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Research Paper

Antimicrobial Analysis of Selected Soft Drinks (Kunun, Zobo, and Brukutu) and Their Antimicrobial Susceptibility Pattern in Lagos, Southwestern, Nigeria.

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ABSTRACT: *The result from the study showed that total bacterial count ranged from 1.3×10^5Cfu/ml to $6.2x10^5$ cfu/ml. The total fungal count ranged from $1.9x10^5$Cfu/ml to $7.4x10^5$Cfu/ml in which sample 11 had the highest count.The samples were microbiologically analysed using standard methods. pH was recorded and the values obtained ranged from 1.40 - 6.40. The Prevalence frequency of the bacterial isolates found present in the screened soft drink samples wasS. aureus (26%), Streptococcus spp. (21%), E. coli (19%), Bacillus subtilis (18%) and Proteus spp. (7%). The isolated fungi were Candida spp. (42%), Aspergillus flavus (35%), A. niger (7%) and Rhizopus spp (16%). Antimicrobial susceptibility was carried subsequently and each result were observed. Staphylococcusaureus exhibited a very high sensitivity to Ampliclox (AMP) with a percentage total of 90%, Bacillus spp.,however, was observed to exhibit a 100% sensitivity to Ciprofloxacin. The isolated fungi were subjected to Antifungal sensitivity test using Griseofulvin 250mg/ml and 500mg/ml respectively and also Ketoconazole 200mg/ml and 400mg/ml.*

KEYWORDS: Zobo, Kunu, Burukutu, Lagos, Antibiotic susceptibility.

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I. INTRODUCTION

Food are classified based on readiness to consumption including fully processed Ready-to-eat such as akara, fried groundnut, rice and bean, fura, bread; and minimally processed ready-to-eat such as tomato, cabbage, ginger drink, lettuce, carrot, kunu zaki and zobo drink [1]. Most snacks are purchased to quench hunger and or thirst. They are sometimes used as an appetizer. Some ready-to-eat foods such as fruit juice are mostly consumed due to their refreshing attributes, nutritive values and health benefits [2]. Fruits are potential source of nutrients, micronutrients, vitamins and fibre for humans. These fruits include watermelon, paw-paw, and pineapples. Some of these fruits are processed into juice/wine such as pineapple juice, watermelon and paw-paw blend orange, apple, pineapple. Other essential nutritional drinks consumed by a large number of the populace in Nigeria is Zobo [2,3].

Zobo drinks are traditional non-alcoholic beverage which is consumed in most parts of Nigeria. It is used as refreshment, entertainment as well as an appetizer before meals are served. It is a reddish liquid drink and taste like fruit punch, served as fair source of vitamin A, Riboflavin, Niacin, calcium and iron; it is also low in sugar content. This drink also contains anthocyanin and vitamin C, among others and it is used in curing minor stomach ailments, sore throat and strengthen the heart among other uses [4]. Due to ease of production and availability of raw material especially H. sabdariffa, they are source of livelihood to several families in both Northern and Southern Nigeria especially in the rural areas [5].

The quality of zobo drink depends mainly on the physicochemical constituents of the raw materials, water used in their production and the hygienic condition of the processors. Water is a major resource used in the production of these drink from their raw materials. Poor quality with regard to both physicochemical (colour, pH, turbidity, total suspended solids, total hardness, total alkalinity salinity, electrical conductivity), heavy metals (lead, cadmium, chromium, iron, zinc, copper, nickel, arsenic) and microbial (total heterotrophic

bacteria, total fungi, total coliform and faecal coliforms) could also impact on the overall quality of the drink [1].

The environment is which the drinks are processed could also influence the quality especially in the microbial perspectives. Zobo drink is a nutritional drink consumed by people in Nigeria. However, the consumption of local beverages could be a potential source of transfer of zoonotic and foodborne diseases including staphylococcus, Salmonellosis, Brucellosis, Tuberculosis, Shigellosis, Listeriosis, E. coli, infections etc. [2].

Kunun is a popular cereal based, non-alcoholic beverage. It is a popular local or indigenous drink consumed throughout Nigeria, mostly in the north for its thirst-quenching properties. Kunun-zaki is prepared from either guinea corn (*Sorghum bicolor*), millet (*Penisetum typhoides*), maize (*Zea mays*) rice (*Oryza sativa*) or wheat (*Triticum aessstivum)* [5]. Traditionally, the production involves steeping of the whole grains for 6-24 hours, wet milling with spices and sweet potato, gelling of about three-quarter (3/4) of the mixture in hot water, pitching with about one- quarter fresh part of the mixture and then allowing to ferment overnight and the supernatant is ready for consumption [3].

Burukutu is a rich non-alcoholic beverage characterized with vinegar-like flavour and a cloudy suspension, it is produced mainly from the grains of either *Sorghumbicolor* or *S. vulgare*[6]. The grains of *Sorghumbicolor* or *S. vulgare* serves as a staple food for the poor and less privileged individuals from the developingcountries in which Nigeria is one and it is constituted by energy and protein rich compounds. The major problem associated with the traditional production of Burukutu includes non-availability of potable water, most often local brewers depends on untreated water supplied by hawkers and such water could be a potential vehicle for the spread and contamination of the brew with pathogenic microorganisms [4].

The current food safety challenges have risen slowly over several years and require strategic efforts to be controlled. In a developing nation like Nigeria, it has not been possible to have control over the processing of hawked foods, because most of the vendors lack the adequate knowledge of food processing and handling practices and this might likely lead to a high risk of chemical and microbial contamination. In most Nigeria cities, the sales and consumption of this locally made beverage is high due to the high cost of other non-alcoholic drinks. These drinks are usually hawked in the motor parks, school premises and market places [7].

II. MATERIALS AND METHODS

2.1 COLLECTION OF SAMPLES

Hawked Burukutu, Kunun and Zobo drinks samples were collected by purchasing randomly from five different locationsalong Ojo Local Government Area. Ojo is located on the eastern section of the Tran-West Coastal Highway, it lies on the Latitude $6^0$27'N and Longitude 3012'E. Samples were collected at different locations of the local government to ensure randomization. The samples were collected in 200mL sterile plastic bottles and immediately transferred to the microbiology laboratory of the Nigerian institute of medical research., in iced parked cooler for microbial analysis

2.2 STORAGE OF SAMPLES

The samples with the spices were stored at refrigeration temperature prior culturing.

2.4 SERIAL DILUTION

Distilled water was used for serial dilution, 9ml each was pipette into a sterile screw cap test tube, the distilled water in the test tube was autoclaved at 121°C for 15 minutes and allowed to cool temperature of 45°C before serial dilution. In each case of dilution, 1ml of Zobo drink was pipette into 9ml tube. The process continues until dilution up to10^{-2} of the dilution factor was made inoculated into well labelled petri dishes.

2.5 BACTERIAL ENUMERATION AND ISOLATION

Nutrient agar medium was used for the enumeration of bacteria in the samples. The total bacteria count was obtained by incubation aerobically at 37℃ for 24hours. Plate count agar was used for the enumeration of total bacterial count (CFU/ml). Plates containing Nutrient Agar and Plate count Agar were incubated for 24 hr at 37℃, while Potatoes dextrose agar (PDA) plates were incubated at 30℃ for 96hours. Pure isolates of bacterial and fungal isolates were obtained and stored onto Nutrient Agar and PDA respectively.

2.6 CHARACTERISATION AND IDENTIFICATION OF BACTERIAL AND FUNGAL ISOLATES

Pure culture of bacterial isolates was identified on the basis of their morphology and biochemical characteristics. The organisms were subsequently characterized according to the taxonomic scheme of Bergey's manual.

2.7 Antibiotic Sensitivity Test.

Antibiotic susceptibility tests on the bacterial isolates were carried out by the Kirby-Bauer method(Bauer *et al*., 1966), and the antibiotics tested included Penicillin (10µg), Amoxicillin (30µg), chloramphenicol (50µg), erythromycin (15µg), gentamicin (10µg), tetracycline (30µg), streptomycin (30µg), erythromycin (10µg), augmentin (30µg), tetracycline (30µg) and peflacin (30µg). Briefly, the test isolate was emulsified in peptone until the turbidity was similar to that 0.5% McFarland standard. A sterile cotton swab was dipped into the suspension and swabbed evenly across the entire surface of the agar plate in order to obtain a semi-confluent

growth. After incubation, the zones of inhibition around the antibiotic disc were measured and interpreted based on the breakpoint criteria of the clinical and laboratory institute (CLSI, 2017).

2.8 Determination of pH

The pH of the fresh home-made juice was determined by measuring 50ml of the fresh sample into 250ml beaker. Thereafter, a portable pH meter (HI 96107model) was inserted into it which was first calibrated using standard buffer solutions of pH 4.0 and 7.0. This procedure was repeated for the other samples of home-made juices.

III. RESULTS

Table 3.1 shows the cultural, morphological and biochemical characteristics of each bacterial isolates found present in all the selected soft drinks from different locations around Lagos, South Western, Nigeria. Also included is the probable organism which was thereafter confirmed by carrying out various biochemical tests.A total of Twelve (12) of the bacterial isolates grew as pink, non-mucoid colonies on MacConkey Agar and there was no blackening of the medium round the growth on Bile Esculin Agar. Biochemical tests showed that they were Gram positive, catalase negative, citrate negative, oxidase negative, urease negative, Voges Proskauer negative, non-motile and indole negative. Based on these characteristics, they are identified as Streptococci.A total of twelve (12) of the bacterial isolates grew as flat, dry, pink, non-mucoid colonies on MacConkey Agar and there was no blackening of the medium round the growth on Bile Esculin Agar. Biochemical tests showed that they were Gram negative, catalase positive, citrate negative, oxidase negative, urease negative, Voges Proskauer negative, motile and indole positive. Based on these characteristics, they are identified as *Escherichia coli.*A total of ten (10) of the bacterial isolates grew as small pink opaque colonies on MacConkey Agar and there was no blackening of the medium round the growth on Bile Esculin Agar. Biochemical tests showed that they were Gram positive, catalase positive, citrate positive, oxidase negative, urease positive, Voges Proskauer positive, non-motile and indole negative. Based on these characteristics, they are identified as Staphylococci.A total of eight (8) of the bacterial isolates grew as pale colonies with swarming and characteristic foul smell on MacConkey Agar and there was no blackening of the medium round the growth on Bile Esculin Agar. Biochemical tests showed that they were Gram negative, catalase positive, citrate positive, oxidase negative, urease positive, Voges Proskauer negative, motile and indole negative. Based on these characteristics, they are identified as *Proteus* spp.

Table 3.2 shows the microscopic and macroscopic characteristics of Fungi isolates present in the samples collected. The following fungi were found to be present; *Aspergillus niger, Aspergillus flavus, Rhizopus* spp. *and Candida* spp.

Figure 3.1: Prevalence frequency of the bacterial isolates found present in the screened soft drink samples was *S. aureus* (23%), Streptococcus spp. (23%), Enterococcus (20%), E. *coli* (17%), Bacillus *subtilis* (15%) and Proteus spp. (6%).Figure 3.2: The Prevalence frequency of the fungi isolates found present in the screened soft drink samples was Candida spp. (42%), *Aspergillus flavus* (35%), *A. niger* (7%) and Rhizopus spp (16%).Both bar chartsshow the diversity of microbial community found to be present in the selected soft drinks samples sold in different local government area of Lagos state.

Table 3.3 shows the pH of Selected Drink Samples Sold in Different Area of Lagos State.The samples were microbiologically analysed using standard methods. pH values ranged from 1.40 - 6.40. The highest

Table 3.4 shows the total microbial count of each samples. Sample 1 was observed to contain $4.8x10^5$ cfu/ml on nutrient agar and $1.9x10^5$ cfu/ml on Potato Dextrose Agar. Sample 8 which was obtained from kunu has the highest bacterial cfu of $6.2x10^5$ cfu/ml. Fungal count ranged from 1.9×10^6 to 7.4×10^6 CFU/ml. The highest colony forming unit of fungi on the hand was observed on Burukutu samples ($7.4x10^5$ cfu/ml).

Table 3.4 shows the antibiotic susceptibility test results for bacteria isolated from each selected soft drink. Antibiotic resistance phenotypes were tested based on the Kirby–Bauer method according to standard recommendations (National Committee for Clinical Laboratory Standards, 2003). Analysis of cross-resistance results revealed that most of the bacterial isolates were multi-drug resistant and exhibited six resistance patterns to commonly used antibiotics.

Table 3.5 shows the Antimicrobial susceptibility pattern of fungi was carried out using Griseofulvin and ketoconazole (both 250mg/ml and 400mg/ml) respectively.

Table 3.1: Cultural, Morphological and Biochemical Characteristics of Bacterial isolates present in each soft drink Samples.

S/N	COLONIAL CHARACTERISTICS	MICROSCOPIC CHARACTERISTICS	PROBABLE IDENTITY
1	Brownish coloured mycelium with dark spores, appeared golden on the reverse side.	Hyphae are septate and hyaline. Conidiophores are long, smooth and hyaline becoming darker at the apex	*Aspergillus niger*
2	Greenish-yellow, white edge, floccose texture, velvety, cream to yellowish on reverse.	Septate hyphae, glucose corridia, rough conidiophores.	*Aspergillus flavus*
3	Cream colour, raised, entire, smooth and butyrous colony.	Round oval cells, purple hyphae, septa appear as smaller round grape-liken clusters	*Candida* spp.
4	Dark coloured, Mucor-like colony	Filamentous, branching hyphae which lacked cross-walls.	*Rhizopus* spp.

Table 3.2: Microscopic and Macroscopic Characteristics of Fungi Isolates present in each drink samples.

Cultural characteristic	Gram reaction	Shape	Catalase test	Oxidase test	Indole test	Coagulase test	Motility test	Citrate	Urease activity	Probable identity
Circular, Smooth, yellow colony.	+ve	Cocci	+	_	_	+	_	_	_	*Staphylococcus aureus*
Slightly yellow	+ve	Rods in chains	+	+	-	-	+	+	-	*Bacillus subtilis*
Whitish-grey.	+ve	Cocci	-	-	+	-	-	-	+	*Streptococcus* spp.
Smooth. shiny/cream, swarming, entire.	-ve	Rods	+	-	-	-	+	-	+	*Proteus mirabilis*
Thick, smooth, slightly raised, mucoid, greyish white colony.	-ve	Rods	+	-	+	-	+	-	-	*Escherichia coli*
Pinhead, smooth, entire, circular, bright-Yellow colony	+ve	Cocci	+	-	-	-	-	-	+	*Micrococcus luteus*

Table 3.3: pH of Selected Drink Samples Sold in Different Area of Lagos State.

Sample code	pH
Zobo Samples	
Sample 1	*4.80*
Sample 2	*6.20*
Sample 3	*2.50*
Sample 4	*1.45*
Sample 5	*5.70*
Kunu Samples	
Sample 6	*5.60*
Sample 7	*1.40*
Sample 8	*6.00*
Sample 9	*6.30*
Sample 10	*5.50*
Burukutu Samples	
Sample 11	*6.40*
Sample 12	*5.50*
Sample 13	*1.90*
Sample 14	*3.20*
Sample 15	*5.00*

Table 3.4: Total Microbial Count of Zobo Drink Samples Sold in Different Area of Lagos State.

Sample code	Bacterial Isolate (Cfu/ml)	Fungi isolates (Cfu/ml)
Zobo Samples		
Sample 1	4.8×10^5	1.9×10^5
Sample 2	2.7×10^5	2.3×10^5
Sample 3	2.5×10^5	3.8×10^5
Sample 4	1.0×10^5	2.9×10^5
Sample 5	1.5×10^5	7.5×10^5
Kunu Samples		
Sample 6	4.1×10^5	3.3×10^5
Sample 7	2.9×10^5	5.1×10^5
Sample 8	6.2×10^5	4.8×10^5
Sample 9	1.5×10^5	3.8×10^5
Sample 10	3.8×10^5	3.8×10^5
Burukutu Samples		
Sample 11	3.6×10^5	4.2×10^5
Sample 12	5.5×10^5	5.1×10^5
Sample 13	2.5×10^5	7.4×10^5
Sample 14	1.7×10^5	4.2×10^5
Sample 15	2.2×10^5	3.9×10^5

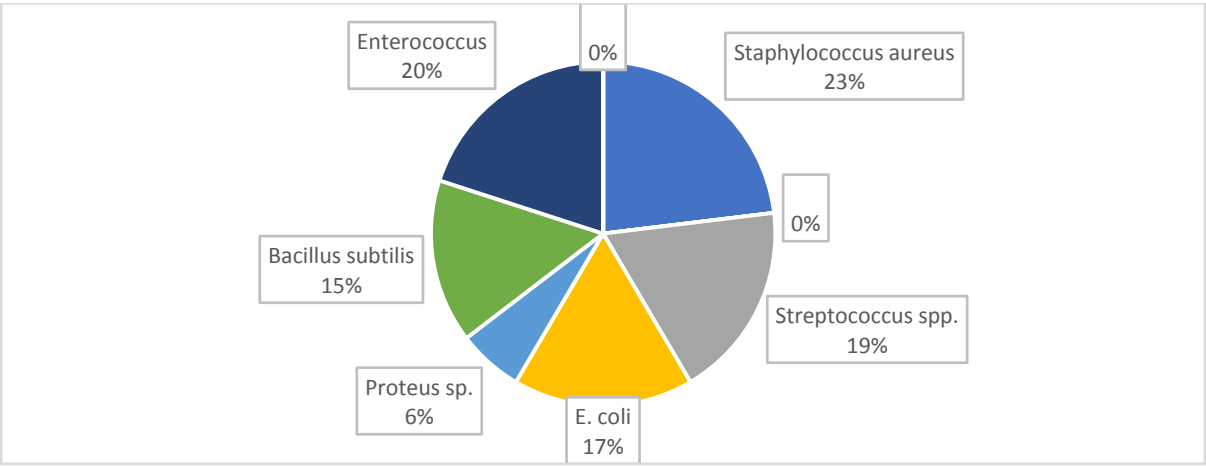

Figure 3.1: Prevalence frequency of the bacterial isolates found present in the screened soft drink samples was *S. aureus* (23%), Streptococcus spp. (23%), Enterococcus (20%), E. *coli* (17%), Bacillus *subtilis* (15%) and Proteus spp. (6%).

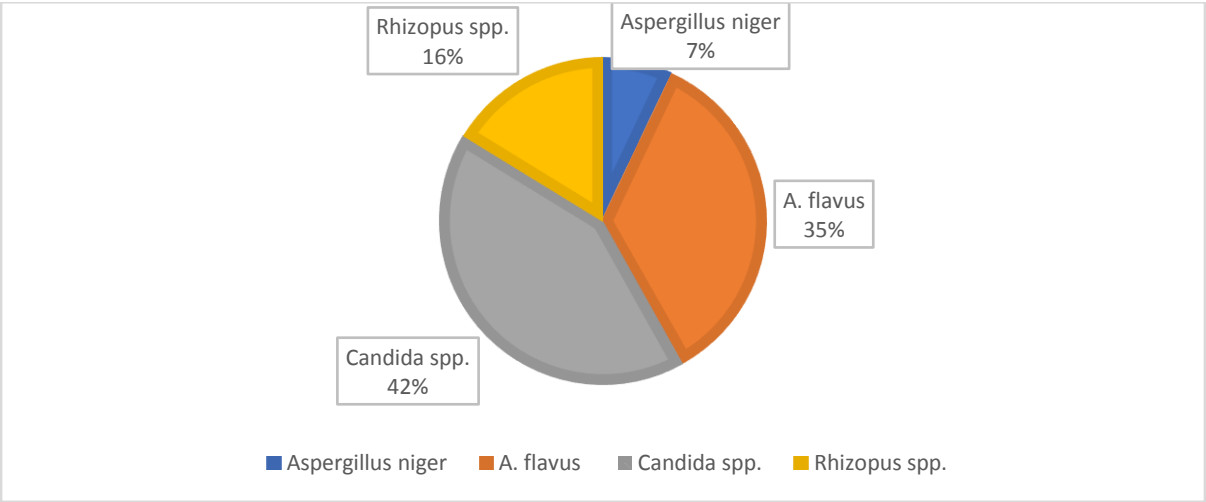

Figure 3.2: The Prevalence frequency of the fungi isolates found present in the screened soft drink samples was Candida spp. (42%), *Aspergillus flavus* (35%), *A. niger* (7%) and Rhizopus spp (16%).

Table 3.4: Antibiotics Susceptibility Test Results for the bacterial isolates from selected soft drinks.

	SXT	E	PEF	CN	APX	Z	AMP	R	CPX
Staphylococcus aureus (n=15)	10(66.7)	6(40.0)	15(100)	6(40.0)	10(66.7)	14(90.0)	3(20.0)	6(40.0)	10(33.3)
Bacillussubtilis (n=15)	9(60.0)	5(33.3)	12(80.0)	5(33.3)	12(80.0)	12(80.0)	6(40.0)	7(46.6)	15(100)
Streptococcus spp. (n=12)	6(50.0)	**1(10.0)**	5(41.7)	8(66.7)	10(83.3)	10(83.3)	7(58.3)	2(16.7)	7(58.3)
Proteus sp. (n=4)	0(0.0)	2(50.0)	3(90.0)	1(10.0)	3(90.0)	4(100)	3(90.0)	2(50.0)	3(90.0)
E. coli (n=11)	3(27.3)	8(72.7)	1(10.0)	5(40.0)	5(40.0)	10(90.0)	7(63.6)	3(27.3)	11(100)

LEGENDS

R – Resistant S – Sensitive SXT- Sulphamethoxazole/trimethoprim Z- ZinnacefE- Erythromycin AMP- Ampiclox R-Rocephin CN- Chloramphenicol CPX- Ciprofloxacin APX- Amoxicillin

Table 3.5: Antimicrobial susceptibility pattern of fungi from selected soft drinks.

	Antifungal Agents							
Isolate	**Griseofulvin (250mg/ml) (%)**		**Griseofulvin (500mg/ml) (%)**		**Ketoconazole (200mg/ml) (%)**		**Ketoconazole (400mg/ml) (%)**	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Aspergillus niger (n=3)	**2(66.7)**	**1(33.3)**	**3(100)**	**0**	**1(33.3)**	**2(66.7)**	**3(100)**	**0**
Aspergillus flavus (n=15)	**6(40.0)**	**9(60.0)**	**13(86.7)**	**2(13.3)**	**11(73.3)**	**4(26.7)**	**13(86.7)**	**2(13.3)**
Candida spp. (n=7)	**2(28.6)**	**5(71.2)**	**6(85.71)**	**1(14.3)**	**3(42.9)**	**4(57.1)**	**7(100)**	**0**

IV. DISCUSSION

The bacterial and fungal counts showed that all the kunu sampled had a high prevalence of these contaminants and spoilage organisms which of course is of great concern. They are therefore considered microbiologically unsafe for human consumption when not hygienically prepared and utensils cleaned regularly. Clusters of infection caused by the organisms have been linked to poorly maintained utensils, poor personal hygiene, improper storage facilities, and poor water supply [8]. Also, it can be linked to unhygienic human handlers, dispensing of the extract into bottles, addition of flavours and sweeteners and process of cooling of the extract [9].

The detection of *S. species* was equally isolated by Akeema*et al*., (2006) from Waraand Kunu, a cereal based, non-alcoholic beverage,also by Aboh and Oladosu, (2014) from Kunu. The presence of organisms like *Bacillus species, Streptococcus* has been reported by Adeyemi and Umar,(1994) and Ayo, (2004) who also isolated theseorganisms from Kunu.

The high occurrence of *E. coli* (17.7%) in kunu is an indication of faecal contamination. Ironically, most food handlers do not practice good personal hygiene and do not follow good manufacturing practices, which could reduce the occurrence of such bacteria in foods [10]. Essien*et al.,* (2011) reported the presence of organisms like *Bacillus cereus,Staphylococcus aureus and Escherichia coli* (PHLS, 1996). It is possible that contamination by these pathogens could have occurred during sieving and packaging, as most of the people involved in the production, packaging and hawking do not take necessary precautions, and as such contamination could be very prominent [11].

A total of three (3) different moulds were isolated from the kunu drink samples in the course of study. These wereCandida spp., *Aspergillus niger andAspergillus flavus.Aspergillus* speciesare ubiquitous, aerobic fungi that can survive in air,dust. The presence of fungi may be attributed to theacidic nature of the sample since it has been observed thatmoulds are capable of utilizing organic acids. Also, thepresence of fungi in the food may lead to food poisoningand contaminated fungi result in the production ofundesirable odour, colour changes and loss of taste of thesample. The presence of these organisms in the samplemay be due to the nutritional composition of the millet;these nutrients are present in different proportions. *Aspergillus flavus* is a common mould in the environment,and can cause storage problems in stored grains. It canalso be a human pathogen, associated with *Aspergillosis* of the lungs and sometimes causing corneal, otomycosisof aflatoxin and nasal-related infections [1,12].

V. CONCLUSION

The microbial content of these hawked marketed Kunu, Burukutu and Zobo drinks was higher and are contaminated with microorganisms which may be potentially pathogenic to human beings. There is therefore the need to maintain adequate hygienic conditions during processing and preparation of these beverages to eliminate these microbial contaminants and to improve on the quality of the final product.

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Conflict of interest

All authors declare that they have no conflict of interest.

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