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Microbiological Analyses of Hawked Kunun and Zobo Drinks within LAUTECH Campus, Ogbomoso, Oyo State, Nigeria

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Abstract: *Food borne intoxication is common in man and the consumption of these local beverages may possibly be the cause of many infectious diseases in human. Therefore, there is need to carry out microbiological analyses on these local beverage drinks. The aim of this work is to carry out microbiological analyses on Kunun and Zobo drinks sold within LAUTECH, Ogbomoso campus. Colony Forming Units per Millilitre of Kunun ranged from 0.2×10^5 to 10.0 ×10^5* while that of Zobo ranged from 0.4×10^5 to 15.0×10^5. *Bacterial isolates identification revealed the presence of Bacillus cereus, Bacillus subtilis, Bacillus laterosporus, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Proteus sp. Escherichia coli, Micrococcus acidophilus and Streptococcus feacium, while the fungal isolates include; Candida castelli, Torulopsis stellata, Geotrichum candidum, Aspergillus flavus, Penicillium citrinum, Rhizopus sp., Penicillium oxalicum, Aspergillus niger and Saccharomyces cerevisiae. The susceptibility patterns of the isolated microorganisms were determined against the commonly used antimicrobial agents. Bacterial isolates showed 100% susceptibility to Ofloxacin, Ciprofloxacin, Gentamycin and Ofloxacin, while they showed 100% resistance to Erythromycin, Clindamycin, Cephalexin, Cloxacillin and Ceftriaxone. Fungal isolates showed different resistant patterns to different concentrations of Griseofulvin and Ketoconazole used as antifungal agents.*

The results obtained from this study showed that both the Kunun and Zobo drinks sold within the study area were contaminated and also contained different pathogenic microorganisms which can serve as sources of infections to human. Therefore, proper hygienic and sanitary measures need to be enforced during processing and packaging of these local beverage drinks.

Keywords: Kunun, Zobo, Microbiological analyses, Antimicrobial agents, Bacteria, Fungi, Resistance

I. Introduction

Kunun is a popular cereal based, non-alcoholic beverage {1}. It is a popular local or indigenous drink consumed throughout Nigeria {2}, mostly in the north for its thirst quenching properties {3}. Kunun-zaki is prepared from either guinea corn [*Sorghum bicolor*], millet [*Penisetum typhoides*], maize [*Zea mays*], rice [*Oryza sativa*] or wheat [*Triticum aessstivum*]. 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 [1/] fresh [ungelled] part of the mixture and then allowing to ferment overnight and the supernatant is ready for consumption {4}. Zobo drink is also a non-alcoholic beverage drink prepared from dried calyces of Roselle [*Hibiscus sabdariffa]*. The production of Zobo involves the boiling of the calyces in water for 1-2 hours, cooling and then sieving. This is followed by the addition of ginger, flavour and sugar; and finally chilling in a refrigerator before consumption {5}. The spices usually added being agricultural commodities may contain high level of microbial impurities {6}, which can serve as a source of pathogenic microorganisms {7}. A large number of lactic acid bacteria, coliforms, moulds and yeast have been reportedly implicated in food spoilage as they use the carbohydrate content for fermentation processes to produce undesirable compounds {8}. Food borne intoxication is common in man {9} and local beverage drinks may be the common sources of infection. The consumption of these local drinks is of public health significance. Hence, local drinks may serve as vehicles for zoonotic and food-borne diseases or pathogens such as Staphylococcosis, Salmonellosis, Brucellosis, Tuberculosis, Shigellosis, Listeriosis, *Escherichia coli* infection, etc. The current food safety challenges have risen slowly over several years and require strategic efforts to be controlled {10}. In 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 {11}. This research was therefore conducted to investigate the microbiological analyses of non-alcoholic drinks called Kunun and Zobo within Ladoke Akintola University of Technology [LAUTECH] campus, Ogbomoso.

II. Materials and Methods

2.1. Collection of samples

Hawked Kunun and Zobo drinks samples were collected by purchasing randomly from five different locations on LAUTECH {Ladoke Akintola University of Technology} campus, Ogbomoso. Samples of kunu and Zobo drinks were purchased and properly labelled in sterile plastic containers. The samples were brought to laboratory on ice for microbiological respectively. The raw materials for laboratory preparation of both Kunun and Zobo drinks were purchased at a nearby market to the campus. The drinks were prepared at the laboratory using aseptic methods and the drink samples were also subjected to the same analysis.

2.2. Isolation of bacteria and fungi and Determination of aerobic plate Count

Samples were serially diluted with sterile distilled water aseptically before inoculation. The media used for bacteria isolation were Nutrient Agar, Mannitol Salt Agar, and MacConkey agar while Potato dextrose agar was used for fungal isolation. The pour plate method was used for the isolation of bacteria and fungi {12}.

Plates containing Nutrient Agar, Mannitol salt agar, and MacConkey agar were incubated for 24 hour at 37^0C, while PDA plates were incubated at 30^0C for 96 hr. Pure isolates of bacterial and fungal isolates were obtained and stored onto Nutrient Agar and PDA respectively. The stock cultures were then preserved in a refrigerator at 4 ^{0}C and used for further analyses of the organisms. The average microbial loads of the samples obtained from the different locations were expressed as Colony Forming Units per millilitre {CFU/mL} of Kunun and Zobo drinks.

2.3. Identification of bacteria and fungi isolates

The identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests {12}. Fungal isolates were identified using colonial appearance and microscopic characteristics.

2.4. Antimicrobial susceptibility Testing

Antibiotic susceptibility was determined on Mueller Hinton agar using the disc diffusion method {13}. All the isolates were tested for their sensitivity against the antibiotics. Inoculum was prepared by both direct colony suspension and growth methods depending on whether the bacterium is Gram-positive or Gram-negative. The standardized inoculum was spread over Mueller Hinton agar plate using sterile cotton swabs. The standard antibiotics discs were placed on the surface of inoculated agar plates. The susceptibility patterns of the isolates were determined by measuring the zone of inhibition {14} in millimetre and interpreted accordingly.

Antibiotics used for bacteria in this test were Ceftriaxone, Amoxicillin, Ampicillin, Chloramphenicol, Cefuroxime, Gentamicin, Tetracycline, Ofloxacin, Nitrofurantoin, Ciprofloxacin, Cloxacillin, Cephalexin, Cotrimoxazole, Clindamycin, and Erythromycin.

Yeasts were cultured in Potato Dextrose broth for 24 hours. This was used to inoculate fresh PDA plates and after sometimes, the antifungal discs containing Griseofulvin and Ketoconazole at different concentrations were placed at equidistant point on the plates. In the case of mould, a little cut was made from the already grown plates and put at the centre of the fresh plates. This was allowed to stay for about 6-10 hours before antifungal disc was placed on the plate. The plates were incubated at 23^0 C for 48 hours and the zone of inhibition was measured if there is any.

III. Results

Table 1 shows the total microbial count of Kunun and Zobo drinks on different culturing media at different locations in LAUTECH community. The count range from 0.1×10^5 to 4.0×10^5 on MacConkey, 0.6×10^5 cfu/ml to 15.0×10^5 cfu/ml on Potato Dextrose Agar (PDA), 0.1×10^5 cfu/ml to 10.0×10^5 cfu/ml on Mannitol Salt Agar (MSA) and 0.4×10^5 cfu/ml to 10.0×10^5 cfu/ml on Nutrient agar (NA). The highest (15.0×10^5 cfu/ml) microbial count was observed in Zobo at point 3 on PDA medium while the lowest (0.1×10^5 cfu/ml) was observed in both the Zobo and Kunun prepared in the laboratory on MacConkey and MSA media.

Table 2 shows the occurrence of microorganisms isolated from Kunun drinks. The occurrence of *B. subtilis* in Kunun was 66.67%, *B. cereus*: 83.33%, *S. feacium*: 33.33%, *S. epidermidis*: 100%, *M. acidophilus* and *E. coli* had 50% occurrence; *G. candidum* and *A. niger* had 33.33% occurrence too. *S. cerevisiae* and *P. oxalicum* had 100% and 66.67% occurrence respectively.

The occurrence of microorganisms from Zobo drinks is shown in Table 3. *B. cereus, P. aeruginosa* and *A. flavus* had 66.67% occurrence in the Zobo drink. *B. subtilis* had 83.33% occurrence, *Proteus* sp, *E. coli* and *B. laterosporus* had 33.33% occurrence. But *S. aureus, S. pyogenes, C. castelli, T. stellata, G. candidum, P. citrinum* and *Rhizopus* sp had 50% occurrence in the Zobo drink.

The susceptibility patterns of bacteria isolated from Zobo is shown in Table 4. Some antibiotics demonstrated levels of effectiveness against the bacteria while others showed total or some level of resistance

against the bacteria. In Table 5 however, some bacteria isolated from Kunun were resistant to erythromycin, clindamycin, cephalexin, Cloxacillin, ceftriaxone, Nitrofurantoin, norfloxacin, amoxicillin and chloramphenicol. In Table 6, the antimicrobial susceptibility pattern of fungi isolated from Zobo drinks using Griseofulvin and Ketoconazole was shown. *P. citrinum* and *Rhizopus* sp were sensitive against the antifungal drugs while *G. candidum* was resistant to the drugs. Table 7 shows the susceptibility pattern of fungi isolated from Kunun drinks samples. Two isolates *P. oxalicum* and *G. candidum* were resistant to antifungal drugs while *S. cerevisiae* was sensitive to the drugs.

3.1. **Table 1:** Total microbial count of Kunun and Zobo drinks prepared at different locations in LAUTECH.

Location	**Kunu**			
	MacConkey (cfu/ml)	PDA (cfu/ml)	MSA (cfu/ml)	NA (cfu/ml)
Point 1	2.1×10^5	5.1×10^5	0.2×10^5	2.2×10^5
Point 2	0.2×10^5	10.0×10^5	10.0×10^5	4.0×10^5
Point 3	5.0×10^5	9.0×10^5	2.5×10^5	1.0×10^5
Point 4	3.2×10^5	5.3×10^5	3.4×10^5	3.5×10^5
Point 5	1.6×10^5	4.6×10^5	5.7×10^5	2.6×10^5
Laboratory Prepared	NG	0.6×10^5	0.3×10^5	0.5×10^5
	Zobo			
Point 1	1.0×10^5	2.5×10^5	NG	2.5×10^5
Point 2	1.6×10^5	2.0×10^5	0.5×10^5	1.5×10^5
Point 3	4.0×10^5	15.0×10^5	1.0×10^5	10.0×10^5
Point 4	2.8×10^5	3.6×10^5	0.6×10^5	3.5×10^5
Point 5	2.1×10^5	6.3×10^5	0.4×10^5	3.0×10^5
Laboratory Prepared	NG	1.4×10^5	0.1×10^5	0.4×10^5

3.2. Table 2: Occurrence of microbial isolates in Kunu drinks

Isolates	Samples						Percentage Occurrence
	Point 1	Point 2	Point 3	Point 4	Point 5	Laboratory Prepared	
B. subtilis	+	-	+	+	-	+	66.67
B. cereus	+	+	+	+	+	-	83.33
S. feacium	-	-	-	+	+	-	33.33
S. epidermidis	+	+	+	+	+	+	100
M. acidophilus	-	+	+	-	_	-	33.33
Proteus sp	+	+	-	+	-	-	50.00
E. coli	+	+	-	+	-	-	50.00
G. candidum	-	-	+	-	+	-	33.33
A. niger	-	+	-	+	-	-	33.33
P. oxalicum	+	-	+	+	-	+	66.67
S. cerevisiae	+	+	+	+	+	+	100

Keys: + = Present, - = Absent

3.3. Table 3: Occurrence of microbial isolates in Zobo drinks

Isolates	Sample						
	Point 1	Point 2	Point 3	Point 4	Point 5	Laboratory Prepared	Percentage Occurrence
B. cereus	+	+	+	+	-	-	66.67
B. subtilis	+	+	+	-	+	+	83.33
Proteus sp	-	+	-	+	-	-	33.33
P. aeruginosa	+	+	+	+	-	-	66.67
S. pyogenes	-	+	+	+	-	-	50.00
E. coli	-	+	-	-	+	-	33.33
B. laterosporus	-	+	-	+	-	-	33.33
S. aureus	-	+	+	-	+	-	50.00
C. castelli	+	+	-	-	+	-	50.00
T. stellata	+	+	-	+	-	-	50.00
G. candidum	-	+	+	+	-	-	50.00
A. flavus	+	+	+	+	-	-	66.67
P. citrinum	-	+	-	+	-	+	50.00
Rhizopus sp	-	+	+	-	+	-	50.00

3.2. Table 4: Antimicrobial Susceptibility Patterns of Bacteria from Zobo drink.

Isolates	Antibacterial Agents for Gram Positive								
	OF	E	CIP	CD	GN	CX	CO	AP	FX
B. cereus	S	R	S	R	S	R	R	R	R
B. subtilis	S	R	S	R	S	R	R	R	R
S. aureus	S	R	S	R	S	R	S	R	R
B. laterosporus	S	R	S	R	S	R	R	R	R
% of Resistance	0	100	0	100	0	100	75	100	100
	Antibacterial Agents for Gram Negative								
	N	CIP	TE	NB	AX	OF	C	CF	AM
P. aeruginosa	R	R	R	R	R	S	R	S	R
Proteus sp	R	S	S	S	R	S	S	R	R
E. coli	S	R	S	R	S	S	R	R	R
% of Resistance	66.7	66.7	33.3	66.7	66.7	0	66.7	66.7	100

R- Resistance, S- Sensitive, FX- Ceftriaxone, AP- Cloxacillin, CX- Cephalexin, CF- Cefuroxime, N- Nitrofurantoin, GN- Gentamycin, OF- Ofloxacin, CO- Cotrimoxazole, CD- Clindamycin, E- Erythromycin, TE- Tetracycline, NB- Norfloxacin, AX- Amoxicillin, C- Chloramphenicol, CIP- Ciprofloxacin and AM- Ampicillin.

3.3. Table 5: Antimicrobial susceptibility pattern of bacteria from Kunu drinks.

Isolates	Antibacterial Agents for Gram Positive								
	OF	E	CIP	CD	GN	CX	CO	AP	FX
B. cereus	S	R	S	R	S	R	R	R	R
S. epidermidis	S	R	S	R	S	R	R	R	R
M. acidophilus	S	R	S	R	S	R	S	R	R
S. feacium	S	R	S	R	S	R	R	R	R
% of Resistance	0	100	0	100	0	100	75	100	100
	Antibacterial Agents for Gram Negative								
	N	CIP	TE	NB	AX	OF	C	CF	AM
Proteus sp	R	S	S	R	R	S	R	S	S
E. coli	R	R	S	R	R	S	R	S	S
% of Resistance	100	50	0	100	100	0	100	0	0

3.4. Table 6: Antimicrobial susceptibility pattern of fungi from Zobo drinks.

Isolates	Antifungal Agents			
	Griseofulvin (250mg/ml)	Griseofulvin (500mg/ml)	Ketoconazole (200mg/ml	Ketoconazole (400mg/ml)
C. castelli	-	-	+	+
T. stellata	-	-	+	+
G. candidum	-	-	-	-
A. flavus	-	-	+	+
P. citrinum	+	+	+	+
Rhizopus sp	+	+	+	+

- = Resistance, + = Sensitive

3.5. Table 7: Antimicrobial susceptibility pattern of fungi from Kunun drinks.

Isolate	Antifungal Agents			
	Griseofulvin (250mg/ml)	Griseofulvin (500mg/ml)	Ketoconazole (200mg/ml	Ketoconazole (400mg/ml)
P. oxalicum	-	-	-	-
S. cerevisiae	+	+	+	+
A. niger	+	-	-	-
G. candidum	-	-	-	-

IV. Discussion

Kunun and Zobo drinks are two non-alcoholic beverage drinks prepare and consume in large quantities in Ogbomosho and its environs. The drinks are well accepted by all in these areas, and hence are being produced as supplements and complements to soft drinks in schools and during occasions like birthday celebrations, weddings and naming ceremonies. The production systems are sometimes done under unhygienic conditions {4} with no authority to monitor their microbial quality and safety. In this study, these two hawked Kunu and Zobo drinks were examined for the presence of microbes which can probably cause infections in humans.

The results revealed that the microbial load of both hawked kunu and Zobo was higher compared to the one prepared in the laboratory. Hawked kunu and Zobo drinks are highly contaminated with bacteria and fungi

which may be potentially pathogenic to human beings. The occurrence of *S. aureus, S. pyogenes, P. aeruginosa, A. flavus, P. citrinum, Rhizopus* species, *C. castelli, T. Stellata, B. laterosporus, B. subtilis, B. cereus, S. feacium, S. epidermidis, M. acidophilus, Proteus sp, E. coli, G. candidum, A. niger, P. oxalicum* and *S. cerevisiae* in Kunun and Zobo drinks are considered detrimental to the health of the consumers {15}. The results obtained from this study was similar to the one reported by {16} who isolated *S. aureus, Proteus* sp, *Streptococcus* sp, *Bacillus* sp, *E. coli* and Yeast from fresh milk and fermented milk product.

Kunun preparation involves cooking, a process that would eliminate all the isolates reported in this work except the heat-resistant spore former (*Bacillus* sp.). The presence of these organisms in Kunun thus suggests that it must have been contaminated after cooking process and after the drink had cooled down. Contamination of Kunun and Zobo could come from the syrup, fermentation vessels, storage containers, sieves used for filtration, hands of the handlers and even the polythene bags or bottles in which it was packaged for sale {17}, this was also observed in the laboratory prepared drinks which were also contaminated. Therefore, there is need for high degree of sanitation during the processing of these beverages.

The occurrence of *E. coli* in kunu and Zobo is an indication of faecal and environmental contamination and a signal for the presence of other enteric pathogens. Therefore, their presence may be linked to faecal, environmental and human contaminations {16} which may occur probably through the use of water. *Bacillus* sp. has been implicated in food poisoning especially in cereals that have been cooked and stored at warm temperature {17}. These *Bacillus* species can produce toxin that cause pneumonia and bronchopneumonia, and besides *Bacillus cereus* is known to produce heat-resistant spores that cannot be eliminated by boiling. The isolation of yeasts from these drinks may be linked to contamination through air/dust, contaminated packaging material or poor hygiene and sanitation of the processing environment. Yeasts can grow at a wide range of temperature and pH and some of these fungi can produce mycotoxins which can cause mycotoxicosis in humans {5}. Microorganisms obtained from this study showed some level of resistance to commonly used antimicrobial agents and this may be as a result of use and misuse of drugs in the society {18}.

V. Conclusion

The microbial content of these hawked marketed kunu 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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