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Microbiological Analysis of Zobo Drink Preserved with Scent Leaves (*Ocimum gratissimum*)

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Authors' contributions

This work was carried out in collaboration among all authors. Author CGU designed the study and wrote the protocol. Authors EKA and CEU wrote the first draft of the manuscript. Author CVN performed the statistical analysis. Authors CWN and UDN helped with the analyses of the work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the microbiological quality of zobo drink preserved with scent leaves.
Methods: The zobo drink and scent leaves were prepared and evaluated using standard microbiological techniques.
Results: Twenty three (23) bacteria species and fourteen (14) fungi species were identified from zobo drink preserved with scent leaves samples. This reveals the major bacterial species to be Enterobacter spp, *Staphylococcus aureus*, *Bacillus* spp, and *Micrococcus* spp. and fungi species to be *Aspergillus niger*, *Rhizopus* spp and *Penicillium* spp. The bacterial and fungal counts decreased as the days increased with day 1 having the highest bacterial and fungal counts at 1.41x105 (cfu/ml) and 3.1x104 (cfu/ml) respectively. The control samples were generally higher than the counts recorded on the bacterial and fungal counts. Zobo + scent leaves (ZSC) recorded the highest bacterial count at 1.41x105 (cfu/ml), while the least was recorded for (ZSA) at 1.01x106 (cfu/ml). Zobo + Scent (ZSC) recorded the highest fungal counts at 3.1x104 (cfu/ml), while the

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least was recorded for ZSA at 1.2x105 (cfu/ml). From this study, *Bacillus* spp and *Staphylococcus aureus* were the most frequently occurring bacterial isolates with a high percentage occurrence of 8(21.6%) and 6(16.2%), while *Penicillium* spp was the most frequently occurring fungal isolate.
Conclusion: The association of these microorganisms with foods such as the commercial zobo drinks may be as a result of poor hygiene or poor sanitary condition. The microbial counts showed that among the zobo drink preserved with scent leaves samples, zobo + scent leaves (ZSC) is the most predisposed product to microbial population due to the high microbial counts recorded. Therefore, the result revealed that the samples of zobo drink were directly and indirectly contaminated with high levels of pathogenic bacteria, but can be reduced by the addition of scent leaves as a preservative.

Keywords: Zobo drink; scent leaves; Staphylococcus aureus; Bacillus spp; Penicillium spp.

1. INTRODUCTION

Zobo drinks as a beverage made traditionally which is consumed in all parts of Nigeria, mostly the northern and southern parts [1]. Being a cheap drink, the economic status of Nigeria has made the drink gain wide and general acceptance. It is widely sold, taken as appetizers or served in parties. Zobo drink chemically contains anthocyanins and Vitamins C, among others and it is used in curing minor stomach complications, sore throat and strengthening the heart [2]. Zobo drink is extracted from the dried reddish purple calyces of the plant *Hibiscus sabdriffa*. The calyces are used to produce herbal teas and other food products. The juice drink can be produced by extraction of the calyx of Hibiscus plant. The drink contains some microorganisms which can cause food spoilage [3]. At present, the production processes are neither mechanized nor standardized.

Furthermore, the mode of production, packaging and dispensing of zobo juice in nylon or plastic container before retailing, i.e the poor hygienic practices as well as lack of running potable water, toilet, proper storage and waste disposal facilities at preparation and services point has led to poor sanitary conditions exposure to potential contaminants and an increased risk to public health [4]. Drinks sold in streets and foods safety has been a major health concern globally, and more importantly in Nigeria and some part of Africa were regulatory policies of this critical sector is inadequate, making street foods and drinks hazardous source of nutrition [5].

Foods frequently serve as routes for spreading of several microorganisms some of which are pathogenic and harmful in nature [6]. Many picnic suppers and eateries have come to a halt which home prepared foods and drinks serves not only as food and drinks for guest, but also as the vehicle for transmitting *Staphylococcus* food poisoning. The microorganisms which have been implicated with the deterioration and spoilage of zobo drink include; *S. faecalis, Proteus* spp*, E. coli, Bacillus* spp*, S. aureus, Enterobacter* spp, *Klebsiella* spp, *Micrococcus* spp *Aspergillus* spp*, Penicillium citrinum, Fusarium oxysporum, Rhizopus* spp and *Mucor* spp [7].

A review by Lin et al. [8] stated that specific extract of *Hibiscus sabdariffa* exhibits activities against atherosclerosis, liver disease, cancer, diabetes and other metabolic syndromes. Zobo is becoming acceptable in social gathering because it is economically affordable and attractive to many people more than soda [9]. Increase in religious and health campaigns against alcoholic beverages in Nigeria and the consequent decrease in the consumption of alcoholic beverages in certain areas has afforded Zobo drink great potential as a local alternative to imported red wines in particular and alcoholic beverages in general [10].

Recently, zobo drink has become a main source of income in many homes both in rural communities and in the urban areas where small scale business has increased due to support from the government through the poverty alleviation schemes, thereby alleviating poverty among the people [11].

Ocimum gratissimum is popularly known as scent leaf. It is a full developed flowering plant with root, stem and leaves systems [12]. The plant is naturally used in the treatment of different diseases like diarrhea, headache, fever, ophthalmic, skin disease and pneumonia [13]. In many parts of the world, especially Africa and Asia, plant parts are used for the treatment of various health complications such as inflammation, fever, gout (Krawinkel). The leaf of *Ocimum gratissimum* is used for prevention and

treatment of gout, catarrh, fever and malaria which has been found to be associated with free radical generation [14].

Scent leaf is a major spice used in the production of Zobo drink. Typically, scent leave reduces the microbial density of the zobo drink [15]. Like moringa, scent leave reduced the population of *M. lutens, M. roseus, S. aureus, B. subtilis, Enterobacter faecalis, R. stoloifer, A. flavus, F. poae* and *P. caseicolum*, but do not have effect on the population of *S. cerevisiae, S. ellipsoideus* [16]. Typically, the ability of scent leave to have effects on the microbial quality of zobo could be due to the presence of secondary metabolites found in them. Also blended scent leave and moringa has superior effect on the bacterial density of zobo as when compared to separate blends [15]. Therefore, this study was to determine the microbiological quality of zobo drink preserved with scent leaves.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh zobo leaves were purchased from five (5) different locations namely; Gate Six Market, Ahieke Market, Umuariga, Ndoru and Orieugba Market, while scent leave samples were obtained from National Roots Crops Research Institute, Umudike and confirmed at the Plant Science and Biotechnology Laboratory, Umudike. Each sample was collected separately in sterile plastic containers, labelled according to locations and transported to the laboratory for microbial analysis.

2.2 Preparation of Extracts

The freshly collected leaves were cleared of dirt's in the laboratory. The plants were grind using electric blender (Banitone BLG-450). This was soaked in water to extract the soluble ingredients.

2.2.1 Preparation of zobo with scent leaves

The zobo drinks preserved with scent leaves were prepared in four (4) ratios

1. ZC (control): 100% zobo,
2. ZS_A: (95% zobo: 5% scent leaves),
3. ZS_B: (90% zobo + 10% scent leaves),
4. (ZS_C): (85% zobo + 15% scent leaves).

The mixtures were vigorously stirred with a stirrer and then allowed to stand for 5 days. The mixtures were analyzed at Day 3 and Day 5 for enumeration (microbial counts) and isolation of microorganisms.

2.3 Media Used

Media used includes; Nutrient Agar Medium, MacConkey Agar, and Sabauroud Dextrose Agar. They were prepared according to the manufacturer's instruction.

2.4 Isolation of Microorganisms

Ten-fold dilutions were prepared under aseptic conditions from each of the mixtures using 9ml of distilled water as diluents. Diluted suspensions of 1ml samples were plated over Nutrient Agar Medium, MacConkey Agar, and Sabauroud Dextrose Agar using a pour plate method as described by Oboh and Elusiyan, [17]. Each of the plates containing the extracts mixtures were incubated at room temperature from 3 to 5 days at room temperature (fungi incubation) and 24 to 48 hours (bacteria incubation). After incubation colonies appearing on the Agar surfaces were counted, and the colony forming units (CFU/g) were calculated.

2.5 Identification of Bacterial Isolates

Isolates were analyzed based on morphological features, Gram staining and biochemical characterization. Catalase, oxidase, coagulase, citrate, motility, indole and urease tests were carried out to verify the identity of the organisms. The bacterial isolates were identified and confirmatory identities of bacteria were made using Bergey's manual of determinate bacteriology [17].

2.5.1 Gram staining techniques

A thin smear was made by emulsifying a little portion of organism picked from grown colony of 24 hours old pure culture into a drop of sterile distilled water on a grease free slide. The smear was allowed to air- dry and then heat- fixed by passing it slightly over flame. The slide was carefully placed on the staining rack, and flooded with the primary stain (crystal violet) for 60 seconds. Grams iodine was added (mordant) for 60 seconds. The smear was gently rinsed with tap water. Alcohol (70% ethanol) was applied to decolorize it for 60 seconds. It was then rinsed with tap water again and allowed to dry. The smear was examined under the microscope using oil immersion, objective lens (x100). Gram

positive organisms appeared purple while Gram negative organisms appeared red [18].

2.6 Identification of Fungal Isolates

Fungal isolates were identified based on their colonial morphology and cell morphology using a procedure described by De-hoop in Atlas of clinical fungi as a guide.

2.6.1 Wet preparation

A small portion of fungal growth was isolated with sterile wireloop and placed on a grease free glass slide and teased with a drop of distilled water. A drop of lactophenol cotton blue stain was added and covered with a grease free cover slip. The slide was observed using X10 and X40 objective lenses.

2.7 Determination of Percentage Occurrence of Isolates from the Zobo Drinks Samples

The occurrence of the bacteria and fungi species isolates from the test samples were determined as a percentage ratio of their prevalence relative to the total number of samples examined [19]. The formula below was used

$$\% \text{ occurrence} = \frac{\text{No of positive test}}{\text{Total No tested}} \times \frac{100}{1}$$

3. RESULTS

Table 1 shows the total viable microbial mean count from the Zobo Preserved with Scent leaves for 5days. The samples had Total heterotrophic bacterial count (THBC) which ranges from $1.01x10^6$ cfu/ml to $1.41x10^5$ cfu/ml, Total coliform count (TCC) which ranges from $1.14x10^5$ cfu/ml to $1.78x10^4$ cfu/ml, while the Total Fungal count (TFC) ranges from $1.2x10^5$ cfu/ml to $3.1x10^4$ cfu/ml. The bacterial and fungal counts decreased as the days increased with day 1 having the highest bacterial and fungal counts at $1.41x10^5$ (cfu/ml) and $3.1x10^4$ (cfu/ml) respectively. The control samples were generally higher than the counts recorded on the bacterial and fungal counts.

Table 2 shows the bacterial isolates from the Zobo Preserved with Scent leaves, which were identified by morphological characteristics, pigmentation on media, microscopy, biochemical and sugar fermentation methods. This reveals the major bacterial isolates to be *Enterobacter* spp, *Staphylococcus aureus*, *Bacillus* spp, and *Micrococcus* spp. respectively.

Table 3 Shows the fungal species isolated from the Zobo Preserved with Scent leaves, which were identified by their cultural characteristic and microscopic morphology. These fungi species includes; *Aspergillus niger*, *Rhizopus* spp and *Penicillium* spp respectively.

Table 4 shows the percentage occurrence of bacterial and fungal isolates from the Zobo Preserved with Scent leaves. A total of thirty-seven (37) microbial strains were isolated from the Zobo Preserved with Scent leaves which includes; *Enterobacter* spp (10.8%), *Staphylococcus aureus* (16.2%), *Bacillus* spp (21.6%), and *Micrococcus* spp (13.5%), while the fungal isolates were; *Aspergillus niger* (10.8%), *Rhizopus* spp (10.8%) and *Penicillium* spp (16.2%).

Table 1. Total microbial plate counts on the zobo preserved with scent leaves for 5 days

	Sample code	Day 1	Day 3	Day 5
THBC	ZS_A	$1.14x10^6$	$1.11x10^6$	$1.01x10^6$
	ZS_B	$1.22x10^6$	$1.12x10^6$	$1.07x10^6$
	ZS_C	$1.41x10^5$	$1.18x10^5$	$1.17x10^5$
	CONTROL	$2.42x10^6$	$4.40x10^5$	$4.10x10^7$
TCPC	ZS_A	$1.31x10^4$	$1.25x10^4$	$1.14x10^5$
	ZS_B	-	$1.78x10^4$	$1.75x10^5$
	ZS_C	-	-	$1.4x10^5$
	CONTROL	$4.6x10^6$	$4.9x10^5$	$4.6x10^5$
TFPC	ZS_A	-	-	$1.2x10^5$
	ZS_B	-	-	$1.9x10^5$
	ZS_C	$3.1x10^4$	$2.3x10^5$	$2.1x10^5$
	CONTROL	$2.67x10^6$	$5.3x10^5$	$5.0x10^5$

Key: THBC = Total Heterotrophic Bacteria Count, TCPC = Total Coliform Plate Count, TFPC = Total Fungal Plate Count

Table 2. Biochemical identification, morphological Identification and gram reaction bacterial isolates from zobo drink preserved with scent leaves

Colonial features	Gram reaction	Cell arrangement	Catalase	Oxidase	Coagulase	Indole	Citrate	Motility	Urease test	Hydrogen sulphide	Voges-Proskeur	Suspected bacteria
Tiny Yellow Colonies	+	Cocci	+	+	_	NA	NA	_	_	NA	NA	*Micrococcus* spp
Smooth Golden Yellow colonies	+	Cocci	+	+	+	NA	NA	_	_	NA	NA	*Staphylococcus aureus*
Large creamy colonies	+	Short Rod	_	+	_	NA	_	_	_	NA	NA	*Bacillus* spp
Large pink mucoid colonies	_	Short Rod	+	_	_	_	+	+	_	_	+	*Enterobacter* spp

Key: - = Absent + = Present, NA = Not applicable

Table 3. Cultural, morphology and microscopic characteristics of fungal isolates from zobo drink preserved with scent leaves

S/N	Cultural characteristics	Microscopic characteristics	Probable fungi
1	Dark – brown mycelium	Septate hyphae, irregular branched conidiospore	*Aspergillus niger*
2	Rapidly growing white cottony colonies on SDA plates	Upright sporangiosphore borne on a septate hyphae with numerous oval spores.	*Rhizopus* spp
3	Bright-green colonies with white edges on SDA plates	Long slender conidiosphores branched at the apex with septal conidia and septate hyphae	*Penicillium* spp

Table 4. Percentage occurrence of the various isolates from zobo drink preserved with scent leaves

Isolates	No of isolates	Percentage occurrence (%)
Micrococcus spp	5	13.5
Staphylococcus aureus	6	16.2
Bacillus spp	8	21.6
Enterobacter spp	4	10.8
Aspergillus niger	4	10.8
Rhizopus spp	4	10.8
Penicillium spp	6	16.2
Total	37	100

Table 5 shows the distribution of bacterial and fungal isolates from the Zobo Preserved with Scent leaves. Among the zobo samples investigated for bacterial and fungal contaminants, Zobo + Scent (ZS_A) had the highest number of isolates at 7(18.9%) while least distributed was recorded for ZS_C at 3(8.1%) each.

Table 5. Distribution of bacterial and fungal isolates from zobo drink preserved with scent leaves

Isolates	Samples				
	ZS_A	ZS_B	ZS_C	C_T	Total
Micrococcus spp	+	+	-	-	2
Staphylococcus aureus	+	-	-	+	2
Bacillus spp	+	-	+	+	3
Enterobacter spp	+	-	-	+	2
Aspergillus niger	+	-	-	+	2
Rhizopus spp	+	+	-	+	3
Penicillium spp	+	+	+	+	4
	7(38.9)	3(16.7)	2(11.1)	6(33.3)	18(100%)

4. DISCUSSION

Local nutritional drinks are consumed by a lot of people probably due to their medicinal and nutritional properties. Zobo is produced from the calyces of *H. sabdariffa* and is one of the local drinks consumed in Nigeria irrespective of the socio-economic status. Different products have been severally produced from *H. sabdariffa*. The zobo drink are sold in several public places in Nigeria including market, motor parks, streets, outside schools, hospitals and highway way due to their convenience and low cost. Therefore this study was to evaluate the microbiological quality of zobo drink preserved with scent leaves.

From this study a total of twenty three (23) bacteria strains were isolated and identified using morphological characteristics, pigmentation on media, microscopy, and biochemical methods from zobo drink preserved with scent leaves. This reveals the major bacterial species to be *Enterobacter* spp, *Staphylococcus aureus*, *Bacillus* spp, and *Micrococcus* spp., and a total of fourteen (14) fungal strains to belong to *Aspergillus niger*, *Rhizopus* spp and *Penicillium* spp.

The total microbial counts evaluated in this study varied from one sample to the other. The bacterial and fungal counts decreased as the days increased with day 1 having the highest bacterial and fungal counts at $1.41x10^5$ (cfu/ml) and $3.1x10^4$ (cfu/ml) respectively. The control samples were generally higher than the counts recorded on the bacterial and fungal counts. Among the various zobo drinks preserved with Scent leaves investigate for microbial contamination, zobo + scent leaves (ZS_C) recorded the highest bacterial count at $1.41x10^5$ (cfu/ml), while the least was recorded for (ZS_A) at $1.01x10^6$ (cfu/ml). Zobo + Scent (ZS_C) recorded the highest fungal counts at $3.1x10^4$ (cfu/ml), while the least was recorded for ZS_A at $1.2x10^5$ (cfu/ml).

The control sample also showed increasing degree of contamination at the various days of incubation, with a total bacterial and fungal count recorded as $4.9x10^5$, $4.6x10^5$ and $2.48x10^6$ for 5days, the total bacterial and fungal counts was recorded as $5.3x10^5$, $5.0x10^5$ and $2.67x10^6$. However these values increased as the period of incubation increased but slight variations in the fungal counts. Zobo preserved with scent leaves recorded low counts when compared with zobo only (control). These values depend on the type of flavor, preservatives used and storage duration, it also corresponds with the result reported by Egbere et al. [10].

The findings of this study have some similarity with previous study. For instance, Bukar [20] reported total viable bacterial counts in the range of $\leq$ 30 - $1.23x\ 10^4$ cfu/ml in zobo sold in Kano metropolis, Kano state. Ezeigbo [21] reported total viable counts ($0.3 - 4.4 \times 10^6$ cfu/ml), total coliform ($0.1 - 6.5 \times 10^5$ cfu/ml) in zobo sold in Market in Aba, Abia State, South Nigeria. Zumbes [22] reported total viable counts (5.20 - 7.70 cfu/ml), total coliform ($x10^4$ cfu/ml) in zobo sold in Jos metropolis, Plateau state. Anagu [23] reported total viable bacterial counts in the range of $3.0 \times 10^2 - 1.0 \times 10^5$ cfu/ml in zobo sold in Awka metropolis, Anambra state. Risiquat [24] reported total viable bacterial counts in the range of $1.2 \times 10^2 - 1.2 - 10^6$ cfu/ml in zobo sold in Markets, Osun state. Slight variations that exist in the findings of this study when compared with previous studies could be due to handling period, quality of the materials used for production and hygienic status of the processors and vendors. The isolation of bacterial in all the zobo drinks samples and the unacceptable total bacteria and fungi count of $\geq 10^4$ CFU/ml established in the

screened zobo drink samples implies extreme contamination and potential health risk of these zobo drink samples.

The microbes isolated from this study has some similarity with the findings of other authors on zobo drinks sold in different locations in Nigeria including Kano metropolis [20], Aba metropolis [21], Jos metropolis [22], Awka metropolis [23], Ibadan metropolis [25], Abakaliki Alo, [26] and Abia state Nwachukwu [27]. These microorganisms have been implicated in outbreak of dysentery, diarrhoea and other gastrointestinal diseases.

However, Gram positive bacteria were found to occur more than Gram negative bacteria. Most bacteria contaminants are Gram positive bacteria, which would account for their predominance on the zobo drink. This agrees with previous reports by Raimi [28] who reported the presence of *E. coli, Staphylococcus* spp*, Lactobacillus* spp*, Bacillus* spp and *Pseudomonas* spp. in zobo drink samples. Most of the bacteria found in zobo drink are known to cause several diseases in human. Some potential diseases caused by some of the bacteria found in Zobo drink include enteric fever, food poisoning and bacillary dysentery [29].

The contamination rate and percentage occurrence accessed on different zobo drink preserved with scent leaves revealed that *Bacillus* spp and *Staphylococcus aureus* were the most frequently occurring isolates with a high percentage occurrence of 8(21.6%) and 6(16.2%) respectively, followed by *Micrococcus* spp 5(13.5%) and *Enterobacter* spp 4(10.8%), which might be as a result of inadequate ascetic condition during their preparation and processing. These findings is in agreement with the result obtained in a study by other authors on zobo drinks sold in different locations in Nigeria including Aba metropolis [21]. It is possible that the occurrence of these pathogens occurred during processing, which was reported as the major source of contamination of locally made zodo drinks by Fowoyo [30]. Necessary precautions might have been neglected and as such contamination could be inevitable as reported by Musa and Hamza [31].

Among the various bacteria species isolated from the zobo drink preserved with scent leaves *Micrococcus* spp, *Staphylococcus aureus*, *Bacillus* spp were exclusively associated with ZS_A. From the present study, *Bacillus* spp was present in all the zobo drinks except ZS_B, which is similar to results obtained by Mohammed and Ismail [32] reported that zobo drink harbours bacteria such as the *Bacillus species*. The possible reasons for dominance may be from contaminant from the environment such as soil and processing equipment and are able to withstand high temperature due to their ability to form spore [33].

Also, *Staphylococcus aureus* isolated from the zobo drinks preserved with scent leaves except ZS_B, and ZS_C. *Staphylococcus aureus* is ubiquitous in air, water, milk and on food contact surfaces. *Staphylococcus specie* in zobo drink could possibly be through the processing methods which usually involve the use of hands since the organism is a common flora of the skin. Besides, other sources of contamination might be the packaging materials or containers which are not properly washed and sterilized. This organism may be responsible for staphylococcal food poisoning, which may also cause similar effect in *Zobo* drink. The presence of *Staphylococcus aureus* in *Zobo* drink is a pointer to largely poor hygiene, improper storage facilities and use of low quality raw material [34]. Occurrence of *Enterobacter* spp (coliforms) in the zobo drinks preserved with scent leaves is an indication of a feacal contaminated drink that must have been from the water (feacal contaminated) during the processing of the zobo drink. This is because most vendors are admitted to using water to dilute zobo drink after boiling and this is a possible source of bacterial contamination to the already boiled zobo. *Micrococcus* species which were detected or isolated from ZS_A, and ZS_B are harmless saprophytic bacteria occurring on the skin of humans and animals.

However, there were wide variations in the fungi population, with *Penicillium* spp 6(16.2%) being most predominant and occurring isolates, followed by *Aspergillus niger* and *Rhizopus* spp at 4(10.8%) each. These results corroborate previous studies of Braide et al. [16] who isolated *Aspergillus, Penicillum, Saccharomyces* (Fungi/yeasts) which had high dormancy in different zobo drinks sold in different market in Uyo, Akwa Ibom state, Nigeria. Some species of fungi could cause disease condition especially in immunocompromised patients as well. Some of this notable fungi species such as *Penicillium, Fusarium* and *Aspergillus* species have the tendency to produce toxins that are harmful to human health WHO [35].

The isolation of from these zobo 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 [36].

The three molds isolated, *Penicillium* spp was found to be associated with ZS_A, ZS_B, and ZS_C, indicating that it can grow on any food stuffs irrespective of its variation in nutrient composition, moisture contents and pH. However, *Aspergillus flavus* and *Rhizopus* spp, was exclusively isolated from the ZS_A samples. The trend in variations in the fungal population followed is similar to that of qualitative variations. The presence of three molds genera isolated in the present investigation is similar to those isolated earlier by Joseph and Adogbo [37].

Occurrences of these microorganisms are largely due to their presence in nature. Their association with foods such as the commercial *Zobo* may be as a result of poor hygiene or poor sanitary condition as reported by Raima [28]. The isolation of coliform bacteria in all the *Zobo* samples exceeds the recommended limit of zero coliform/ml in drinks. These coliforms are potential hazard for human especially during food consumption [28]. Coliforms, whose natural habitat is the intestinal tract of man and animal, revealed possible association of these faecal indicators into the commercially procured *Zobo*. Their presence may also indicate the presence of faecal or contamination by sewage introduced into the *Zobo* via the use of contaminated water or from the unsanitary environment during processing [38].

5. CONCLUSION

It may be concluded from the present study that *Bacillus* spp and *Staphylococcus aureus* are the most frequently occurring bacteria isolates from the zobo drink preserved with scent leaves and accounts for the bacteria contamination of zobo drink, while among the fungi species, *Penicillium* spp (molds) is the common genera of molds generally isolated from the fresh zobo drink preserved with scent leaves during the present investigation. Also from the present study, the microbial counts showed that among the zobo drink preserved with scent leaves, zobo + scent leaves (ZS_C) is the most predisposed product to microbial population due to the high microbial counts recorded. Therefore, the result revealed that the samples of zobo drink were directly and indirectly contaminated with high levels of pathogenic bacteria, but can be reduced by the addition of scent leaves as a preservative. However the occurrence of these pathogens can essentially be reduced or prevented by employing the good manufacturing practices (GMP). From this research, the issue of food safety is of paramount importance in developing countries especially in Nigeria. Food borne illness is really preventable by good hygiene and standard food handling techniques.

6. RECOMMENDATIONS

- It is recommended that producers should aim at, wherever possible, to develop formulations which are incapable of microbial growth.
- The level of microbial contamination in the zobo drink preserved with scent leaves, should be made clear in the microbial limit standards and should be maintained in the products during their use and production.
- In spite of the inevitable contamination by the producers, addition of a suitable preservative in the products should be guaranteed to control microbial growth even before they are marketed.
- There is need to educate the producers on good manufacturing practices (GMP) in order to safe guard against the risk of food borne illness.
- Drinks and beverages should be regulated in Nigeria by NAFDAC and other food regulatory bodies, as drinks of low and below minimum safety standard is injurious to health on acute or chronic basis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The authors declare that all experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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