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Bacteriological Assessment of Zobo Beverage Sold at Confluence University of Science and Technology, Osara, Kogi State, Nigeria

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Abstract

Zobo is a popular Nigerian beverage made from Hibiscus sabdariffa. *Often sweetened with sugar or fruit extracts, this refreshing drink is enjoyed by many Nigerians for its good taste and nutrient-rich composition. This study investigated the microbiological quality of Zobo, a locally brewed drink, sold at three different locations at Confluence University of Science and Technology, Osara, Kogi State, Nigeria. The pour plate technique was used to determine the total viable bacterial counts, which ranged from* 8×10^{1} *to* 3.5×10^{2} *CFU/mL across the sampled locations. Among the samples, Sample A*

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(University compound) recorded the highest bacterial count, followed by Sample C (University town, Osara), while Sample B (University gate) showed the lowest count. The bacterial isolates were identified through an analysis of their cultural traits, Gram staining reactions, and biochemical characteristics. The identified bacteria included Klebsiella sp (41.2%), Staphylococcus sp (23.5%), Bacillus sp (23.5%), and Shigella sp (11.8%). The findings reveal varying levels of bacterial contamination in Zobo samples, with potential public health implications, emphasizing the need for improved hygiene practices during production and storage.

Keywords: Zobo, beverage, drink, bacteria contamination,

INTRODUCTION

The drink known as Zobo is a popular local Nigerian beverage made from *Hibiscus sabdariffa*, commonly referred to as Roselle (Ojileh and Okechukwu, 2023). The plant's dried calyces are cleaned, soaked, and boiled to create this deep-red, non-alcoholic beverage. The combination is then filtered to produce an extract.

Often sweetened with sugar or fruit extracts, Zobo has become a refreshing choice for many Nigerians due to its sour taste and nutrient-rich composition (Ezekiel *et al.,* 2016). It contains vitamins, carbohydrates, proteins, calcium, iron, and antioxidants (Bamishaiye *et al.,* 2011). Known by various names globally—like "Bissap" in Senegal and "Sorrel" in the Caribbean—Zobo is particularly valued in Northern Nigeria, where it is also known as "Zoborodo" in Hausa (Ayandele, 2015; Okereke *et al*., 2015).

In Nigeria, the rising advocacy against alcoholic beverages has boosted the popularity of Zobo as a healthier, local alternative to drinks like red wine. The drink also holds potential medicinal benefits, traditionally used to address ailments such as hypertension, urinary tract infections, cardiac, and nerve issues (Bamishaiye *et al*., 2011). With a low price point and numerous health benefits, Zobo appeals widely, especially amid Nigeria's economic challenges (Adamu *et al*., 2016).

However, due to frequent microbial contamination from high microbial loads in the spices and other additives, Zobo can be prone to spoilage. Common contaminants include lactic acid bacteria, molds, and yeasts, which utilize the drink's sugars for fermentation and may produce harmful by-products (Ayandele, 2015). According to research by Amusa *et al. (*2005), Zobo may even carry pathogens, presenting a public health risk if not properly prepared and stored. Concerns about microbial contamination extend to University campuses and other institutions, where hygiene measures might be inconsistently enforced, further emphasizing the need for quality assessment of Zobo in settings like the University of Maiduguri.

Furthermore, contamination of Zobo drink can occur during the cooling of the hot extract, the addition of flavors and sweeteners, or the transfer of the extract into nylons and bottles (Risiquat, 2013). Additionally, utensils and water used during the post-heating stages may also serve as sources of contamination (Risiquat, 2013).

According to Izah *et al*. (2015), various microbes have been widely isolated from Zobo drinks sold in public places across Nigeria. The identified bacterial genera include *Staphylococcus, Escherichia, Proteus, Pseudomonas, Salmonella, Shigella, Enterobacter, Klebsiella, Serratia, Bacillus, Streptococcus, Lactobacillus, Clostridium, Corynebacterium, Aeromonas,* and *Micrococcus*. The

fungal genera reported include *Aspergillus, Saccharomyces, Penicillium, Candida, Rhizopus, Fusarium, Mucor,* and *Geotrichum.*

Zobo is a widely consumed and affordable beverage in Nigeria; however, microbial contamination poses potential health risks. While studies have identified common contaminants, research on Zobo sold in institutional settings, particularly at CUSTECH, Osara, remains limited. This study aims to evaluate the bacteriological quality of Zobo beverages sold at CUSTECH, Osara, Kogi State, Nigeria, by identifying bacterial contaminants.

MATERIALS AND METHODS

Study Area
The research was carried out in Confluence University of Science and Technology, Osara, a small town in Kogi State, north-central Nigeria, within Latitude 7.1608˚N and Longitude 3.3483˚E

Sample Collection
Zobo samples were purchased and transferred aseptically from three randomly selected locations in Osara. Samples in plastic bottles were purchased from each seller for three consecutive days. These were labeled, packed in plastic bags, and transported immediately to the Microbiology Laboratory at **(**CUSTECH, Osara. Kogi State for bacteriological analysis.

Sterilization of Materials
A thorough cleaning, rinsing with water that had been distilled, and sterilization in an autoclave at 121 degrees Celsius for fifteen minutes were performed on the glassware, which included test tubes and conical flasks (Bristone *et al*., 2018)

Preparation of Culture Media
The nutrient agar was made in accordance with the directions provided by the manufacturer, **Oxoid** . The powdered media was mixed in water that was distilled, then autoclaved at 121 degrees Celsius for fifteen minutes to sterilise it (Yimana and Tesfaye, 2022). The resulting mixture was then used for the cultivation of bacteria.

Isolation of Bacteria
One milliliter of each Zobo sample was serially diluted in sterile water in order to determine the total numbers of bacteria, 0.1 millilitres of dilutions were used to inoculate Nutrient Agar using the pour plate method (Umar *et al*., 2016). For twenty-four hours, the plates were kept in an incubator at 37 degrees Celsius.

Purification of Isolates
After growing colonies on Nutrient Agar, the colonies were purified by sub-culturing them onto fresh Nutrient Agar plates and then incubating them at 37 degrees Celsius for twenty-four hours. After that, the colonies that had been isolated were prepared for further examination by being preserved on agar slants.

Identification of Microbial Isolates
Microbial isolates were identified using a combination of morphological characteristics, Gram staining, and biochemical tests such as catalase, oxidase, coagulase, indole production, and citrate utilization.

Gram Staining
Based on the characteristics of their cell walls, Gram staining classified bacteria as either Gram-positive (violet) or Gram-negative (red) (Iyiola, 2022).. The following steps were taken using a thin smear of bacterial cells: crystal violet staining, iodine treatment, alcohol decolorisation, and safranin counterstaining.

Biochemical Tests
Catalase test
The existence of the catalase enzyme, which decomposes hydrogen peroxide (H_2O_2) into oxygen and water, was determined by this assay (Iyiola, 2022). The existence of catalase was confirmed when a few bacterial colonies were immersed in 3% H_2O_2 and bubbles were produced, indicating a favourable outcome.

Indole test
Bacterial cultures were incubated in peptone water at 37°C for 48 hours to determine their ability to synthesise indole from tryptophan, an amino acid (Iyiola, 2022). Once the incubation period was over, the addition of Kovac's reagent confirmed the formation of indole by creating a crimson ring on the surface.

Coagulase test
This test distinguished *Staphylococcus aureus* from other *Staphylococcus* species based on their ability to clot plasma. A bacterial suspension was mixed with human plasma on a slide, and **visible** clumping within a few seconds indicated a positive result.

Oxidase **test**
The ability of bacteria to produce the electron transport chain enzyme cytochrome oxidase was determined by the oxidase test (Umar *et al.,* 2016). The oxidase reagent was soaked onto filter paper and a colony was spread onto it. The growth of a rich purple hue served as confirmation of a favourable outcome.

Citrate utilization test
The capacity of the bacteria to use citrate as the only carbon source on Simmons' citrate agar was evaluated in this test (Iyiola, 2022). As a result of growth on the medium and a shift in colour from green to blue, it was determined that citrate was being utilised.

RESULTS

Total Viable Bacterial Counts
The viable bacterial count results, as presented in Table 1, indicate the total number of living bacteria in the samples. The bacterial counts varied across the three locations: University Compound (Sample A): Ranged from 8×10^1 to 3.5×10^2 CFU/mL, University Gate (Sample B): Ranged from 4×10^1 to 1.9×10^2 CFU/mL, and University Town, Osara (Sample C): Ranged from 1.8×10^2 to 2.5×10^2 CFU/mL. Among the samples, Sample A (University Compound) recorded the highest bacterial count, followed by Sample C (University Town, Osara), while Sample B (University Gate) had the lowest count.

Table 1: Total viable bacteria counts of Zobo drink (CFU/mL)

Sample locations	Day 1	Day 2	Day 3
A	8×10^1	3.5×10^2	3.0×10^2
B	1.9×10^2	1.9×10^2	4×10^1
C	1.8×10^2	2.5×10^2	3×10^1

Keys: A = University compound, B = University gate and C = University town, Osara,

Bacterial Isolate Identification

The morphological features of the macroscopic and microscopic appearances of the bacterial isolates on culture media and under the microscope, using the gram staining technique, were used for identification. Table 2 below shows the results of the biochemical reactions used to confirm the isolates. These assays included catalase, oxidase, citrate, indole, and oxidase.

Table 2: Bacterial Isolate Gram Reaction and Biochemical Tests

S/N	Gram Reaction	Catalase	Citrate	Coagulase	Indole	Oxidase	Probable organisms
1	+	+	+	-	-	-	*Bacillus* sp
2	-	+	+	-	-	-	*Klebsiella* sp
3	+	+	+	+	-	-	*Staphylococcus aureus*
4	-	+	+	-	-	-	*Shiegella* sp

The frequency of bacteria in zobo drink samples

Nine Zobo samples from the three sales points yielded 17 bacterium isolates. Table 3 shows that *Klebsiella* sp had the highest prevalence of 7 (41.2%), followed by *Staphylococcus aureus* 4 (23.5%), *Bacillus* sp 4 (23.5%), and *Shigella* sp 2 (11.8%).

Table 3: Percentage Occurrence of Isolated Bacteria

Bacteria isolated	Number of isolates	Percentage of occurrence (%)
Bacillus sp	4	23.5%
Klebsiella sp	7	41.2%
Staphylococcus aureus	4	23.5%
Shiegella sp	2	11.7%
Total	**17**	**100%**

DISCUSSION

The findings of this study reveal that Zobo drinks sold in Osara contain diverse bacterial populations. However, the microbial load of the analysed Zobo samples ranged from 8×10^1 to 3.5×10^2 cfu/ml, which falls within the acceptable limits according to Risiquat (2013). The presence of bacterial contaminants in Zobo has been linked to poor personal hygiene during production (Ojo *et al.,* 2011), and inadequate preservation techniques (Udensi *et al*., 2020).

Pathogenic microorganisms are commonly transmitted through the fecal-oral route, often via the ingestion of contaminated food, drinks, or water (Rossi *et al.,* 2024). *Klebsiella* sp (41.2%)., the most frequently isolated bacterium in this study, presents significant public health concerns, as it is associated with opportunistic infections such as pneumonia and bloodstream infections (Imai *et al*., 2019). Its presence in food or beverages often indicates possible fecal contamination, which may result from poor personal hygiene during preparation or the use

of contaminated water (Feglo & Sakyi, 2012). These findings highlight the urgent need for proper sanitation measures. This study is consistent with the findings of Mwambete and Peter (2011), who identified *Klebsiella* sp. as the second most isolated bacterium, after *E. coli*, in juice beverages from Dares Salaam.

Isolation of other bacterial pathogens: *S. aureus* (23.5%) and *Shigella* sp. (11.8%) in the Zobo samples highlights concerns about poor handling practices and hygiene deficiencies during preparation and distribution (Bristone *et al.,* 2018). *S. aureus* is a well-documented cause of foodborne illnesses due to its ability to produce enterotoxins that can lead to severe gastrointestinal symptoms (Omeremu *et al*., 2019). The detection of *Shigella* sp. is particularly alarming, as it is a major pathogen responsible for dysentery, which is commonly linked to fecal contamination and inadequate sanitation measures during food handling (Warren *et al*., 2006). The presence of these bacteria strongly suggests that improper personal hygiene, contaminated water sources, and unhygienic processing environments may be contributing factors to microbial contamination in Zobo beverages.

Additionally, the isolation of *Bacillus* sp. (23.5%) points to environmental contamination, as this genus is known for its widespread presence in soil, air, and dust particles (Effiong *et al*., 2023). While some *Bacillus* species are considered harmless, certain strains are capable of producing toxins that may lead to food poisoning if ingested in significant quantities (Li *et al*., 2023). This finding suggests that contamination could occur through exposure to unclean surfaces, airborne spores, or raw materials used during Zobo production.

CONCLUSION

This study highlights the microbiological risks associated with consuming *Zobo* drinks sold in Osara, Kogi State. The bacterial contamination levels and the presence of pathogenic species such as *Klebsiella sp.* and *Shigella sp.* underscore the need for improved hygiene and sanitary practices throughout the *Zobo* production and distribution chain. Public health authorities should enforce stricter regulations and promote awareness among producers and vendors to minimize contamination risks. Enhanced quality control measures and regular monitoring are essential to ensure the safety of locally produced beverages like Zobo.

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Conflicts of interests

The authors declare no conflict of interest.

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