

Antimicrobial Activity of Some Seed Extracts on Bacteria Isolated from Maize Slurry (*Akamu*) in Port Harcourt Metropolis

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Abstract

Minimal attention has been given to the impact of herbal extracts on food-borne organisms. The aim of this study, therefore, was to ascertain the health hazards associated with the consumption of maize slurry (*Akamu*) as a food diet and the susceptibility of microorganisms isolated from *Akamu* to some seed extracts. The antimicrobial activity of aqueous (cold and hot) and ethanol extracts of *Garcinia kola* (Bitter kola), *Cola nitida* (kola nut) and *Persea americana* (Avocado) on bacteria isolated from *Akamu* was investigated using standard microbiological techniques. Agar well diffusion technique was used to determine the antimicrobial sensitivity and the minimum inhibitory concentration (MIC) of the seed extracts on the bacterial isolates. The results of bacterial counts in the *akamu* ranged from 1.1×10^3 cfu/g to 1.4×10^3 cfu/g; the bacteria isolated from the *Akamu* were *Staphylococcus aureus*, *Enterobacter aerogenes* and *Klebsiella* sp. All the isolates were susceptible to ethanol extract of bitter kola with MIC values of 0.005 µg/ml. Only *Enterobacter aerogenes* was susceptible to ethanol extract of kola nut with MIC value of 0.005 µg/ml. Pairwise comparisons of the different seed extracts at $p > 0.05$ showed that there was no significant difference among the seed extracts. Ethanol extract of bitter kola was the most active against the test organisms with mean zones of inhibition of 15.33mm. It can be inferred thus that seeds of bitter kola, kola nut and avocado have antimicrobial activities with the type of extracting solvent playing a major role in their level of antimicrobial activity. This implies that an antimicrobial seed or herb extract should be prepared in the most suitable solvent that could elicit its highest efficacy.

Keywords: Maize slurry, bacteria, seed extracts, antimicrobial activity, efficacy.

Introduction

Any substance that destroys or inhibits the growth of microorganisms is said to have antimicrobial activity, while the extent to which such lethal or inhibitory activity occurs is referred to as the efficacy of the antimicrobial agent (Roto *et al.*, 2015). Antimicrobial agents comprise of antibiotics, chemotherapeutic substances and herbal extracts. Antibiotics are produced from microorganisms and inhibit their growth. Antibiotics are used to treat infections caused by bacteria and to prevent bacterial

infection. Chemotherapeutic agents are compounds synthesized from elementary substances that are used in the treatment of diseases (Roto *et al.*, 2015). Herbal extracts are mostly lotions, teas, juices, tinctures or other liquid preparations derived from plant sources for treatment of diseases and infections (WHO, 2016).

Great impacts have been made by the application of herbs in the treatment of ailments and infections in Africa and other continents (Thomford *et al.*, 2015). Apparently, the abundance of antimicrobial agents from herbs in Nigeria is quite vast and adequate, while they are often not dangerous and potentially life-threatening (Thomford *et al.*, 2015). Thus, scientists have attracted attention to herbs and now undertake researches to highlight the authenticity and efficacy of herbs as antimicrobial agents against a vast range of bacteria (Mahmood and Zafar, 2013).

From ancient periods, plants have been a source of real and presumptuous means of healing to man (Gardner, 2013). Plants in the form of fruits, twigs, barks, roots, seeds and leaves, have been useful in the production of portions that are very potent in the treatment of different human illnesses (Gami *et al.*, 2012). The effectiveness of plants in medicine has gained widespread acceptance in Africa, Asia and in the United States of America. Most of the self-treatments are for presumed or actual infectious processes such as colds and flu (Etang, 1999). There are various options available to the physician who wishes to administer herbal products for microbial infections (Etang, 1999).

In China, primary health care depends on herbs, and treatment of diseases with herbs is considered as important as treatment with synthetic drugs (Okigbo and Ajalie, 2007). Herbal treatment accounts for 30 - 50 percent of total consumption of medications. In Nigeria and in Niger Delta in particular, traditional medicine plays a vital role in the health care delivery system (Etang, 1999). In most Nigerian communities, health-care delivery depends to a large extent on traditional medicine brought to us by the availability of herbs (Ayodele, 1983). The study of higher plants for the purpose of detecting antimicrobial agents in their tissues is of comparatively recent origin (Okigbo and Ajalie, 2007).

Nigeria is naturally endowed with a lot of plant resources. This has greatly encouraged researchers to delve into undertaking studies in order to make these plants/herbs with unknown economic importance to be useful for the benefit of mankind, especially as medicines for the treatment of diseases (Okigbo and Ajalie, 2007).

This research is justified because of: increasing threat of bacterial infections to life; increase in the cost and allergic reaction of synthetic drugs; occurrence of resistant strains of bacteria because of misuse of synthetic drugs, and existence of fake and adulterated drugs in our markets today (Obire *et al.*, 2009). Also, bulk of the studies on microorganisms associated with food handling or processing has been more concentrated on the organisms present in the food. Minimal attention has been given to the impact of herbal extracts on the organisms.

As bacterial infections pose more threat to life, research scientists all over the world have continued to increase their quest for solutions to such problems. A problem worthy of mention is that antibiotics and chemotherapeutic agents are today taken with extra caution due to a variety of reasons. Problem of drug resistance to antibiotics has led to the decline in the therapeutic value of most antibiotics in use today (Etang, 1999; Obire *et al.*, 2009). And considering the high cost of antibiotics

and chemotherapeutic agents, there is a need for complementary and alternative medicine (CAM) (Chen, 2004). For these reasons, herbal treatment for bacterial infections have gained acceptance in this part of the world in recent times. Thus, it is still true as herbalists continue to boast about their ability to cure any kind of bacterial infections with herbs.

In Nigeria, consumption of maize slurry popularly known as “akamu” has gained wide acceptance as a form of diet. Nursing mothers use akamu as weaning food for new-borne babies and infants (Obire and Amadi, 2015). The process of preparing *akamu* from raw maize to fermentation stage up to the stage of turning it into food diet could introduce microbes into it. Despite wide use of *akamu* as food diet, information on the microbiological quality and activity of the organisms associated with it are still scarce (Obire and Amadi, 2015).

The objectives of this study, therefore, were: To isolate and identify bacteria and fungi associated with maize slurry (akamu); to determine the efficacy by means of Minimal Inhibitory Concentration of *Garcinia kola*, *Persea americana* and *Cola nitida* seed extracts on the isolated organisms *in vitro*; to determine the extraction capacity of different solvents (hot water, cold water and local gin, “*kaikai*”) by means of percentage yield and to determine the antimicrobial activity of the different seeds extract on the test organisms.

Materials and Methods

Collection of Maize Slurry “Akamu” and Seed Samples

Samples of freshly prepared maize slurry (*Akamu*) were purchased from three locations (Borokiri, Mile 3 and Eagle Island) in Port Harcourt metropolis and immediately taken to the laboratory for analysis. Sample A was purchased directly from local producers of *Akamu* in New Road of Borokiri area. Sample B was purchased from sellers in Mile 3 Market in Diobu area. Sample C was purchased from sellers in Eagle Island area. Samples were collected weekly for a period of three weeks.

The seeds of *Garcinia kola* (Bitter kola), *Cola nitida* (Kola nut) and fruits of *Persea Americana* (Avocado) used in this study were purchased from the Fruit Garden Market located in D line, Port Harcourt, during August and September, 2015. Materials purchased were immediately taken to the laboratory where the seeds of avocado pear were then removed from the pulp. All the seeds of *Garcinia kola* (Bitter kola), *Cola nitida* (Kola nut) and *Persea americana* (Avocado) were properly identified by Professor Edith Chuku of the Botany unit in the Department of Applied and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt.

Microbiological Analysis of Maize Slurry

Cultivation, Isolation and Enumeration of Bacteria in maize slurry

Isolation of bacteria and fungi in the Maize slurry (*Akamu*) were carried out using the spread plate method. Serial dilution was done on each sample of maize slurry (*Akamu*). One gram (1.0g) each of the maize slurry (*Akamu*) samples was added to

separate 9.0ml of sterile normal saline (diluent) and further dilutions were made up to 10^{-3} according to the method of Obire and Amadi (2015).

Aliquot (0.1ml) of 10^{-3} dilution of the sample was then inoculated onto nutrient agar plates for the isolation of bacteria. The spread plate method was done using sterile bent glass spreader to spread the sample evenly on the agar plates. Plates were inoculated in duplicates, and incubated at 37°C for 24 hours for bacteria according to the method of Obire and Amadi (2015). Colonies that developed after incubation were counted and the average counts for the duplicate plates were recorded as total viable bacteria enumerated.

Selection of Bacteria Isolates

Pure cultures of bacteria were obtained by aseptically streaking representative colonies of different morphological types which appeared on the cultured plates onto freshly prepared nutrient agar plates and were further incubated at 28°C for 24 hours. Pure cultures were transferred onto the surface of sterile agar slants in McCartney bottles and incubated at 28°C for 24 hours. Weekly working cultures were usually 24 hours old cultures plated out on nutrient agar from the frozen sterile agar slants in McCartney bottles that were left on the laboratory bench to warm up as to activate the bacterial cells before being plated out. These agar slant preparations served as stock cultures for long-term storage and a source for weekly working cultures.

Determination of Morphological and Biochemical Characteristics

After incubation, the pure cultures were stored in the refrigerator at 4°C and served as pure stock cultures used for subsequent characterization tests. For the purpose of identification of bacteria, the following characterization tests were performed: Gram staining, motility test, catalase test, coagulase test, sugar fermentation test, methyl red test, indole test and starch hydrolysis. The isolates were identified on the basis of their cultural, morphological and biochemical characteristics in accordance with methods described by Cruickshank *et al.*, (1975) and with reference to Holt (1977).

Processing of Seed Samples (Preparation of Macerate and Pulverization of Dry Seeds)

Each of the seeds of *Garcinia kola* (Bitter kola), *Cola nitida* (Kola nut) and *Persea Americana* (Avocado) were separately chopped into lumps, dried in an oven at 50°C and subsequently ground into powder using a pre-cleaned mortar (thoroughly washed with detergent and rinsed with 95% alcohol), and were kept wrapped in filter paper cartridges according to the method of Okigbo and Ajalie (2005). The powdered form of each seed was then soaked in 500ml of the different solvents (cold aqua, hot aqua, and ethanol) for 48 hours.

Batch-wise, 50g of seeds (Balance used: Digital Scout Pro balance (Model SPU601) was pulverized using a sterile mortar to obtain a powdery sample. The dry seed powder was sieved with a hand sieve following the method of Okigbo and Ajalie (2005). The dried seed extracts obtained from the above procedure were clas-

sified as cold aqueous extract (CAE), hot aqueous extract (HAE), and ethanol extract (EE).

Tests for Sterility of Extracts

Each of the extracts was streaked onto the surface of nutrient agar plates and incubated at room temperature for 48hrs to observe for growth of bacteria. No growth of organisms for 48 hours was considered as meeting the condition for sterility of extract (Nester *et al.*, 2008).

Drying of Seed Extract into Concentrate and Preparation of Various Concentrations from the Concentrates Obtained

The crude extracts (filtrate) were filtered using Whatman no 1 filter paper. The supernatant was discarded and residue was put in 100 ml beaker and later transferred to an evaporator where the aqueous solvent was evaporated at low temperature to obtain constant weight of powder (Flores *et al.*, 2009). The standard extract powder (concentrate) which was obtained in the process was stored in a refrigerator at 4°C until required for use. The extract obtained was dispensed into universal bottles as two sets of cold and hot aqueous extracts, except the extract of ethanol. The hot aqueous extract was obtained by autoclaving at 121°C for 15 minutes. The yields of the extracts; cold aqueous extract (CAE), hot aqueous extract (HAE) and ethanol extract (EE) were recovered and calculated as percentage of the quantity of initial pulverized or macerated plant materials following the procedure of Okigbo and Igwe (2007).

To obtain various concentrations the concentrate of each extract was separately diluted into five concentrations (0.25g/ml to 0.01µg/ml) using the two-fold dilution method. A twofold dilution reduces the concentration of a solution by a factor of two. That is, it reduces the original concentration by one half. 0.50g of the concentrate was dissolved in 2ml of the diluents and subsequently 1ml was pipette into tubes already containing 1ml of the diluents according to the method of Okigbo and Ajalie (2005).

Determination of Antimicrobial Activity of Various Concentrations of Seed Extracts and Application of Controls (Negative and Positive Controls)

Agar well diffusion and susceptibility testing was carried out by employing the method of Borgio *et al.*, (2008). Sterile cotton swab was taken and dipped into 24 hours old culture of each test organism. The entire surface of the nutrient agar (Fluka Biochemical, Germany) was seeded, first horizontally and vertically to ensure even distribution of organism over the agar surface using the above swab. The seeded agar surface was allowed to dry for 5 to 10 minutes. The tip of a 16mm customized well cutter was sterilized by heating on Bunsen burner flame and used for well preparation after seeding the nutrient agar plates with the test organisms. Four (4) wells were prepared in each plate (as four replicates of the same test). As soon as the wells were prepared, 1ml of reformulated plant extract (using the initial solvent of

extraction) was poured into each well using sterile micro-tip. The same procedure was followed for all test bacteria following the method of Borgio *et al.*, (2008).

The following were used as negative controls: Cold Distilled Water (CTRL 1), Hot Distilled Water (CTRL 2), and locally produced alcohol (CTRL 3). Four agar wells of 16 mm were bored on each Petri dish, using a 16mm well borer. Each dish was impregnated with a test organism and aliquots of 1ml of control solvent was poured into the agar wells after seeding the test organism following the method of Borgio *et al.*, (2008). The antimicrobial agent (tetracycline (250mg/ml) (CTRL 4) used as a positive control. It was poured into prepared agar wells on nutrient agar plates. All these controls served as checks in the experiment.

The cultured plates of test organisms and set-up of the controls were incubated at 37°C for 24 to 48 hours. After incubation, the zone of inhibition was measured. The results of sensitivity tests were used as basis for estimating activity levels of the extracts. The standard zones of inhibition for tetracycline according to Talaro (2008) and Cheesbrough (2010) was used to determine whether any of the test bacteria was sensitive, intermediate or resistant with respect to the antimicrobials (seed extracts) that they were tested against.

Determination of Minimal Inhibitory Concentration (MIC)

Minimal Inhibitory Concentrations (MIC) of the crude extracts on the test organisms was determined by using agar well diffusion technique. In the well diffusion tests, three (3) sets of five agar plates containing crude extracts of variably formulated concentrations of 0.125g/ml to 0.005µg/ml were used. The different concentrations were derived by double fold dilution. Each set of agar plates was inoculated with one of the test organisms to determine the minimal inhibitory concentration. The lowest concentrations of the crude extracts resulting in no growth after 24 hours' incubation at 37°C was considered as the MIC (Prescott *et al.*, 2008).

Calculation of Percent (%) Activity Index of the Seed Extract

The zone of inhibition in values of extracts and those of standard antimicrobial agents were used to calculate the activity index of the extracts according to the formula of Borgio *et al.*, (2008).

$$\text{Activity Index (Ai)} = \frac{\text{Zone of Inhibition by Extract}}{\text{Zone of Inhibition by Standard Antimicrobial Agent}}$$

$$\% \text{ Activity Index} = \text{Activity Index} \times 100$$

Data Analysis

Completely randomized design (CRD) comprising of three treatments (extracts) groups, each group of extract obtained from three seeds were considered.

The data obtained from the experiments were structurally arranged in Microsoft Excel 2007 and analyzed using SPSS for Windows Version 20.0. Analysis of Variance (ANOVA) and Scheffe's post hoc Test for pairwise comparison was used to

test for significance. Graphical presentation was done on some relevant aspects of data generated using MS Excel 2007.

Results

The results of bacterial count in maize slurry samples ranged from 1.2×10^3 cfu/gm to 1.4×10^3 cfu/gm with a mean value of 1.3×10^3 cfu/gm for Borokiri samples; from 1.1×10^3 cfu/gm to 1.2×10^3 cfu/gm with a mean value of 1.17×10^3 cfu/gm for Eagle Island samples; from 1.1×10^3 cfu/gm to 1.2×10^3 cfu/gm with a mean value of 1.13×10^3 cfu/gm for Mile 3 market samples. The population of bacteria was higher in the Borokiri samples than in the Eagle Island and Mile 3 samples.

The bacteria that were isolated from the maize slurry were as *Staphylococcus aureus*, *Enterobacter aerogenes* and *Klebsiella* sp. Subsequent tests were carried out using these bacteria owing to literature evidence describing them as pathogens.

Results of the antimicrobial sensitivity showed that all the seed extracts showed some level of antimicrobial activity against the bacterial isolates. CTRL1 (cold distilled water) and CTRL2 (hot distilled water) did not demonstrate any activity against the test organisms.

Comparison of the bacteria with respect to mean inhibition zone showed that *Klebsiella* species and *Enterobacter aerogenes* were sensitive to the action of the extracts than *Staphylococcus aureus* which exhibited resistance to the extracts. *Enterobacter aerogenes* was the most sensitive.

The amount of concentrate obtained from each crude extract of 50g of dried pulverized seed is presented in Table 1. Ethanolic extract of Bitter Kola gave the highest yield of 4.95g, representing 9.9% of the weight of the original pulverized sample used, while the least yield of 2.7g, representing 5.4% was recorded with the cold aqueous extract of Bitter Kola. Hot aqueous extract of Avocado produced yield of 4.9g (9.8%) which is next in quantity to ethanol extract of Bitter Kola.

Table 1: Percentage yield of crude extracts of 50g of pulverized/macerated plant seed.

Plant Seed	Solvent Extraction	of Colour of Extract	Weight of ex- tract (g)	Yield of extract (%)
Bitter Kola	Cold aqua	Pale milkish	3.7	7.4
	Hot aqua	Pale milkish	4.85	9.7
	Ethanol	Dark milkish	4.95	9.9
Kola nut	Cold aqua	Light brown	2.7	5.4
	Hot aqua	Light brown	3.9	7.8
	Ethanol	Dark brown	4.7	9.4
Avocado	Cold aqua	Light Brown	4.75	9.5
	Hot aqua	Brown	4.9	9.8
	Ethanol	Dark Brown	4.70	9.4

The zone of inhibition of 0.125 μ g/ml concentration which represents the highest concentration of the seed extracts on the test bacteria are presented in Table 2. These figures were used to compare with those of standard antimicrobials to determine whether an organism is resistant, susceptible or intermediate. It shows Ethanol extract elicited the highest zones of inhibition.

Table 2: Zone of inhibition (mm) at 0.125 μ g/ml dilution of the various extracts of bitter kola, kola nut and avocado seeds on the test bacteria

Bacterium	Extract	Bitter kola	Kola nut	Avocado
<i>E. aerogenes</i>	HA	13	12	12
<i>E. aerogenes</i>	CA	12	10	9
<i>E. aerogenes</i>	EE	16*	15*	14
<i>S. aureus</i>	HA	14	12	13
<i>S. aureus</i>	CA	12	8	10
<i>S. aureus</i>	EE	16*	14	14
<i>Klebsiella</i> sp	HA	13	11	13
<i>Klebsiella</i> sp	CA	10	9	11
<i>Klebsiella</i> sp	EE	15*	12	14

Key: HA –Hot Aqua, CA –Cold Aqua, EE –Ethanol Extract; *=Susceptible

The minimal inhibitory concentration (MIC) of the different seed extracts on the test bacteria are presented in Table 3. It shows Ethanol extract elicited the lowest MIC.

Table 3: The minimal inhibitory concentration (MIC) of the various seed extracts on the test bacteria

Bacterium	Extract	Bitter kola	Kola nut	Avocado
<i>E. aerogenes</i>	HA	0.02	0.02	0.02
<i>E. aerogenes</i>	CA	0.08	0.08	0.08
<i>E. aerogenes</i>	EE	0.005	0.005	0.005
				0.02
<i>S. aureus</i>	HA	0.02	0.02	0.02
<i>S. aureus</i>	CA	0.08	0.08	0.08
<i>S. aureus</i>	EE	0.005	0.005	0.005
<i>Klebsiella</i> sp	HA	0.02	0.02	0.02
<i>Klebsiella</i> sp	CA	0.08	0.032	0.08
<i>Klebsiella</i> sp	EE	0.005	0.02	0.02

Key: HA –Hot Aqueous, CA –Cold Aqueous, EE –Ethanol Extract

The percentage (%) activity index for different extracts of the various seeds on bacterial isolates is presented in Figure 1 below. The ethanol seed extracts had the highest % activity index while the cold aqua seed extracts had the lowest % activity index.

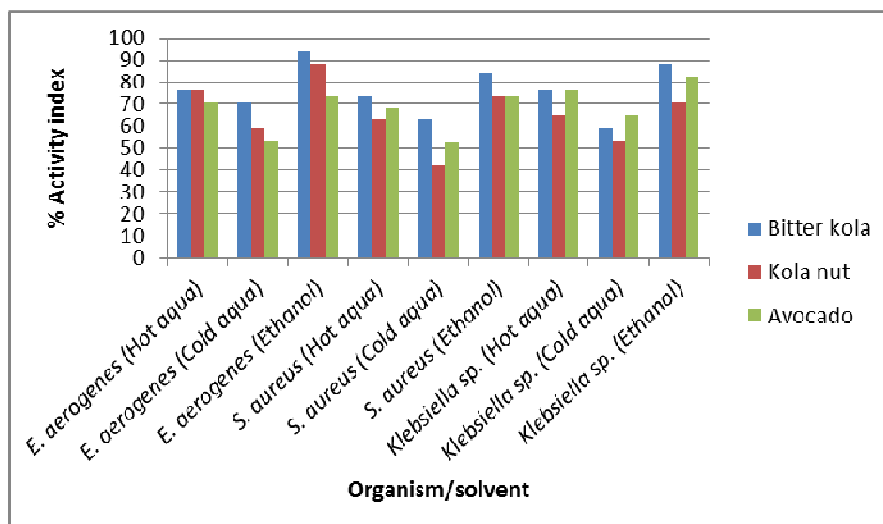


Fig. 1: Percentage (%) activity index for different extracts of seeds on bacteria

The Percent (%) susceptibility of isolated bacteria to the various seed extracts is presented in Table 4.

Table 4: Percent (%) susceptibility of the various seed extracts

Organisms	% Susceptibility		
	Bitter Kola	Kola nut	Avocado
<i>E. aerogenes</i>	66.7	66.7	33.3
<i>S. aureus</i>	66.7	33.3	33.3
<i>Klebsiella sp.</i>	66.7	0	66.7

Discussion

This present study has revealed efficacy of the extracts of seeds of *Garcinia kola* (Bitter kola), *Cola nitida* (kola nut) and *Persea americana* (Avocado) on bacteria isolated from *Akamu* and has contributed in some ways to the knowledge of the science of antimicrobial herbs which include that these seeds which have no reports of allergy, are readily available and cheap, have much more value than just food in the treatment of infection. Also, that the extracting solvent is an important factor in determining the extent of antimicrobial activity of most seed extracts; and primarily, the extracts of these seeds possessed antimicrobial properties. This finding agreed with that of Okeke *et al.*, (1999).

The bacteria isolated in this study were *Staphylococcus aureus*, *Enterobacter aerogenes*, and *Klebsiella* sp. *Staphylococcus aureus* was more susceptible to ethanol extract of bitter kola. Whereas, the same organism was less susceptible to cold aqueous extract of the same seed with a higher MIC. This implies that, antimicrobial effects vary among the different types of extracts of the same seed or plant Okigbo and Ajalie (2005). The variation in the effect of extract type on test organisms was also reported by Okeke *et al.*, (2001), who reported that hot water extracts contain higher amount of plant, with an implied higher activity against bacteria. Iloki-Assanga *et al.*, (2015) reported that hot water extract has high activity due to higher extractability of the active agents in hot water, which in turn, affects its concentration per unit volume of the extract. This implies a seed or herb even if it is known to possess some antimicrobial properties should be prepared in the most suitable extract type of the plant that could elicit its highest efficacy.

The study also revealed, as a corollary with the above findings, that certain extract types may not be cost effective with respect to their efficacy on certain test organisms. For instance, ethanol extract of Avocado seed did not show much activity against *Klebsiella* species than hot aqueous extract of the same seed. Thus, procuring local ethanol at a more expensive rate to prepare such an extract for therapeutic use on patients, as may be done by tradomedical practitioners would not be necessary.

With respect to the seeds in general, bitter kola showed more efficacy than other seeds when applied using its most suitable extract in a given treatment. Bitter kola was most active in dry hot aqueous and ethanol extracts. *Enterobacter aerogenes* and *Klebsiella* species were less resistant to hot aqueous extracts of bitter kola whereas, *Staphylococcus aureus* was more resistant to dry hot aqueous extracts of bitter kola but less resistant to ethanol extract of the same seed. The higher activity of ethanol extracts may be due to synergistic effect with the phytochemical properties of the extracts while the activity of hot water extract may be due to the high extractability (Iloki-Assanga *et al.*, 2015).

Statistical analysis showed that there was no significant difference at $p = 0.05$ on the zones of inhibition of the three extracts on bacteria. However, Bitter kola extract had the highest mean and would be the most potent in treating bacterial infection while kola nut was the least active on bacterial isolates. Nonetheless, it is statistically backed-up fact that Bitter kola is very potent in treatment of bacterial infections since it maintained the highest zone of inhibition.

Staphylococcus aureus although not always pathogenic has been implicated as the causal agent of a range of illnesses from minor skin infections such as pimples, impetigo, boils, folliculitis, scaled skin syndrome and abscesses to more serious, life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, and toxic shock syndrome (Ansari *et al.*, 2016). Long term carriers of *S. aureus* of human population are estimated to be around 20% which includes normal skin flora and in the nostrils (Brown *et al.*, 2014). *S. aureus* is a normal inhabitant of the lower reproductive tract of women (Rampersaud *et al.*, 2011). *Enterobacter aerogenes* is also a pathogenic and opportunistic bacterium and have also been implicated in infections resulting from specific antibiotic treatments, venous catheter insertions and/or surgical procedures. *E. aerogenes* is not pathogenic in healthy individuals. Its habitat includes various wastes, hygienic chemicals and soil (Sanders, 1997). *Klebsiella* species are routinely found in the human nose, mouth, gastrointestinal

tract as normal flora, however they have opportunistic features (Martin *et al.*, 2014). *Klebsiella* organisms have been implicated in a wide range of disease conditions notably pneumonia, urinary tract infections, meningitis and diarrhea (Alshammari *et al.*, 2016). The use of herbal medication which is a cheap alternative to chemotherapeutic agents will sure assist the general public in the reduction in the cost of treatment of these infections.

Conclusion

This study has shown that seed extracts of bitter kola, kola nut and avocado have antimicrobial activities, with varying efficacies against certain bacteria. It is established in this study that when considering treatment of bacterial infections with these seed extracts, the type of extracting solvent is important because the same seed may exhibit different levels of efficacy with respect to the type of solvent used. The study has also revealed that the hygiene level especially for those who produce and market maize slurry (akamu) has improved considering the number of organisms isolated, but the need to upgrade the handling and processing methods cannot be over emphasized. This is so, owing to the pathogenic species of bacteria that were isolated from the product. The result of this research has further equipped policy makers to make policies that will enhance food safety and by extension the public health of the general population.

Recommendations

Public health institutions and agencies should effectively monitor the sanitary conditions of the environment where the maize slurry is produced and marketed to ensure they comply with acceptable standards. Determination of actual oral or prenatal doses needs to be carried out by performing bioassays of any extract to be used therapeutically. In attempting to use any seed or its extract, the practitioner should endeavor to determine and use the most suitable extract type, since not all extract of the same seed yields the same result in every situation.

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