 250 mL of solvents (distilled water) in conical flasks plugged and with cotton plugs, respectively. It was observed on a shaker for 48 and 72 hrs. The

stock concentration was 400 mg/mL. The extracts were filtered through a Whatman No.1 filter paper and Muslin cloth severally and concentrated to dryness with the aid of a rotary evaporator. The stocks were kept at 4°C in a refrigerator until further use. The concentrated extracts of osu seed, osu epicarp and bitter kola were varied into different concentrations; the quantities used were 100 mg and 200 mg, according to the methods of Adeogun *et al.* (2016).

Figure 3. *Hunteria umbellata* (left) and *Garcina kola* (right)

Antimicrobial screening

Agar well diffusion method was used to screen the antibacterial activities of different solvent extracts (Daoud *et al.,* 2015). One mL of fresh bacterial culture was pipetted in the center of sterile Petri dish. Molten cooled (MHA) was then poured into the Petri dish containing the inoculum and mixed well. Upon solidification, wells were made using a sterile cork borer (10 mm in diameter) into agar plates containing inoculums. Then, 100 µl of each extract 20 % (w/v) was added to respective wells. The concentration of extracts 20 % (w/v) has been selected based on our pre-experiments, and previous literature. The plates were placed in the refrigerator for 30 mins to let the extracts diffusion well into the agar. Then, the plates were incubated at 37OC for 18 hours. Antimicrobial activity was detected by measuring the zone of inhibition (including the wells diameter) appeared after the incubation period.

Determination of minimum inhibitory concentration

The tested extracts which exhibited antimicrobial activity at a concentration of 20 % (w/v) were used to determine their minimum inhibitory concentrations (MIC) using agar well diffusion method, and to evaluate their effectiveness in

pathogens. Different concentrations, 100, 75, 50, 25, 12.5, 6.25, 3.12 and 1.56 mg/mL, were prepared by two-fold serial dilution. One ml of each prepared inoculum was pipetted into sterile Petri dishes followed by the addition of molten agar and mixed well. One mL of the inoculum was also transferred into tubes containing the extracts. Then, wells were made on each plate, and 100 µl of 100, 75, 50, 25, 12.5 6.25, 3.12 and 1.56 mg/mL concentrations were transferred to the respective wells. Plates were kept in the refrigerator for 30 mins and then incubated at 37°C for 18 hours. The MIC was considered as the lowest concentration which inhibited the growth of the respective microorganisms.

Determination of minimum bactericidal concentration

A modification of the dilution method for the determination of MBC was used. Various concentrations of 200, 100, 50, 25, 12.5 and 6.25 mg were placed in sterile nutrient broth in test tubes. Using standard wire loop (Merck), a loopful (10 µl) of isolates, 0.5 McFarland standards was inoculated into test tubes containing 1 mL of the various concentrations. The tubes were incubated at 37°C for 18 to 24 hours and thereafter observed for growth or turbidity. Subsequently, a loopful of broth from each test tube not showing growth was inoculated into nutrient agar plate. Thereafter, equal volumes of sterile nutrient broth were added into the test tube cultures and incubated further for 24 hours at 37°C. Then, the tubes and agar plates were examined for turbidity or growth (Daoud *et al.,* 2015).

Results

The results obtained from the bacteriological analyses of the water samples are shown in Figures 4 and 5. The total heterotrophic bacterial counts were highest in EJ water at (3.72±0.50) $x10^2$ cfu/mL and least in IB water at (0.00±0.0) $x10^2$cfu/mL. For the total coliform count, the highest count was recorded as (3.62±0.30) $x10^2$cfu/mL for EJ water, while the least was (0.00±0.0) $x10^2$cfu /mL in OL, IB, NOS and UNI waters.

Five bacterial species were isolated and identified in the water samples, namely: *Aeromonas* sp. (EJ, OL and FM), *Bacillus* sp. (SB, FL and NOS), *Pseudomonas* sp. (SB and UNI), *Staphylococcus aureus*. (OJ) and *Enterobacter* sp. (NL).

The percentage frequency of occurrence of the various bacterial isolates identified in the samples is shown in Fig. 6. The highest value (30%) was recorded for *Aeromonas* sp. and *Bacillus* sp. each while the lowest value (10%) was recorded for *Staphylococcus aureus* and *Enterobacter* sp.

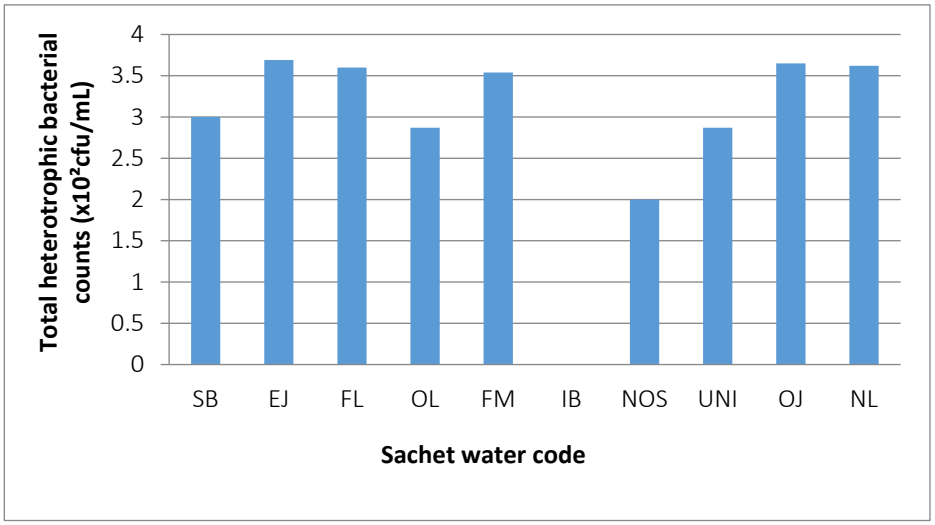

Figure 4. Total heterotrophic bacterial counts

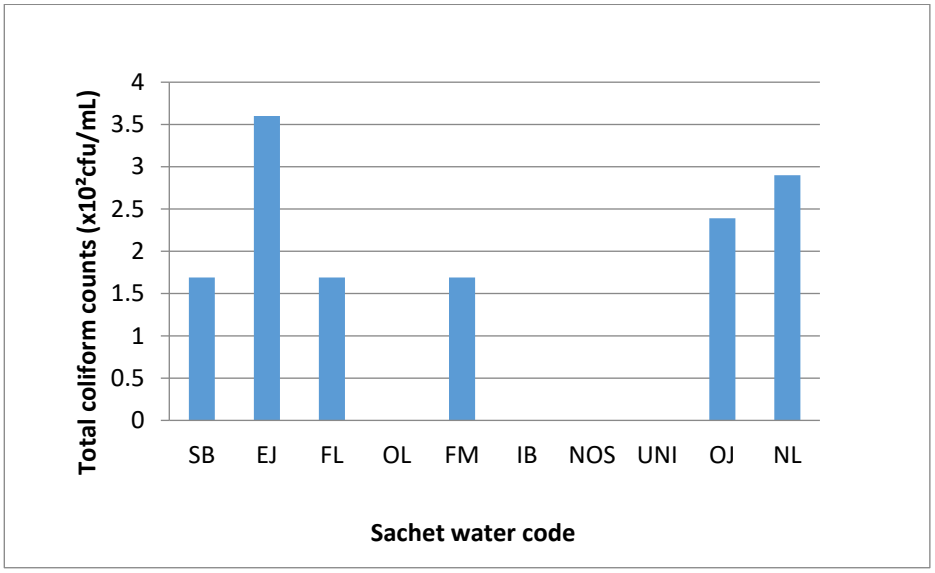

Figure 5. Total coliform counts

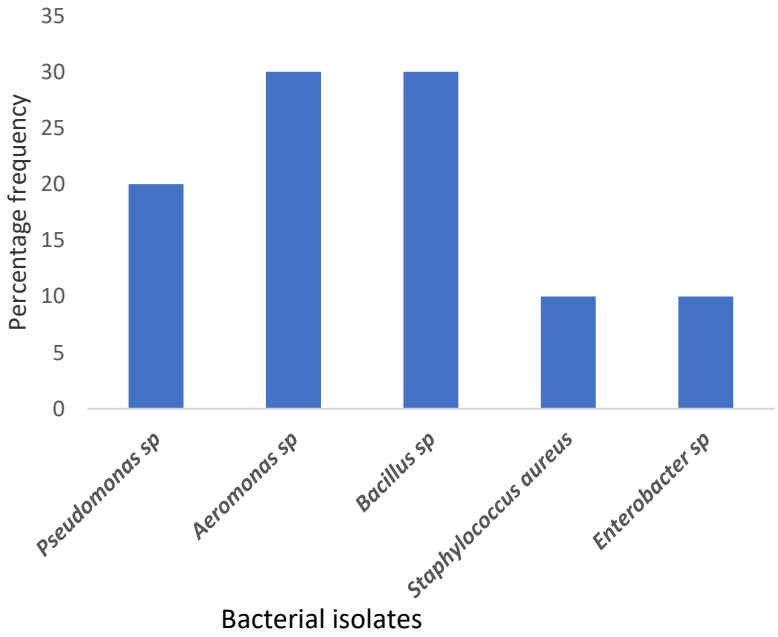

Figure 6. Percentage frequency of occurrence of isolates

Antibacterial activity of *Garcinia Kola* (bitter kola) and *Hunteria umbellata* (osu) extracts was also investigated (Tabs. 1-5). Table 1 shows the zone of inhibition of the various extracts, (*Hunteria umbellata)* (osu) seed, epicarp and bitter kola extracts. There was no zone of inhibition for *Hunteria umbellata* (osu) seed extract effective against the various isolates. The highest zones of inhibition for (*Hunteria umbellata* (osu) epicarp extract, 20mm, was effective against *Staphylococcus aureus, Bacillus* sp. and the lowest zone of inhibition, 14mm, was recorded against *Aeromonas* sp. *Garcinia Kola* had its highest and lowest zones of inhibition recorded against *Staphylococcus aureus* and *Aeromonas* sp., 14 and 4mm, respectively.

Tables 2 and 3 reveal the minimum inhibitory concentrations of *Hunteria umbellata* (osu) epicarp and *Garcinia kola* extracts. The minimum inhibitory concentration of *Hunteria umbellata* (osu epicarp) of the various isolates (*Staphylococcus aureus, Enterobacte*r sp. and *Bacillus* sp.) was 6.25mg/mL except for *Aeromonas sp.* which had 50mg/mL. For bitter kola extract, the MIC for *Staphylococcus aureus, Enterobacte*r sp. and *Bacillus* sp. was 12.5mg/mL while *Aeromonas* sp. had 50mg/mL. The tubes containing the extract inoculated with *Pseudomonas sp.* were turbid, indicating that the extract

was not effective against the isolates. The minimum bactericidal concentration results obtained for the various extracts are shown in Tables 4-5. Bitter kola (*Garcinia kola*) result from the non-turbid tubes (clear tubes) plated on nutrient agar showed growth after 24 hours. *Staphylococcus aureus* and *Bacillus sp.,* both had MBCs of 100 mg/mL, while *Enterobacte*r sp. had a MBC of 12.5 mg/mL. The results showed growths on agar plates and tubes from those of *Pseudomonas* sp. and *Aeromonas* sp., indicating that the extract did not have any bactericidal effect on the organisms (Tab. 4). Minimum bactericidal concentration results obtained for *Hunteria umbellata* (osu epicarp) extract were 6.25, 100, 100, 50 mg/mL from tubes of extract inoculated with *Staphylococcus aureus, Enterobacte*r sp., *Aeromonas* sp. and *Bacillus* sp., indicating that the extract had bactericidal effects against all isolates except *Pseudomonas* sp. (Tab. 5).

Table 1. Zones of Inhibition of *Hunteria umbellata* (osu seed, epicarp) and *Garcinia kola* (bitter kola) extracts

Isolates	**Osu seed (100mg)**	**Osu epicarp (100mg)**	**Bitter kola (200mg)**
Staphylococcus aureus	0mm	20mm	14mm
Bacillus sp.	0mm	20mm	12mm
Aeromonas sp.	0mm	14mm	4mm
Enterobacter sp.	0mm	18mm	10mm

Table 2. Minimum Inhibitory Concentration (MIC) for *Hunteria umbellata* (osu epicarp) extract on the various isolates from sachet water (mg/mL)

Isolates	**100**	**75**	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.56**
Bacillus sp.	-	-	-	-	-	-	+	+
Staphylococcus aureus	-	-	-	-	-	-	+	+
Aeromonas sp.	-	-	-	+	+	+	+	+
Enterobacter sp.	-	-	-	-	-	-	+	+
Pseudomonas sp.	+	+	+	+	+	+	+	+

KEY: - = no growth; + = growth

Table 3. Minimum inhibitory concentration for *Garcinia kola* extract on the various isolates from sachet water (mg/mL)

Isolates	**100**	**75**	**50**	**25**	**12.5**	**6.25**	**3.12**	**1.56**
Bacillus sp.	-	-	-	-	-	+	+	+
Staphylococcus aureus	-	-	-	-	-	+	+	+
Aeromonas sp.	-	-	+	+	+	+	+	+
Enterobacter sp.	-	-	-	-	-	+	+	+
Pseudomonas sp.	+	+	+	+	+	+	+	+

KEY: - = no growth; + =growth

Table 4. Minimum bactericidal concentration for *Garcinia kola* extract on the various isolates from sachet water (mg/mL)

Isolates	200	100	50	25	12.5
Bacillus sp	-	-	+	+	+
Aeromonas sp.	+	+	+	+	+
Enterobacter sp.	-	-	-	-	+
Staphylococcus aureus.	-	-	+	+	+
Pseudomonas sp.	+	+	+	+	+

KEY: - no growth, + = growth

Table 5. Minimum bactericidal concentration for *Hunteria umbellata* (osu epicarp) extract on the various isolates from sachet water (mg/mL)

Isolates	200	100	50	25	12.5	6.25
Bacillus sp.	-	-	-	+	+	+
Aeromonas sp.	-	-	+	+	+	+
Enterobacter sp.	-	-	+	+	+	+
Staphylococcus aureus.	-	-	-	+	+	+
Pseudomonas sp.	+	+	+	+	+	+

KEY: - = no growth, + = growth

Discussion

The health risk implication that drinking of contaminated water could cause to man cannot be overemphasized, hence this study was conducted to investigate the microbial contamination of sachet water, otherwise called 'pure' water, and to see how potent using natural remedies (osu and bitter kola) could be.

The result obtained from the bacteriological analysis of sachet water revealed the presence of heterotrophic and total coliform counts in some of the sampled water which were otherwise branded as 'pure water'. EJ water had the highest counts in both the heterotrophic and total coliform count. This could be due to unhygienic practices leading to the contamination of the environment and production process corroborating with the report of Idu *et al.* (2011) and Ademoroti (1996), who indicated that contamination by microbes may be the result of the environment in which they were produced, improper handling or storage.

The bacteriological investigation revealed the presence of five isolates: *Bacillus* sp. *Pseudomonas* sp.*, Staphylococcus aureus, Enterobacter* sp *and Aeromonas* sp. This agrees with the work of Funmilayo *et al.* (2021), who revealed the presence of microorganisms in packaged sachet water as a result of post contamination as some of the organisms are commensals in groundwater and are present in the environment; it also conforms to the work done by Daniel and Daodu (2016),

who isolated eight bacterial species (*Staphylococcus aureus, Pseudomonas* sp.*, Aeromonas* sp.*, Corynebacterium* sp.*, Bacillus* sp.*, Bacillus badius, Proteus vulgaris* and *Escherichia coli*) from sachet water.

The antimicrobial investigation of *Hunteria umbellata* (osu) seed extract showed little or no zones of inhibition against the various isolates indicating its non-effectiveness against these isolates. This result, although, does not correlate with work done by Udinyiwe *et al.* (2017), who reported an MIC/MBC of 15mg/mL and 50mg/mL for *Hunteria umbellata* seed extract bacteriostatic and bactericidal effect against *Staphylococcus aureus*, *Micrococcus* sp., *Bacillus* sp., *Proteus* sp. and *Streptococcus pneumoniae*. The variations in the diameter for zone of inhibition for plant extracts might be due to the alterations in the chemical composition of their extracts (Issah *et al.,* 2020).

Both *Hunteria umbellata* (osu) epicarp and bitter kola (*Garcinia kola*) revealed that the extract was effective against the various bacterial species isolated, especially *Staphylococcus aureus, Bacillus* sp. and *Aeromonas* sp. There was no zone of inhibition seen for *Pseudomonas sp.* This agrees with the findings of Ogu *et al.* (2017), who recorded high resistance of *Pseudomonas aeruginosa* to various antibiotics such as Norfloxacin (NF), Amoxicillin (AX), Cafuroxime (CF) and Ampicillin (AM). Gram-negative bacteria are known to be more resistant to regular antibiotics, especially some nosocomial strains such as *Acinetobacter baumannii, P. aeruginosa* and *Klebsiella pneumonia*, due to the presence of a peptidoglycan layer (Li *et al.,* 2006; Abdallah *et al.,* 2015).

The MIC/MBC of bitter kola (*Garcinia kola)* extract revealed a value of 12.5-50 mg/mL and 12.5-100 mg/mL, respectively, and that of *Hunteria umbellata* (osu) epicarp extract recorded for the various extract was between 6.25-50 mg/mL and 6.25-100mg/mL, which correlates with the work done by Anibijuwon *et al.* (2011) who reported a range of 20 to 100 mg (MIC/MBC) at treatment with *Hunteria umbellata* fruit extract on bacterial isolates.

Conclusion

The antimicrobial potential of *Hunteria umbellata* (osu epicarp) and *Garcinia kola* (bitter kola) extract has been demonstrated in this study. Both extracts have the potential as natural therapeutic agent against four out of the five isolates investigated with greatest impact on *Bacillus* sp., *S. aureus and Enterobacter* sp. The effect of *H. umbellata* epicarp extract against both Gram Positive and Gram Negative bacteria indicates it will be a useful tool for drug synthesis with a broad spectrum of activity with the possibility of developing therapeutic substances which will be active against multi-drug resistant organisms.

The health risk of drinking contaminated water cannot be overemphasized, hence regular water quality monitoring, combined with community-led intervention with a focus on sanitation, hygiene education, better source water protection strategies and source water treatment is recommended to avoid contamination of pathogenic bacterial isolates in consumed sachet water.

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