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Research Article

DETECTION OF BACTERIAL CONTAMINANTS IN READY-TO-DRINK BEVERAGE (*ZOBO*) SOLD ON FEDERAL UNIVERSITY DUTSE CAMPUS, NORTHWEST NIGERIA

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ABSTRACT

Introduction: Consumption of ready-to-drink beverages is a norm in the world over. However, the presence of total coliform in any beverage renders it unfit for human consumption.

Objectives: Due to non-existence of scientific data on the safety status of the ready-to-drink *Zobo* which is a popular local beverage, we did conduct this study to assess the bacteriological quality of the lots sold to consumers on the campus of Federal University Dutse (FUD), Nigeria.

Methods: Purposive sampling was employed to sample nine (9) locally packaged *Zobo* in three (3) different outlets; FUD Bakery (BAK) where three (3) were assayed (BAKA, BAKB, and BAKC), FUD Backside (BS) where three (3) samples were assayed (BSA, BSB, and BSC), and Morocco Girls' hostel (MGH) three (3) samples were assayed (MGHA, MGHB, and MGHC). Standard methods were used to assay the sampled *Zobo*.

Results: Results obtained indicate that samples from MGH (8.0×10^4 CFU/mL) had the lowest total viable count (TVC), while samples from BAK (2.8×10^7 CFU/mL) recorded the highest TVC. However, samples assayed from MGH recorded no total coliform count. The number of bacterial colonies recorded in BAKA and BAKB was not significantly different from each other ($p>0.05$). However, the number of bacterial colonies recorded from MGHA was significantly different from those obtained in MGHB and MGHC ($p<0.05$).

Conclusion: Due to the results obtained, we can conclude that *Zobo* samples assayed in the sampling points were not fit for human consumption during the conduct of our study.

Keywords: *Zobo*, Ready-to-drink, Total viable count, Coliform count, Federal university dutse.

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INTRODUCTION

A red, non-alcoholic beverage from the region called *Zobo* is produced from various dried petal and succulent aqueous acid extracts of *Roselle calyx* (Zumbes *et al*., 2014). The term *Zobo* originated from its Hausa native name *Zoborodo* (Ayandele, 2015). There are other names for *Hibiscus sabdariffa*, including *Gongura* in Hindi, *Krajeab* in Thailand, *Bissap* in Senegal, and *Sorrel* in the Caribbean (Okeke *et al*., 2015). Particularly in Northern Nigeria, this non-alcoholic beverage is highly well-liked (Egbere *et al*., 2007). *Zobo* is becoming widely accepted as a great potential local substitute for imported drinks like red wine in various areas of Nigeria due to the rise in religious, economic, and health campaigns against alcohol and the ensuring decrease in alcohol consumption in northern Nigeria.

Vitamin A, carbohydrates, protein, riboflavin, niacin, calcium, iron, minerals, and other antioxidants are all abundant in *Zobo* (Ehsemokha, 2020). In addition to these uses, it has been employed in traditional medicine as a diuretic, a mild laxative, a therapy for heart and nerve disorders, and a cancer management strategy (Bristone *et al*., 2018). In addition, *Zobo* is said to be an effective traditional medication for treating a number of illnesses, including hypertension, urinary tract infections, and excruciating menstrual cramps and equally help out in instances of infertility in both men and women (Bamishaiye *et al*., 2011).

The Hibiscus flower's dried, completely developed calyx, which can generate a range of colours in Zobo and can be extremely red, brilliant crimson, or brown in colour (Amusa *et al.*, 2005). The *H. sabdariffa* plant's calyx is an annual herb that is extensively grown in Africa and India (Balakarami *et al*., 2016). The low cost and health benefits of *Zobo* have led to an increase in demand for *Zobo* calyx, which is used to produce Zobo (Bristone *et al*., 2018). Due to the country's economic downturn, millions of Nigerians from all socioeconomic backgrounds now find *Zobo* to be a suitable substitute for other beverages (Balakarami *et al*., 2016). These days, several parties and social gatherings employ chilled *Zobo* bottles as a source of refreshment (Bristone *et al*., 2018).

Many demographics typically drink zobo due to its pleasant flavor and non-alcoholic composition (Okeke *et al*., 2015). Given that *Zobo* is an agricultural product, spices that are typically added to it may have a high amount of microbial load (Ayandele, 2015). Research has indicated that consumers' preferences for certain drinks are influenced by their color. For example, ginger (*Zingiber officinale*), one of the ingredients used to produce *Zobo*, contains anti-oxidative compounds that can slow down the oxidation process of food color (Bristone *et al*., 2018).

Many questions are raised by the way *Zobo* juice is now produced, packaged, and dispensed in nylon or plastic containers in Nigeria before retail sale. Poor hygienic practices, exposure to possible contaminants, and an elevated risk to public health have resulted from the lack of drinkable water, toilets, appropriate storage, and waste disposal facilities at Zobo's preparation and point of sale (Omemu *et al*., 2006). Unfortunately, the consumption of home-prepared food and beverages has led to the spread of *Staphylococcus* food illness, causing several picnic suppers and dining establishments to abruptly close (Raimi,

2013). *Streptococcus faecalis*, *Proteus* spp., *Escherichia coli*, *Bacillus* spp., *Staphylococcus aureus*, *Enterobacter* spp., *Klebsiella* spp., *Micrococcus* spp., *Aspergillus* spp., *Penicillium citrinum*, *Fusarium oxysporum*, *Rhizopus* spp., and *Mucor* spp. are among the microorganisms linked to the deterioration and spoilage of *Zobo* (Udensi *et al*., 2020). Furthermore, it is probable that infections including Salmonellosis, Brucelosis, *E. coli*, Tuberculosis, and Staphylococcosis could be transmitted through *Zobo*, as well as zoonotic and food-borne illnesses (Ayandele, 2015; Healthlink, 2021). A particular *H. sabdariffa* extract shows potential protection against diabetes, cancer, liver disease, atherosclerosis, and other metabolic syndromes (Lin *et al*., 2011).

There have been numerous reports of lactic acid bacteria, mold, and yeast in *Zobo* (Bristone *et al*., 2018). These microorganisms have implications for food deterioration and potential infections because they use the carbohydrate content of *Zobo* for fermentation, which produces unwanted substances and raises questions about the drink's potential impact on public health (Bristone *et al*., 2018). In the event that *Zobo* is free of microbiological contaminants, it is anticipated that its consumption will have a positive impact on human health. Due to the established background information and the recent food poisoning-related cases reported on the Federal University Dutse (FUD) campus, this study was conducted to assess the bacteriological quality of ready-to-drink *Zobo* that is popularly consumed by students and workers on the university campus.

METHODS

Study area

Dutse is a city located in northern Nigeria. It has a latitudinal and longitudinal location of 11.7333N and 9.2875E, respectively. It is the capital of Jigawa state. It is home to FUD which was established in November 2011.

Sampling of Zobo

Purposive sampling was employed to sample nine (9) locally packaged *Zobo* in the three (3) different outlets on FUD campus where it is currently sold. The sampled *Zobo* was packaged in reusable plastic bottles. During sampling, the *Zobo* samples were bought from FUD Bakery (BAK), FUD Backside (BS) and Morocco Girls' hostel (MGH). These sampling points are designated as BAKA, BAKB, BAKC, BSA, BSB, BSC, MGHA, MGHB, and MGHC (Table 1). These sampling points were considered for sample collection because of the fact that they are highly patronized by consumers and are the only outlets where the beverage is sold the most on the campus. From each sampling point, *Zobo* samples were purchased at different times. Having collected the samples, it was then transferred immediately to the Laboratory for bacteriological analysis. Each of the nine (9) samples collected from the sampling points was assayed and the average was taken (Sylvester *et al*., 2016).

Enumeration of total viable count (TVC)

The presence of TVC in the sampled *Zobo* was detected through pour plating (American Public Health Association [APHA] [2005]). This was done by making serial dilution of the sampled *Zobo*. However, dilution factors of 10^4 and 10^6 were transferred into 0.1% buffered peptone water (oxoid) and a duplicate of 1 mL aliquot was inoculated into 10 mL sterilized molten plate count agar into sterilized bottles. Subsequently, it was comprehensively variegated and dispensed into sterile Petri dishes. The Petri dishes were later subjected to incubation at 37°C for 24 h. After incubation, the representative Petri dish was visualized and counted using an electric colony counting machine. Results were expressed as colony forming unit per millimeter (CFU/mL) at the end of the count (Olayemi *et al*., 2011).

Table 1: Acronyms ascribed to the sampling points

Sampling points	Acronyms
MGH A	MGHA
MGH B	MGHB
MGH C	MGHC
FUD Bakery A	BAKA
FUD Bakery B	BAKB
FUD Bakery C	BAKC
FUD Backside A	BSA
FUD Backside B	BSB
FUD Backside C	BSC

FUD: Federal University Dutse, MGH: Morocco girls' hostel

Membrane filtration technique

Each of the *Zobo* samples collected, 100 mL was aseptically taken out of which the necessary dilution was made (Acharya, 2022). Tryticase soy agar was prepared according to the instructions of the manufacturer and dispensed into a sterilized petri dishes ensuring it was evenly distributed. With a flamed forcep the membrane filter was placed into the membrane funnel assembly and the sample was dispensed into the funnel after flaming the tip of the conical flask. The vacuum was turned on and the sample was allowed to draw out completely through the filter. The membrane filter was removed from the funnel using a flamed forcep and carefully placed in the prepared media in the Petri dishes. The plates were subsequently incubated at 37°C for 24 h. Colonies that developed after incubation were counted and recorded accordingly. The CFU/100 mL derived from the assayed *Zobo* was generated using the formula depicted below;

Colonies counted ÷ Volume of Zobo sample filtered (mL) × 100

Determination of total coliform count in the sampled Zobo

We conducted the test by placing the filter paper from membrane filtration on sterilized violet red bile agar (VRBA) so as to generate discrete colonies (Ochei and Kolhatkar 2008; Nisha, 2022). This was attained by aseptically transferring a loopful of culture from positive test tubes into plates containing sterilized VRBA and test tubes containing sterilized peptone water. The plates and test tubes were subsequently incubated at 37°C for 24 h. After respective incubation periods, production of gas and indole production in peptone water was recorded as a positive result for the presence of *E. coli* while growth of pink colonies with metallic sheen, bleaching at center on VRBA confirmed the presence of coliform. The metallic sheen colonies on the agar plates were counted using the electric colony counter and further calculated the number of colonies and documented as the number of colonies present in the *Zobo* samples.

Gram staining and biochemical characterization tests for the identification of coliform

Gram staining was done following the procedure reported by Olutiola *et al*. (2000). Having Gram stained the bacterial isolates, various biochemical tests were conducted following the protocol reported by Barrow and Feltham (1993). Biochemical characterization tests ranging from indole (Ayandele, 2015; Aryal, 2018), catalase (Chessbrough, 2006), methyl red (Ochei and Kolhatkar 2008; Raimi, 2013), voges proskauer (Aryal, 2018), citrate (Sylvester *et al*., 2015), and oxidase (Chessbrough, 2006) were further conducted with a view to confirming the identity of the bacterial isolates.

Data analysis

Results obtained from the TVC of the *Zobo* were consecutively summarized in Tables. The CFU obtained from the membrane filtration technique was calculated using the formula reported by Pakpour and Horgan (2021). Results obtained from the TVC and total coliform counts across the sampling points were subjected to one way analysis of variance using Proc. GLM of Genstat Version 17. Significant means were separated accordingly.

RESULTS

Results generated from the TVC of the assayed *Zobo* depicted in Table 2 show that in the dilution factors (10^4 and 10^6) employed for the *Zobo* assay across all the dilution strengths, MGHC (8.0×10^4 CFU/mL) had the lowest TVC, while BAKA (2.8×10^7 CFU/mL) recorded the highest TVC. However, based on the number of colonies recorded in this study,

Table 2: Total viable counts detected in the assayed *Zobo* samples

Dilution factor	Number of colonies	TVC (CFU/mL)
BAKA 10^4	296	2.9×10^5
10^6	280	2.8×10^7
BAKB 10^4	288	2.8×10^5
10^6	276	2.7×10^7
BAKC 10^4	268	2.6×10^5
10^6	244	2.4×10^7
BSA 10^4	292	2.9×10^5
10^6	270	2.8×10^7
BSB 10^4	288	2.8×10^5
10^6	280	2.8×10^7
BSC 10^4	276	2.7×10^5
10^6	244	2.4×10^7
MGHA 10^4	172	1.7×10^5
10^6	76	7.6×10^6
MGHB 10^4	88	8.8×10^4
10^6	72	7.2×10^6
MGHC 10^4	80	8.0×10^4
10^6	56	5.6×10^6

TVC: Total viable count. Each value is a mean of triplicate determination

Table 3: Variation of bacterial colonies in the assayed *Zobo* samples

Sampling points	Number of colonies (CFU/mL)
BAKA	285.3^a
BAKB	278.0^{ab}
BAKC	252.0^b
BSA	284.0^a
BSB	283.0^{ab}
BSC	254.7^{ab}
MGHA	108.0^c
MGHB	77.3^d
MGHC	69.3^d

Means with different letters in each column are significantly different using Duncan's multiple range test (DMRT). ($p<0.05$). BAK: FUD bakery, BS: FUD backside, MGH: Morocco girls' hostel, CFU: Colony-forming unit

variation was witnessed across the sampling points (Table 3). Notably, in the first sampling point, the number of bacterial colonies recorded in BAKA and BAKB was not significantly different from each other ($p>0.05$). In the same vein, the number of bacterial colonies recorded in BSA, BSB, and BSC was not significantly different from each other ($p>0.05$). Furthermore, the number of bacterial colonies recorded in BAKA and BAKC was significantly different from each other ($p<0.05$).

However, the number of bacterial colonies recorded from MGHA was significantly different from those obtained in MGHB and HC ($p<0.05$). Results generated from the sampled *Zobo* regarding the total coliform count indicate that the MGH samples had no coliform count (Table 4). However, BS had the lowest mean total coliform count (1.4 CFU/mL) while BAK had the highest mean total coliform count (3.1 CFU/mL), While the variation in the mean values of the total coliform count from the sampling points are depicted in Table 5. Each *Zobo* sample had a mean total coliform count during the conduct of this study except the sample from MGH which had no growth indicating its fitness for human consumption. However, it can be observed that samples from BAK recorded more bacterial growth compared to that of BS (Table 5).

Results obtained from the colonial characteristics of the bacterial isolates show that *Zobo* samples collected from the BAK had dark pink shiny colonies with clustered heavy growth while samples from BS Zobo had pink colonies with scattered scanty growth except the sample from MGH which had no visible growth (Table 6). Bacterial isolates obtained from the assayed *Zobo* samples in BAK were seen under the microscope to be spherical, flat rod shape with pink color while that of BS were seen to be rod shape with pink color (Table 6).

Table 4: Mean total coliform count of the assayed *Zobo* samples in the sampling points

Sampling points	Mean values (CFU/mL)
BAKA	2.06
BAKB	2.16
BAKC	3.1
BSA	1.4
BSB	1.6
BSC	1.8
MGHA	0
MGHB	0
MGHC	0

BAK: FUD bakery, BS: FUD backside, MGH: Morocco girls' hostel, CFU: Colony-forming unit

Table 5: Variation in the mean total coliform counts in the sampling points

Sampling points	Mean value (CFU/mL)
BAK	2.44±0.33
BS	1.60±0.12
MGH	0.00±0.00

BAK: FUD bakery, BS: FUD backside, MGH: Morocco girls' hostel, CFU: Colony-forming unit

From the biochemical characterization tests conducted on the bacterial isolates from BAK and BS *Zobo* samples indicate that the bacterial isolates to be positive for catalase, indole and methyl red tests while it was negative for oxidase, Voges Proskauer and citrate utilization tests (Table 7). For all the biochemical tests conducted, the bacterium identified was found to be *E. coli*.

DISCUSSION

The detection of total coliform in the assayed *Zobo* is in agreement with the reports of Mbaeyi-Nwaoha and Egbuche (2012); Raimi (2013); and Mkpuma *et al.* (2022). The presence of bacterial contaminants in *Zobo* has been attributed to lack of personal hygiene on the part of the producer coupled with the poor quality of the water employed for the production (Ojo *et al*., 2011). Interestingly, many authors (Nwachukwu *et al*., 2007; Braide *et al*., 2009), have reported a shelf life of 4–14 days for a good quality *Zobo*. This implies that bacterial contaminants detected in this study might have emanated due to the assayed *Zobo* exceeding the expected shelf life.

Some authors (Ilondu *et al*., 2007; Nwachukwu *et al*., 2007) have reported the spicing of *Zobo* with the juices of some fruit flavor with a view to enhancing better taste. The spicing of the assayed *Zobo* in this study might have led to the detection of bacteria therein. The results generated in this study are in agreement with the report of Nwafor and Ikenebomeh (2009) who detected high number of yeasts in the *Zobo* assayed in their study. In addition, other research findings have confirmed the presence of several other microscopic organisms in the calyces and *Zobo* including fungi (*Aspergillus flavus*, *F. oxysporum,* and *P. citrinum*); yeasts (*Saccharomyces cerevisiae*) and lactic acid bacteria, namely, *Lactobacillus planetarium* and *Streptococcus lactis* (Nwachukwu *et al*., 2007; Braide *et al*., 2009; Nwafor and Ikenebomeh, 2009; Ruiz-Ramírez *et al*., 2015; Adeoye *et al*., 2018).

Furthermore, studies have shown that spoilage of *Zobo* within a short time can be attributed to the presence of *S. aureus*, *Pseudomonas* spp., *Klebsiella* spp., and some *Bacillus* spp. causing both food spoilage and food poisoning (Dogan and Boor, 2003; Bulakarima *et al*., 2017; Obasi *et al*., 2018). The detection of *E. coli* in the sampled *Zobo* in this study corroborates the reports of many authors (Braide *et al*., 2009; Ojo *et al*., 2011; Adeoye *et al*., 2018) who attributed the deterioration of *Zobo* assayed in their respective studies to the presence of microorganisms,

Table 6: Colonial and morphological attributes of the bacterial isolates

Sampling points	Colonial attributes	Microscopic	Gram reaction
BAKA	Pink shiny colonies with heavy growth	Spherical rod shape with pink colour	-
BAKB	Dark pink colonies with clustered heavy growth	Shiny flat rod shape with pink color	-
BAKC	Pink Spherical colonies with heavy growth	Rod shape with pink color	-
BSA	Dark pink colonies with heavy growth	Rod shape with pink color	-
BSB	Pink colonies with heavy growth	Rod shape with pink color	-
BSC	Pink colonies with very scattered scanty growth	Rod shape with pink color	-
MGH A, B and C	No visible growth	ND	ND

-: Gram-negative, ND: Not done

Table 7: Biochemical tests conducted on the bacterial isolates

Biochemical tests	BAK	BS
Catalase	+	+
Oxidase	-	-
Indole	+	+
Methyl red	+	+
Voges Proskauer	-	-
Citrate utilization	-	-
Identity	*E. coli*	*E. coli*

+: Positive test result, -: Negative test result, *E. coli: Escherichia coli*

processing method, contamination from the sorrel calyces, ingredients and poor quality water used for production, and lack of personal hygiene from the home producers resulting to microbial activities and growth. According to the WHO (2021), all the sampled *Zobo* from MGH that recorded 0 CFU/mL can be certified fit for human consumption. Unfortunately, the detection of heterotrophic bacteria in the assayed *Zobo* samples makes it a potential reservoir of bacterial infections. However, *Zobo* samples from the remaining sampling points were entirely unfit for human consumption due to the detection of total coliform and heterotrophic bacteria.

CONCLUSION AND RECOMMENDATIONS

It can be concluded since all the assayed *Zobo* samples from BAK and BS recorded total coliform counts and TVC, it is unfit for human consumption. However, *Zobo* samples purchased from MGH did not record any total coliform count but it did record substantial TVC. These results have established that the consumption of *Zobo* sold on the university campus could be a source of public health crisis due to the detection of total coliform in the beverage. Based on the results obtained in this study, we recommend that array of locally produced drinks and beverages in the category of *Zobo* should be regulated in Nigeria by the National Agency for Food and Drug Administration and Control and other food regulatory bodies, as drinks of low and below minimum safety standards have been established to be injurious to health on acute or chronic basis.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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