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Bacteriological Assessment of Hawked Sorrel Drink (Zobo Drink) in Aba, South-East Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author ORE designed the study, wrote the first draft of the manuscript and managed literature searches. Author SU performed the statistical analysis and wrote the protocol while authors JAN and MUE managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Bacteriological investigation of hawked Sorrel drink (zobo drink) was carried out in Aba, South-east Nigeria, between January and March, 2014. Zobo drink is a non-alcoholic local beverage made from different varieties of dried petals, acid succulent aqueous extract of calyx of roselle, *Hibiscus sabdariffa*. It is consumed in Nigeria and other parts of the world. Samples of zobo drink were randomly purchased from the open markets, for a period of three months and analyzed for bacteriological qualities using standard methods. The result revealed a total aerobic bacterial count ranging from 0.3×10^6 cfu/ml to 4.4×10^6 cfu/ml while the total coliform count ranged from 0.1×10^5cfu/ml to 6.5×10^5 cfu/ml. The control sample gave 0.8×10^6 cfu/ml and 0.1×10^5cfu/ml for aerobic bacterial and coliform count respectively. All the screened samples including the control sample had total aerobic bacterial count above the acceptable limit of $<10^4$cfu/ml. The contamination of the control samples could likely come from the spices (grounded) and additives which are usually added raw, since the appropriate hygienic standards were maintained during preparation. A total of five bacterial isolates were identified, which include *Escherisha coli, Staphylococcus* spp, *Lactobacillus* spp, *Bacillus* spp and *Pseudomonas* spp. Personal and environmental hygiene is required during production, packaging and preservation of zobo to avoid food borne illnesses.

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1. INTRODUCTION

Zobo drink is a non-alcoholic local beverage made from different varieties of dried petals, acid succulent aqueous extract of calyx of roselle, *Hibiscus sabdariffa* which is an annual herb that is widely cultivated in India and Africa [1]. It is a red liquid drink and tastes like fruit punch. Zobo is a name derived from "zoborodo" which is the local Hausa (Northern Nigeria) name for *Hibiscus sabdariffa*. The non-alcoholic drink or zobo is quite popular especially in Northern Nigeria and is usually served chilled at various social gatherings [2,3]. There is an increase in the demand of zobo due to its low price, nutritional and medicinal properties [4]. The economic situation in Nigeria has made zobo drink gain more acceptance in different occasions. It is being consumed by several millions of people from different socio-economic classes and backgrounds in the West African sub-region especially among the youths, who also see zobo as an alternative source of cheap and relaxing non-alcoholic drink in social gatherings [5]. It is used for refreshment, entertainment in parties or as appetizers before the main dish is served and also sold in the markets to various consumers [6].

Zobo drink is prepared by boiling the dry calyces (sepals) of *Hibiscus sabdariffa* in water for about 10-15 minutes from which the pigments embedded in the flower is extracted. The extract may be taken hot as tea or taken as a refreshing drink when chilled. The sharp sour taste of the raw extract is usually sweetened with sugar cane, granulated sugar, pineapple, orange or other fruits depending on choice [7]. However, the variety and preparation of zobo vary from one locality to another; thereby leading to variation in the quality attributes especially the nutrients and microbial qualities as well as the appearance of the products [8].

Zobo is found to be rich in vitamins, natural carbohydrates, protein, calcium, iron, other antioxidants [9,10] and minerals [11]. The leaves of the plant are used as vegetables and the seeds as source of oil [12]. Hibiscus, especially roselle is used in folk medicine as a diuretic mild laxative treatment for cardiac and nerve diseases and cancer. Roselle is associated with traditional medicines and is used as treatment for several diseases such as hypertension and urinary tract infection [13]. The green leaves are used as spicy version of spinach [14]. The seeds are said to be diuretic and tonic in action and the brownish-yellow seed oil is claimed to heal sores on camels [15]. A recent review stated that specific extract of *Hibiscus sabdariffa* exhibits activities against atherosclerosis, liver disease, cancer, diabetes and other metabolic syndromes [16].

The greatest limitation for large-scale production of zobo drink is the rapid deterioration of the drink. Its shelf-life is approximately twenty-four hours following production, if not refrigerated. The drink contains some microorganisms which can cause food spoilage [17]. Furthermore, the mode of packaging the juice in nylon or plastic containers before retailing, and the poor hygienic practices as well as lack of portable water, toilets, proper storage and waste disposal facilities at preparation and service points have resulted in poor unsanitary conditions and thus served as potential contaminants and increased risk to public health [18]. The microorganisms associated with the deterioration of zobo include *E. coli, Bacillus* spp*, S. aureus, S. faecalis, Proteus* spp*, Enterobacter* spp, *Klebsiella* spp, *Micrococcus* spp *Aspergillus* spp*, Penicillium citrinum, Fusarium oxysporum, Rhizopus* spp and *Mucor* spp [19,20]. The present study is aimed at evaluating the bacteriological quality of zobo drinks hawked in Aba, South-east Nigeria. This will serve as an important working tool for the food safety authorities in ensuring that good manufacturing process is maintained.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The study was conducted in Aba, Abia State, Southeast Nigeria. Samples were randomly collected from five popular markets in Aba (coded A-E). Sampling lasted for three months, with each market sampled ten times on different dates. The samples were collected with capped sterile bottles and taken to the Abia State Polytechnic Microbiology Laboratory for analysis. Dried calyces of roselle were purchased from one of the local markets and taken to the laboratory in sterile cellophane bag to prepare the control sample for comparative analysis. The control sample was prepared by maintaining the appropriate hygienic standards.

2.2 Preparation of Zobo Drink

Zobo drink is prepared by boiling the dry calyces of *Hibiscus sabdariffa* in water for 10-15 minutes to extract the pigments. After extraction, the filtrate is spiced and may be taken hot as tea or allowed to cool and packaged in plastic sachet containers and taken as refreshing drink when chilled. The sharp sour taste of the raw extract is sweetened with sugarcane, granulated sugar, pineapple, orange or other fruits, depending on choice.

2.3 Preparation of Media

Two different media were used for the analysis, which include Nutrient agar and MacConkey agar. The media were prepared according to the manufactures' procedures.

2.4 Microbiological Analysis

One milliliter of each sampled zobo drink was put in 9ml of sterile distilled water in sterile test tubes, shaken and then serially diluted. From the appropriate dilution, 0.1ml was inoculated separately on to Nutrient agar and MacConkey agar plates and spread evenly using sterile bent glass rod. Each experiment was carried out in duplicates to get a mean standard value of the colony forming units (cfu/ml) on the plates. The inoculated Nutrient agar and MacConkey agar plates were incubated at 30ºC and 35ºC for 24 hours. After the period of incubation, the colonies on the plates were counted and recorded as colony forming unit per milliliter (cfu/ml) and coliform respectively [21]. Each of the bacterial colonies on the agar plates was sub-cultured and the pure culture obtained. Isolates were identified by carrying out tests which include Gram staining, spore staining and biochemical tests such as catalase, coagulase, oxidase, citrate utilization, indole, methyl red, urease, Voges-Proskauer and sugar fermentation [22,23,24].

3. RESULTS

The total coliform and aerobic bacterial counts are shown in Table 1. The result revealed that the total aerobic bacterial count ranged from 0.3 × 10^6 cfu/ml to 4.4 × 10^6 cfu/ml while the total coliform count ranged from 0.1 × 10^5 cfu/ml to 6.5 × 10^5 cfu/ml. Each count represents an average value for each test sample. The control sample also showed some degree of contamination, with total aerobic bacterial count of 0.8 × 10^6 cfu/ml and the total coliform count of 0.1 × 10^5 cfu/ml.

The morphological and biochemical characteristics of the bacterial isolates are shown in Table 2. A total of five bacterial isolates were identified, which include *E. coli, Bacillus* spp, *Staphylococcus* spp*, Lactobacillus* spp and *Pseudomonas* spp*.*

Table 3 shows the percentage occurrence of the different isolates in each sample. *Staphyloccus* spp and *Lactobacillus* spp had 100.0% occurrence respectively, *E. coli* has 90.0% occurrence, *Bacillus* spp had 50.0% occurrence while *Pseudomonas* spp had the least percentage occurrence of 33.3%.

4. DISCUSSION

The results obtained from the analysis showed that bacteria isolated from the sampled zobo drinks include *E. coli, Staphylococcus* spp*, Lactobacillus* spp*, Bacillus* spp and *Pseudomonas* spp*. E. coli, Staphylocuccus* spp and *Lactobacillus spp* occurred in all the samples. This result agrees with the findings of some authors [19,20,25].

Table 1. Total aerobic bacterial and coliform counts (cfu/ml)

Sample	Aerobic bacterial count (cfu/ml)	Total coliform count (cfu/ml)
A	0.3× 10^6	0.1 × 10^5
B	1.4 × 10^6	4.7 × 10^5
C	2.1 × 10^6	1.3 × 10^5
D	5.0 × 10^5	6.5 × 10^5
E	4.4 × 10^6	3.1 × 10^5
Control	0.8 × 10^6	0.1 × 10^5
Average total count	1.6 × 10^6	2.6 × 10^5

Note: A-E represents the five different markets where the zobo drinks were purchased

Table 2. Morphological and biochemical characteristics of the bacterial isolates

Morphology	Gram Reaction	Catalase	Oxidase	Coagulase	Spore stain	Citrate	Indole	V-P	Methyl red	Urease	Glucose	Lactose	Sucrose	Manitol	Probable isolates
Short rods	-	+	-	-	-	-	+	-	-	-	A/G	A/G	-	-	*E. coli*
Long rods	+	-	-	-	+	+	-	NA	-	NA	A	-	-	A/G	*Bacillus* spp
Cocci in cluster	+	+	-	+	-	-	-	-	+	+	-	-	-	A	*Staphylococcus* spp
Rods in cluster	-	+	-	-	-	-	-	+	+	-	A	-	-	A	*Lactobacillus* spp
Rods in cluster	-	+	+	-	+	-	-	+	-	-	A/G	A	A/G	-	

Key: V-P= Voges-ProsKauer; A/G=Acid and Gas production; A= Acid production; NA= Not applicable

Table 3. Percentage occurrence of the isolates in each sample

Sample	*E.coli*	*Staphylococcus* spp	*Lactobacillus* spp	*Bacillus* spp	*Pseudomonas* spp
A	+	+	+	-	-
B	+	+	+	+	-
C	+	+	+	+	+
D	+	+	+	-	-
E	+	+	+	+	+
Control	-	+	+	-	-
Total (%)	90	100	100	50.0	33.3

Surprisingly, the control sample prepared under standard hygienic conditions was also contaminated. Presumably, the source of contamination of the control sample may have come from the raw materials (dried calyces and additive) which were purchased from the open market. In the open market, *Hibiscus sabdariffa* calyces are displayed in large bowls and polyethylene bags for prospective consumers and in the process exposed to microbial contamination. The calyces are usually boil or soaked in hot water to extract the red pigments, and raw spices (grounded) added to the drink after boiling, might serve as sources of contamination. The occurrence of the different bacterial isolates in zobo drinks is of public health importance. The presence of these bacteria is an indication of contamination. The presence of *E. coli, Basillus* spp and *Staphylococcus* spp obtained from this study is an indication of poor hygienic handling of the beverage. These microorganisms are contaminants from contaminated containers or from untreated water that is normally used in the preparation of zobo [26,27].

Average total count of 1.6×10^6 cfu/ml and 2.6×10^5 cfu/ml for aerobic bacterial and coliform counts respectively were obtained. The range of bacterial count is above the acceptable limit of $>10^4$ [28]. All the sampled zobo drinks were contaminated with varying levels of bacterial counts that can be classified as unsatisfactory. The average coliform count is well above the zero value recommended for safety. The following is therefore recommended:

- In processing zobo drinks, the environmental and personal hygiene should be maintained.
- Packaging materials and additives should be adequately sterilized.
- Potable water should be used during processing.
- Producers of zobo should be educated on the importance of adherence to quality control measures.

5. CONCLUSION

Results obtained in this study have shown a high contamination of zobo drink beyond the acceptable limit. This is dangerous to public health, as these microorganisms can cause varying levels of diseases from food poisoning. The control sample prepared under hygienic conditions also indicated contamination. Sources of contamination in all the screened samples could come from the sources of water used, contamination of the raw materials and lack of personal and environmental hygiene. Therefore, drinks like zobo consumed by all and sundry in Nigeria should be regulated by National Agency for Food and Drug Administration and Control (NAFDAC) and other regulatory bodies, as the drink is commonly consumed among the youths, who also see zobo as an alternative source of cheap, non-alcoholic drink. To effect regulation, producers of both the dried calyces and the drinks, should be adequately educated on personal and environmental hygiene. The spices and additives should be boiled before use. Proper monitoring of producers is very necessary and punitive measures should be enforced against non-compliers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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