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Microbial Content of Zobo Drink from Five Different Producers within Yenagoa City Bayelsa State, Nigeria

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ABSTRACT

The Microbial content of the local beverage Zobo prepared from five distinct sources in Yenagoa Local Government Area, Bayelsa State, Nigeria was investigated in order to determine the quality of the consumed drink and its overall implication on human health. The drink samples from the five sources were labeled alphabetically from letters A to E. A control sample drink was also prepared under hygienic condition. All zobo drink samples were prepared by processing the dried calyces of Roselle. All the samples and control were screened for aerobic bacterial and fungal count using standard techniques. The result from the study showed that all samples and control had total aerobic and bacterial count above the acceptable limit of <104 cfu/ml. The total bacterial count ranged from 4.1 X 105 cfu/ml to 8.6 X 105 cfu/ml in which the highest count was obtained from the D sample. The total fungal count ranged from 4.9 X 105 cfu/ml to 6.1 X 105 cfu/ml in which sample B had the highest. The control sample had the lowest bacteria and fungi count. A total of eight bacterial and six fungal isolates were identified. The isolated bacteria are Staphylococcus aureus, Pseudomonas aeruginosa, Micrococcus luteus, Escherichia coli, Bacillus subtilis, Enterobacter aerogenes, Enterobacter cloacae and proteus vulgaris. The isolated fungi were Aspergillis flavus, Mucor spp, Cladosporium spp, Penicillium spp, Candida spp and Rhizopus spp. Based on the findings of this work, it can be concluded that locally prepared zobo drink possess a serious risk to public health.

Key words: Microbial, Zobo Drink, Yenagoa.

1. INTRODUCTION

In Nigeria, there is a growing disinterest in the consumption of conventional carbonated drinks as a result of the perceived health implications of excess sugars. Therefore locally prepared beverage drinks are taking preeminence. Zobo drink is one of the traditionally prepared drinks that have gradually taken the consumption vacuum left by erstwhile consumers of carbonated drinks. That aside, it is cheap and easily affordable and provides a ready means of hydration in yenagoa where portable water supply is a daunting challenge. Zobo drink is prepared by either steeping or boiling the calyx of the sorrel plant *Hibiscus sabdariffa* in portable water and usually sweetened with sugar and served chilled to consumers [1, 2]..The preparation of Zobo drinks varies from community to community and from person to person [3, 4]. Therefore the issue of hygiene and sterilization is of immense importance in the preparation of the drink. Sadly, zobo drink is often prepared for commercial purposes by the lower income and socially subjugated group of Nigerians that hardly have access to portable water and proper hygiene. Therefore there is an acute societal need to investigate its microbial content, suitability for consumption and the wider implication of its consumption on human health. This will provide information for regulatory purposes as well as information for researchers and health professionals

2. MATERIALS AND METHODS

2.1 Preparation of Control Zobo Drink Sample

Dried calyces of roselle were purchased from one of the local markets and taken to the laboratory in a sterile cellophane bag to prepare the control sample for comparative analysis. The control sample was prepared by maintaining the appropriate hygienic standards. Zobo drink was prepared by boiling the dry calyces of *Hibiscus sabdariffa* in water for 10 to 15 minutes to extract the pigments. After the extraction, the sharp sour taste of the raw extract (filtrate) was sweetened with pineapple fruit, cola flavor and pineapple flavor. The prepared drink was put in sterile bottle and labelled "control".

2.2 Collection of Samples (A – E)

Prepared zobo drink samples were randomly collected from five different producers in Yenagoa city, Bayelsa State, Nigeria. The samples were collected with capped sterile specimen bottles and taken to the Microbiology laboratory of the Niger Delta University for analysis. The sterile bottles were coded (A – E) to differentiate them.

2.3 Preparation of Media

Five different media were used in this study. These are Nutrient Agar, MacConkey Agar, Mannitol Salt Agar (MSA), Cetrimide Agar, Sabouraud Dextrose Agar (SDA). The media were prepared according to manufacturer's procedure.

2.3.1Nutrient Agar

28 g of nutrient agar was transferred into a conical flask containing 100 ml of distilled water. The medium was then left to soak for 10 minutes before swirling to dissolve property.

2.3.2 MacConkey Agar

53.5g of MacConkey Agar was suspended in 1000 millimeters distilled water. It was heated to boiling point to dissolve the medium completely and autoclaving at 121^0C for 15 minutes. It was cooled at 45 – 50^0C before it was poured into a sterile petri-plates

2.3.3 Mannitol Salt Agar (MSA)

Mannitol Salt Agar (111g) was suspended in 1000 millimeter of distilled water to prepare the medium. The medium was boiled to dissolve completely and then sterilized in an autoclave at 121^0C for 15 minutes. It was cooled at 45 – 50^0C before it was poured into a sterile petri-plates.

2.3.4 Cetrimide Agar

45.5g of Cetrimide Agar was suspended in 1000millimeter of distilled water. 10ml of glycerol was added and boiled to dissolve completely. The medium was sterilized by autoclaving at 121^0C for 15minutes and cooled to 50^0C before being poured into sterile petri-dishes.

2.3.5 Sabouraud Dextrose Agar (SDA)

65g of Sabouraud Dextrose Agar was suspended in 1000millimeters to prepare the medium. The medium was allowed to soak for 10 minutes. Swirl to mix in an autoclave at 121^0C for 15minutes and cooled to 50^0C before being poured into sterile petri-dishes.

2.4 Sterilization

All glassware include pipettes, petri-dishes, flask, measuring cylinders and test tubes, distilled water, media, sample bottles and slides were sterilized at 121^0C for 15 minutes at 15pst.

2.5 Viable Count of Bacteria and Fungi

Nine (9) mm of the diluent (distilled water) was dispensed into test tubes and 1ml of the zobo sample to be analyzed were taken with the aid of 5ml sterile syringe and transferred to the first set of test tubes (dilution 10^{-1}). The 10^{-2} dilution was prepared by transferring 1ml of dilution 10^{-1} into test tubes containing 9ml diluent. Similarly, 1ml of dilution 10^{-2} was dispensed into 9ml diluent to obtain dilution 10^{-3}. The same procedure was used to make serial dilution of 10^{-4} to 10^{-6}. The pour plate method was used in determining the viable count of the various samples of zobo drink.

2.5.1 Bacteria

One millimeter (1ml) of the various dilutions were placed or transferred in the center of sterile Petri dishes using 5ml sterile syringe. Molten cool agar i.e. (Nutrient agar for bacteria count) 20ml was poured into the Petri dishes containing the inoculums (samples) and mixed well. After the solidification of the agar, the plates were inverted and incubated at 37^oc for 24-48 hours. Different bacterial colonies present on the plates were counted and multiplied by the reciprocal of the appropriate dilution factor to obtain the viable count.

2.5.2 Fungi

The method stated above was used in determining the fungal count of the various samples of zobo drink but molten cooled Sabouraud Dextrose Agar (SDA) was used instead of Nutrient Agar (NA) as it is selective medium primarily used for the isolation of dermatophytes, other fungi and yeasts. After the solidification of the agar, the plates were incubated at room temperature for five days. Different fungal colonies present on the plates were counted and multiplied by the reciprocal of the appropriate dilution factor to obtain the viable count.

2.6 Isolation of Bacteria and Fungi and Maintenance of Pure Culture

The pour plate method was used in isolating pure culture. Colonies that appeared on agar plate (Nutrient agar plates and Sabouraud Dextrose Agar plates) were sub-cultured aseptically by streaking into newly prepared agar plates using sterile wire loop. The streaked plates for bacteria were incubated for 24hrs at 37^0C while the fungi colony morphology was identified directly from the plate after incubating for 5days at room temperature. Pure colonies of bacteria were inoculated into slants and incubated for 24hrs at 37^0C and kept in a refrigerator to keep the culture viable for further use.

2.7 Identification of Bacteria and Fungi from Zobo Drink Samples

Cultured isolates of bacteria cells were characterized and identified by using biochemical tests, as well as observing the characteristic colony morphology of bacteria colonies. Gram staining was also carried out on bacteria isolates to ascertain their response to gram staining.

2.7.1 Morphological Characteristics

The morphological characteristics of isolated bacteria colonies on growth media were observed after growth. Few of the morphological characteristics observed were those relating to bacteria colony shape, color, texture, elevation and margin.

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2.7.2 Gram Stain

In this procedure, a thin smear of bacteria isolate was made on a clean glass slide using a sterile wire loop to transfer a small portion of distilled water unto the slide and aseptically transferring a portion of bacteria isolate to the slide and gently smearing into the water. The smear was then air dried and heat fixed by quickly passing the slide two to three times through a Bunsen burner flame. The smear was then flooded with crystal violet and allowed to stand for one minute before being gently washed off with tap water. The smear was again flooded with lugos iodine solution and allowed to stand for one minute before being again gently washed off with tap water. 95% alcohol was then used to decolorize the dyes before being washed by gently flooding the smear with tap water. Safranin was poured over the smear and allowed for one minute before being washed with running tap water and allowed to air dry. The smear was then observed under oil immersion with (100x) objective using a light microscope.

2.8 Biochemical Test

Some biochemical tests used to identity bacteria isolates include catalase, coagulase, indole, motility, and oxidase. These tests create a platform from which isolated bacteria colonies were identified.

2.8.1 Catalase Test

A loopful of 3% hydrogen peroxide was transferred unto a clean glass slide, and a small portion of selected bacteria of pure culture was smeared onto the drop of hydrogen peroxide. An observed bubbling effect indicates oxygen gas production. Such organism is said to be catalase positive.

2.8.2 Coagulase Test

Blood plasma was dropped on a grease free glass slide, and the bacteria isolate was aseptically smeared on it using a sterile wire loop. Clothing indicates positive result, while non-clotting indicates organisms without coagulase enzyme, and are therefore termed negative.

2.8.3 Indole Test

In this test, 10 g of peptone and 8 g of sodium chloride were dissolved in 1 litter of distilled water. It was then heated and dispensed to the test tubes and sterilized. The isolates were inoculated into the test tubes and incubated at 37 ^{o}C for 2-5 days. About 2-3 drops of kovac's reagent was then added into the test tubes. The appearance of a red layer or red ring on the culture indicates a positive result.

2.8.4 Motility Test

This test was done to determine the non-flagellated bacteria. The isolates were inoculated into the liquid motility medium after cooling and incubated at 370C for 2 – 4days. Motile organisms grow diffusely from the top to the bottom of the test tubes while non-motile bacteria only grow on top the motility medium.

2.8.5 Oxidase Test

1.1 g of oxidase reagent (tetra-methyl-p-phenylene diamine dehydrochloride) was dissolved in 10 ml of distilled water, which was added into filter paper in a petri dish. The isolated test culture was then smeared unto the filter paper using a glass Rod. A positive result shows the development of dark purple color within 10 seconds.

2.9 Fungal Identification

The selected fungal isolates were culturally characterized by observing the colour, texture and margin of the colonies on sabouraud dextrose agar. The microscopic morphology characterization was done using potassium hydroxide treatment (KOH).

2.9.1 Use of Potassium Hydroxide (KOH) Treatment

Fungi isolates were identified using KOH reagent. This reagent and digest the debris surrounding the fungi so that hypae and conidia (spores) can be seen. A drop of KOH solution was dropped on a clean slide with the smear of the suspected fungi organism on it. The slide was covered with a clean cover slip. The slide was placed in a petri dish and was allowed to stand for 30minutes to digest the debris. The slide was examined microscopically using the 10x and 40 x objective views with the condenser and iris diaphragm closed sufficiently to give good contact (Onyeagba, 2004).

3.0 RESULT AND DISCUSSION

3.1 Microbial Load of Various Zobo Drink Samples

The microbial load of various zobo drink samples are shown in Tables 1 and 2. The total bacteria count ranged from 6.1 X 10^5 cfu/ml to 4.9 X 10^5 cfu/ml in which sample B had the highest microbial load. The control sample had the lowest bacteria and fungi count.

3.2 Bacteria and Fungi Isolated From Zobo Drink Samples

The cultural and biochemical characteristics of bacterial isolates of zobo drink are shown in Table 3-8. Isolate 1 (identified organism) *Staphylococcus aureus* occurred in six samples. Isolate 2 (identified organism) *Pseudomonas aeruginosa* occurred in two samples. Isolate 3 (identified organism) *Micrococcus luteus* occurred in three samples. Isolate 4 (identifies organism) *Escherichia coli* occurred in four samples. Isolate 5 (identified organism) *Bacillus subtilis* occurred in one sample. Isolate 6 (identified organism) *Enterobacter aerogenes* occurred in one sample. Isolate 7 (identified organism) *Enterobacter cloacae* occurred in one sample. Isolate 8 (identify organism) *proteus vulgaris* occurred in two samples. Only isolate one was isolate from the entire zobo drink sample.

The colonial (Macroscopic) and Microscopic charactertics of fungi isolates of zobo drink are shown in Tables 9-14.

F-1 (Probable genera) *Aspergillus flavus occurred in two samples.*

F-2 (Probable genera) *Mucor spp occurred in one sample.*

F-3 (Probable genera) Cladosporium spp occurred in one sample.

F-4 (Probable genera) Penicillium spp occurred in two samples.

F-5 (Probable genera) Candida spp occurred in two samples.

F-6 (Probable genera) Rhizopus spp occurred in one sample.

Only isolate F-2 was isolated from five sample.

Table 1: The Total Bacterial Count of Zobo Drink Sample

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Zobo Drink Samples	Dilutions	Colonies On Plates	Bacterial Count (Cfu/ml)
A	10^{-6}	86	8.6×10^5
B	10^{-6}	69	6.9×10^5
C	10^{-6}	63	6.3×10^5
D	10^{-6}	88	8.8×10^5
E	10^{-6}	54	5.4×10^5
CONTROL	10^{-6}	41	4.1×10^5

Table 2: The Total Fungal Count Of Zobo Drink Sample

Zobo Drink Samples	Dilutions	Colonies On Plates	Fungal count (cfu/ml)
A	10^{-6}	61	6.1×10^5
B	10^{-6}	73	6.9×10^5
C	10^{-6}	58	6.3×10^5
D	10^{-6}	68	8.8×10^5
E	10^{-6}	59	5.4×10^5
CONTROL	10^{-6}	49	4.1×10^5

KEY: CFU-Colonies forming units.

:A-E represents the five different producers whom the zobo drinks were purchased.

Determination were done in duplicates

Table 3: Characteristics of Bacteria Isolated from Zobo drink Sample A

ISOLATE NUMBER	COLONIAL CHARACTERISTIS	GRAM STAIN	CELLULAR MORPH	MOT	COAG	INDO	CATA	OXID	IDENTIFIED ISOLATE
1	Circular pinhead, smooth, entire, convex, yellow colony	+	Cocci in grape-like clusters	-	+	-	+	-	*Staphylococcus aureus*
6	Round, shiny, entire, convex, yellow colony	-	Rods	+	-	-	+	-	*Entererobacter aerogenes*
8	Round ,entire ,convex mucoid, yellowish colony	-	Rods	+	-	+	+	-	*Proteus Vulgaris*
4	Circular, entire, slightly raised, mucoid, Spink colony	-	Rods	+	-	+	+	-	*Escherichia coli*

Table 4: Characteristics of Bacteria Isolated from Zobo drink Sample B

ISOLATE NUMBER	COLONIAL CHARACTERISTIS	GRAM STAIN	CELLULAR MORPH	MOT	COAG	INDO	CATA	OXID	IDENTIFIED ISOLATE
4	Circular, entire, slightly raised, mucoid, pink colony	-	Rods	+	-	+	+	-	*Esherichia coli*
3	Circular, pinhead, smooth, entire, convex, bright-yellow colony	+	Cocci	-	-	-	+	-	*Micrococus lutus*
1	Circular, pinhead, smooth, entire, convex, yellow colony	+	Cooci in grape-like clusters	-	+	-	+	-	*Staphylococcus aureus*

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7	Round, shiny, entire, convex, cream-pink colony	-	Rods	+	-	+	+	-	*Entererobacter cloacae*

Table 5: Characteristics of Bacteria Isolated From Zobo Drink Sample C

ISOLATE NUMBER	COLONIAL CHARACTERISTIS	GRAM STAIN	CELLULAR MORPH	MOT	COAG	INDO	CATA	OXID	IDENTIFIED ISOLATE
2	Oval, mucoid, wavy, umbonate, diffusible green colony	-	Rods	+	-	-	+	+	*Pseudomonas aeruginosa*
5	Irregular, wavy, lobate, flat, white and dull colony	+	Rods in chains	+	-	-	+	-	*Bacillus subtilis*
1	Circular, pinhead, smooth, entire, convex, yellow colony	+	Cooci in grape-like clusters	-	+	-	+	-	*Staphylococcus aureus*

Table 6: Characteristics of Bacteria Isolated From Zobo Drink Sample D

ISOLATE NUMBER	COLONIAL CHARACTERISTIS	GRAM STAIN	CELLULAR MORPH	MOT	COAG	INDO	CATA	OXID	IDENTIFIED ISOLATE
1	Circular, pinhead, smooth, entire, convex, yellow colony	+	Cooci in grape-like clusters	-	+	-	+	-	*Staphylococcus aureus*
4	Circular, entire, slightly raised, mucoid, pink colony	-	Rods	+	-	+	+	-	*Esherichia coli*
3	Circular, pinhead, smooth, entire, convex, bright yellow colony	+	Cooci	-	-	-	+	-	*Micrococus lutus*

Table 7: Characteristics of Bacteria Isolated from Zobo drink Sample E

ISOLATE NUMBER	COLONIAL CHARACTERISTIS	GRAM STAIN	CELLULAR MORPH	MOT	COAG	INDO	CATA	OXID	IDENTIFIED ISOLATE
2	Oval, mucoid, wavy, umbonate, diffusible green colony	-	Rods	+	-	-	+	+	*Pseudomonas aeruginosa*
1	Circular, pinhead, smooth, entire, convex, yellow colony	+	Cocci in grape-like clusters	-	+	-	+	-	*Staphylococcus aureus*
8	Round, entire, convex, mucoid, yellowish colony	+	Rods	+	-	+	+	-	*Proteus vulgaris*
4	Circular, entire, slightly raised, mucoid, pink colony	-	Rods	+	-	+	+	-	*Escherichia coli*

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Table 8: Characteristics of Bacteria Isolated From Zobo Drink Control Sample

ISOLATE NUMBER	COLONIAL CHARACTERISTIS	GRAM STAIN	CELLULAR MORPH	MOT	COAG	INDO	CATA	OXID	IDENTIFIED ISOLATE
1	Circular, pinhead, smooth, entire, convex, yellow colony.	+	Cocci in grape-like clusters	-	+	-	+	+	*Staphylococcus aureus*
1	Circular, pinhead, smooth, entire, convex, yellow colony	+	Cocci	-	-	-	+	-	*Micrococcus luteus*

Table 9: Characteristics of Fungi Isolated From Zobo Drink Sample A

ISOLATE NUMBER	COLONIAL (MACROSCOPIC) CHARATERISTICS	MICROSCOPICAL CHARACTERISTICS	PROBABLE GENERA
F-1	Greenish-yellow colour, white edge, floccose texture, velvety cream to yellow on reverse.	Septate h hyphae, globose conidia, rough conidiophores.	*Aspergillus Flavus*
F-2	Filamentous, white-gray cotton-candy colour darkening with time, not rhizoids, pale-white on reverse	Broad non-septate hyphae, long and branched conidiophores, large round sporangia.	*Mucor Spp*

Table 10: Characteristics of Fungi Isolated From Zobo Drink Sample B

ISOLATE NUMBER	COLONIAL (MACROSCOPIC) CHARATERISTICS	MICROSCOPICAL CHARACTERISTICS	PROBABLE GENERA
F-6	Filamentous, fluffy, cotton-candy like growth, whitish in colour but turns brown with age, reverse remains white.	Unbranched sporangiophores, large rhizoids	*Rhizopus spp*
F-5	Cream colour, raised, entire, smooth and butyrous colony	Round o oval cells, purple hypae, septa appears as smaller round grape-like clusters.	*Candida spp*

Table 11: Characteristics of Fungi Isolated From Zobo Drink Sample C

ISOLATE NUMBER	COLONIAL (MACROSCOPIC) CHARATERISTICS	MICROSCOPICAL CHARACTERISTICS	PROBABLE GENERA
F-3	Velvety texture, grayish-brown colour, dark brown to black on reverse.	Erect dark septate hypae, dark pigmented conidiophores, round conidia.	*Cladosporium spp*
F-2	Filamentous, white-gray cotton-candy colour darkening with time, not rhizoids, pale-white on reverse	Broad non-septate hyphae, long and branched conidiophores, large round sporangia.	*Mucor spp*

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Table 12: Characteristics of Fungi Isolated From Zobo Drink Sample D

ISOLATE NUMBER	COLONIAL (MACROSCOPIC) CHARACTERISTICS	MICROSCOPICAL CHARATERISTICS	PROBABLE GENERA
F-2	Filamentous, white-gray cotton-candy colour darkening with time, no rhizoids, pale-white on reverse	Broad non-septate hyphae, long and branched conidiophores, large round sporangia.	*Mucor spp*
F-4	Blue-green colour, white edge, pale-cream to yellow on reverse.	Septate hyaline hyphae, simple conidiophores, brush-like conidia	*Penicillium Spp*

Table 13: Characteristics of Fungi Isolated From Zobo Drink Sample E

ISOLATE NUMBER	COLONIAL (MACROSCOPIC) CHARACTERISTICS	MICROSCOPICAL CHARATERISTICS	PROBABLE GENERA
F-5	Cream colour, raised, entire, smooth and butyrous colony	Round to oval cells, purple hyphae, septa appears as smaller round grap-like clusters.	*Candida spp*
F-4	Blue-green colour, white edge, pale-cream to yellow on reverse.	Septate hyaline hyphae, simple conidiophores, brush-like conidia	*Penicillium Spp*

Table 14: Characteristics of Fungi Isolated From Zobo Drink Control Sample

ISOLATE NUMBER	COLONIAL (MACROSCOPIC) CHARACTERISTICS	MICROSCOPICAL CHARATERISTICS	PROBABLE GENERA
F-1	Greenish-yellow colour, white edge, floccose texture, velvety, cream to yellowish on reverse.	Septate hyphae, globose conidia, rough conidiophores.	*Aspergillus Flavus*
F-2	Filamentous, white-gray cotton candy colour darkening with time, no rhizoids, pale-white on reverse.	Broad non-septate hyphae, long and branched conidiophores, large round sporangia.	*Mucor Spp*

Table 15: Percentage of occurrence of the bacteria isolated from zobo drink samples

Sample	Saureus	P.aeruginose	M.luteus	E.coil	B.subtilis	E.aerogenes	E.cloacae	P.vulgaris
A	+	-	-	+	-	+	-	+
B	+	-	+	+	-	-	+	-
C	+	+	-	-	+	-	-	-
D	+	-	+	+	-	-	-	-
E	+	+	-	+	-	-	-	+
CONTROL	+	-	+	-	-	-	-	-
TOTAL (%)	30%	10%	15%	20%	5%	5%	5%	10%

Table 16: Percentage of occurrence of the Fungi Isolated from zobo drink samples

Sample	A. flavus	Mucor Species	Cladosporium species	Penicillium species	Candida Species	Rhizopus species
A	+	+	-	-	-	-
B	-	-	-	-	+	+
C	-	+	+	-	-	-
D	-	+	-	+	-	-
E	-	-	-	+	+	-
CONTROL	+	+	-	-	-	-
TOTAL (%)	16.66%	33.33%	8.33%	16.66%	16.33%	8.33%

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4.0 DISCUSSION

The result obtained from the analysis show that bacteria isolated from the Zobo sample include *Staphylococcus aureus, Pseudomonas aeruginosa, Micrococcus luteus, Escherichia coli, Bacillus subtillis, Enterobacter aerogenes, Enterobacter cloacae and Proteus vulgaris*. This is in agreement with the findings of some earlier works carried out by reference [5] and reference [6] where they identified microorganisms associated with the plant.

The fungi isolates were *Aspergillis flavus, Mucor spp, Cladosporium spp, Penicillium spp, Candida spp and Rhizopus spp*. Considering the bacterial isolates, *Staphylococcus aureus* has the highest occurrence of 30% while *Bacillus subtillis, Enterobacter aerogenes and Enterobacter cloacae* had the least occurrence of 5%. Among the fungal isolates, *Mucor spp* had the highest occurrence of 33.33% while *Cladosporium spp* and *Rhizopus spp* had the least occurrence of 8.33%.

Surprisingly, the control sample prepared under hygienic conditions was also contaminated. Presumably, the source of contamination of the control sample may have come from the raw materials (dried calyces and additives) which were purchased from the open market.

One reason for this is the fact that *Hibiscus sabdariffa* calyces are displayed in large bowls and polyethylene bags for prospective consumers and in the process exposed to microbial contamination. Also, the calyces are usually boiled or soaked in hot water to extract the red pigments and raw spices (grounded) added to the drink after boiling might have served as sources of contamination.

The occurrence of the different bacterial isolates (*Micrococcus luteus, Escherichia coli, Bacillus subtillis and Staphylococcus aureus*) in the zobo drinks as obtained in 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.

Staphylococcus aureus in zobo could be possible through the processing methods which usually involves the use of hands, since the organism is a common flora of the skin. This organism is also responsible for *Staphylococcal* food poisoning [7]. Reference [8] reported that *Staphylococcus aureus* at levels of 108ml are considered potentially hazardous to consumers. The occurrence of *Pseudomonas spp* also could be as a result of contamination from food handlers or water since the organism is found in water and on skin surface as flora to the skin [9]. Generally, *Escherichia coli* are an indication of water pollution [9] and therefore the presence of the organism in zobo drink is probably related to the source or quality of water used for the processing. In addition, *Escherichia coli* isolated from water may have some health implication [5].

The occurrence of *Bacillus subtilis* could also be as a result of the prevalence of their spores in the environment [10]. *Bacillus* species forms spores which could survive high temperature of processing. *Bacillus* have been isolated from non-alcoholic beverages [11, 12]. *Proteus vulgaris* which occurred in sample A and E might have come from either the soil, water used or from the ingredients used because it is found in water, soil and fecal matter.

The occurrence of *Mucor spp, Cladosporium spp, Penicillium spp and Rhizopus spp* might have come from the soil and plant surface as they are cosmopolitan and ubiquitous organisms found in the soil or growing on mature fruits, plant debris, decaying vegetation, crops seeds, grains leaf surfaces and food stuffs. The fungal isolates therefore found in the plant beverage could be traced to the time when the petals were either being harvested or stored. This may have produced spores

which were attached to the petals and overcome adverse condition during the preparation and finally germinated in the finished product. This is also attributable to the prevalence of their spores in the atmosphere. The liberated spores can easily settle on food and ceilings of rooms and then be germinated [13]. Reference [14] has shown that Aspergillus occurred highest in the number of colonies identified from air spora of some localities. Reference [15] isolated and identified *A. flavus* and *A. niger* from zobo drink.

The isolation of *Aspergillus flavus* in this study is of the highest concern because it is known to produce aflatoxin and can grow at low water activities [15]. Therefore in order to avoid such growth and possible production of toxic metabolites, care should be taken to dry the product quickly before these moulds have the chance of establishing any significant growth.

5.0 CONCLUSION

Consumption of zobo drinks from local drink vendors poses a serious risk to human health as has been demonstrated in this study due to the high prevalence of pathogenic bacteria and fungi. Therefore it is recommended that government and established authority should ensure greater regulatory control in the production, distribution and consumption of zobo and indeed locally prepared beverage drinks as their unregulated production and consumption portends a grave public health concern.

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