



## MICROBIOLOGICAL ASSESSMENT AND PROXIMATE COMPOSITION OF SORREL (ZOBO) DRINKS SOLD IN IKOT EKPENE METROPOLIS, AKWA IBOM STATE, NIGERIA

\*<sup>1</sup>Ekanem J.O, <sup>2</sup>Daniel U.J, <sup>2</sup>Akpan B.C. and <sup>2</sup>Akpan E.A.

<sup>1</sup>Department of Microbiology, University of Uyo, PMB 1017, Uyo, AkwaIbom State, Nigeria.

<sup>2</sup>Department of Science Laboratory Technology, Foundation College of Technology Ikot Idem PMB 1166, Ikot Ekpene, Akwa Ibom State Nigeria.

\*Correspondent author: [johnnykoko01@yahoo.com](mailto:johnnykoko01@yahoo.com); +2348063011636

### ABSTRACT

*This research was conducted to evaluate the proximate composition and microbiological quality of zobo drinks produced and sold locally. Three zobo drink samples were randomly purchased from three markets in Ikot Ekpene metropolis, which include Ator market, White market and Edet Mbat market. The nutritional quality of the zobo drink samples showed high moisture content (85.16%), ash (0.65%), crude fat (0.87%), protein (2.12%) and carbohydrate (11.17%). Their microbiological quality was assessed according to standard methods. Bacteria isolated from the zobo drink samples include *Staphylococcus aureus*, *Lactobacillus* sp, *Pseudomonas* sp, *Bacillus* sp and *Escherichia coli* while the fungal isolates include; *Penicillium* sp, *Aspergillus* sp and *Saccharomyces* sp. The associated bacteria were isolated and identified by following standard microbiology methods; morphology, gram staining, biochemical test. The samples were found to be high in microbial counts of *Escherichia coli*, *Bacillus* sp and *Staphylococcus aureus* as shown in their percentage occurrence. The microbial load ranged from  $2.3 \times 10^5$  to  $8.5 \times 10^5$  for bacteria and  $5.2 \times 10^5$  to  $7.5 \times 10^5$  for fungi. The present findings revealed the nutritive and microbiological quality of zobo drinks retailed and sold in these markets. There is a high potential for these drinks to serve as vehicles for the transmission of food borne illness. Hence, need to employ good manufacturing processes in the production and packaging of these drinks.*

**Key words:** Bacteria, Fungi, Zobo, Microbial load, proximate

### INTRODUCTION

*Hibiscus sabdariffa* is commonly named as “red sorrel” or “roselle”. Even though permeable soil is the best, Roselle can adapt to a variety of soil in a warmer and more humid climate (Robert, 2005). *Hibiscus sabdariffa*, a member of Malvaceae family, is a known medicinal plant with a worldwide fame (Abbas *et al.*, 2011) and the plant can be found in almost all warm countries such as India, Saudi Arabia, Malaysia, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt and Mexico. It is mainly cultivated to be consumed and the main producers of Roselle blossoms are Egypt, Sudan, Mexico,

Thailand and China. Other hibiscus varieties are planted for their fibers they produce (Naturland, 2002). Roselle is believed to be a native plant of West Africa and from there it was carried to other parts of the world such as Asia and America (Cobley, 1975).

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 extract is usually sweetened with sugarcane, granulated sugar,

pineapple, orange or other fruits depending on choice (Osueke and Ehirim, 2004). 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 (Babalola, 2000). At present, the production processes in neither mechanized nor standardized. Consequently, the shelf life of the drink is less than two days (Samy, 1980).

The consumption of 'Zobo', a drink made from the dried calyces of *Hibiscus sabdariffa* has gained prominence in almost every part of Nigeria. Zobo is still locally prepared through indigenous technology and has become accepted in most social gatherings because it is affordable, nutritious and attractive to many people as compared to carbonated drinks (Olayemi *et al.*, 2011). Zobo is rich in natural carbohydrate, protein, antioxidants, vitamin C, calcium, magnesium and zinc. It is a non-alcoholic beverage with medicinal value and has low glycemic index (Wong, 2002; Osuntogun, 2004).

Zobo drink has become an important source of income in many homes both in rural communities and more in the urban areas where cottage business has increased due to support from the government through the poverty alleviation schemes, thereby alleviating poverty among the people (Essien *et al.*, 2011). Owing to the popularity of zobo drink in the area under research and for the fact that it has not been subjected to any post-production treatment, there is need to ascertain the microbiological and nutritive composition of zobo drinks sold in Ikot Ekpene metropolis as several studies has been done with regards to the microbial quality of zobo drinks consumed in other parts of Nigeria.

## MATERIALS AND METHODS

**Study Location:** Ikot Ekpene is a city in Akwa Ibom State, South-South, Nigeria. Its area is approximately 125 km<sup>2</sup>. The city's population is about 254,806. It is situated between 5.18° and 5°10'60.00" North latitude and 7.71° and 7°42'59.99" East longitude and 159 meters elevation above the sea level. The climate here is tropical. Most months of the year are marked by significant rainfall. The short dry season has little impact. The Köppen-Geiger climate classification is Am. The average annual temperature in Ikot Ekpene is 26.4 °C. In a year, the average rainfall is 2349 mm.

### Sample Collection

Three zobo samples from different market sellers at different locations were purchased with a sterile container and taken to laboratory for analysis. The samples collected were labeled with an alphabet for easy identification such as sample A (Ator Market), B (White Market) and C (Edet Mbat Market). The samples were then stored in the refrigerator till the time of use which did not exceed two hours.

### Preparation of Media

Two different media were used for the analysis. The media were Nutrient agar which was used for the isolation of bacteria and Potato dextrose agar which was used for the isolation of fungi. The media were prepared according to the manufactures' procedures.

### Preparation of Nutrient Agar (NA)

Twenty-eight grams (28.0g) of nutrient agar powder was dissolved in 1000 ml of distilled water. The medium was gently heated to dissolve and completely sterilize by autoclaving at 15psi (121°C) for 15 minutes. The autoclaved media was allowed to cool before pouring 15ml each onto sterile Petri dishes and allowed to gel.

### Preparation of Potato Dextrose Agar (PDA)

This was done by suspending 39g of the media in 1000ml of distilled water. The suspended was mixed very well and heated with frequent agitation to dissolve the powder completely. The suspended media was sterilized by autoclaving at 121°C and 15psi, for 15 minutes. The autoclaved media was allowed to cool before pouring 15ml each onto sterile Petri dishes and allowed to gel.

### 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 Potato Dextrose 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 Potato Dextrose agar plates were incubated at 30°C and 35°C for 24 and 48 hours respectively. 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 (Harrigan and McCance, 1976). 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 (Onyeagba, 2004; Cheesbrough, 2006).

The bacteria isolates were characterized using microscopic techniques and biochemical tests. The identities of the isolates were determined by comparing their characteristics with those of known taxa as described by Bergey's manual of Determinative Bacteriology (Holt *et al.*, 1994) and Cheesbrough, (2006). The cultures used for biochemical test were between 18 to 24 hours old.

Fungal identification and enumeration were based on their colony elevation, colour, texture, shape and arrangement of conidia (spherical or elliptical, unicellular or multicellular), branched or unbranched mycelia, presence or absence of cross walls (whether septate or non-septate) and others. They were enumerated by reference to illustrated manual on identification of some seed borne fungi (Kulwant, 1991) and illustrated genera of imperfect fungi (Bernet and Hunter, 1972).

### Proximate Analysis of Zobo Samples

#### Moisture determination

Ten ml of sample was measured in a clean crucible using sensitive balance. The crucible with the sample was placed in an air-dry oven at 105°C and left to stay overnight. Then crucible was transferred to oven again and weighted after 2 hours, this was repeated until constant weight was obtained.

#### Calculation

$$\text{Moisture Content\%} = \frac{(w_2 - w_1) - (w_3 - w_1) \times 100}{w_2 - w_1}$$

Where;

w<sub>1</sub> = weight of empty crucible

w<sub>2</sub> = weight of crucible + sample

w<sub>3</sub> = weight of crucible + dry sample

#### Determination of total ash

The ash content was determined from the loss in weight that occurred during incineration of the evaporated sample at a temperature high enough to

allow all organic matter to be burnt off without allowing appreciable decomposition of the ash constituents. Ashing was carried out in a muffle furnace subjected to heat at 550°C for 6 h (AOAC., 2005).

#### Determination of fat

This was carried out using the method of AOAC (2005). Clean and dried thimble were weighed (W<sub>1</sub>) and 5 g oven dried sample will be added and re-weighed (W<sub>2</sub>). Round bottom flask was filled with petroleum ether (40-60°C) up to <sup>3</sup>/<sub>4</sub> of the flask. Soxhlet extractor was fixed with a reflux condenser to adjust the heat source so that the solvent boiled gently, the sample was put in the thimble and inserted into the soxhlet apparatus and extraction under reflux was carried out with petroleum ether for 6 h. After the barrel of the extractor will be emptied, the condenser was removed and the thimble removed, taken into the oven at 100°C for 1 h and later cooled in the desiccator and weighed again (W<sub>3</sub>):

$$\text{Fat (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

#### Estimation of crude fibre

About 2g of the sample was defatted with petroleum ether for 2 hours. It was then boiled under reflux for some minutes with 200ml of a solution containing 1.25g of H<sub>2</sub>SO<sub>4</sub> per 100ml solution. The solution was filtered through a cloth on fluted funnel and washed with boiling water until they were no longer acidic. The residue was transferred to a beaker and boiled for another 30 minutes with 200ml of a solution containing 1.25g of NaOH per 100ml. The final residue was then filtered and washed with boiling water several times until it was no longer basic. The residue was finally washed twice with methanol and quantitatively transferred into a pre-weighed crucible and dried at 105°C (W<sub>1</sub>). The furnace was incinerated at 550°C and was allowed to stand at this temperature for 2 hours. It was then cooled in a desiccator and weighed as W<sub>2</sub> (AOAC., 2005).

Calculation:

$$\text{Crude fibre (\%)} = \frac{W_1 - W_2}{\text{weight of original sample taken}} \times 100$$

Where; W<sub>1</sub> = weight of empty crucible

W<sub>2</sub> = weight of crucible and its content after incineration

**Determination of crude protein:** One gram of the sample was introduced into micro Kjeldahl digestion flask and one tablet of Selenium catalyst was added. The mixture was digested on an electro thermal heater until a clear solution was obtained. The flask was allowed to cool after which the solution was diluted with distilled water to 50 and 5 of this was transferred into the distillation apparatus, 5 of 2% boric acid was added into a 100-capacity conical flask (the receiver flask) and four drops of methyl red indicator were added. A 50% of NaOH was continually added to the digested sample until the solution turned cloudy which indicated that the solution had become alkaline. Distillation was carried out in the boric acid solution in the receiver flask with the delivery tube below the acid level. As the distillation was going on, the pink colour solution of the receiver flask turned blue indicating the presence of ammonia. Distillation was continued until the content of the flask was about 50 after which the delivery of the condenser was rinsed with distilled water. The resulting solution in the conical flask was then titrated with 0.1 M HCl and the protein content calculated (Pearson, 1970; AOAC., 2005).

**Determination of Carbohydrate:** This was determined as the difference obtained after subtracting total organic nitrogen (protein), Lipid, Ash, Moisture and Fibre from the total dry matter (AOAC., 2005).

Calculation

Carbohydrate % = 100 – value of moisture, protein, lipid, ash and fibre

i.e CHO = (M+P+L+A+F)

**Determination of Caloric Value (Energy Level):**

The caloric value was obtained by multiplying the value of the crude protein, lipid and carbohydrate by 4:9:4 kcal respectively and taking the sum of the product.

**RESULTS**

The proximate composition of the zobo drinks as presented in Table 1 shows a high moisture content of 85.16%, the ash content was 0.652%, the crude fat and crude protein were 0.87% and 2.14% respectively. Crude fibre was not detected while carbohydrate was 11.175%, calorie was 61.1kcal.

**Table 1. Proximate Composition of Zobo**

PARAMETER (%)	ZOBO
Moisture content	85.161
Ash content	0.652
Crude fat content	0.871
Crude fibre content	ND
Crude protein	2.141
Carbohydrate	11.175
Calorie value (kcal)	61.103

ND = non-detected

The morphological, physiological and biochemical characteristics (Table 2) reveals that the bacterial

isolates from the various zobo samples as identified belong to the genera *Pseudomonas*, *Bacillus*, *Lactobacillus*, *Staphylococcus* and *Escherichia*.

**Table2. Morphological and Biochemical Characteristics of the Bacterial Isolates**

Isolated strain	Morphology	Gram's reaction	Catalase	Oxidase	Motility	Citrate	Spore stain	Indole	Methylred	Voges proskauer	H <sub>2</sub> S production	Urease	Glucose	Sucrose	Lactose	Mannitol	Probable organism
A	R	-	+	-	-	-	-	-	+	+	-	-	A	-	-	A	<i>Lactobacillus sp</i>
B	R	-	+	-	+	-	+	-	+	+	-	-	+	-	-	-	<i>Bacillus sp</i>
C	R	-	+	+	+	+	-	-	-	-	+	-	-	+	-	-	<i>Pseudomonas sp</i>
D	R	-	+	+	+	-	-	-	-	-	+	-	+	+	-	-	<i>Pseudomonas sp</i>
E	C	+	+	-	+	-	-	-	-	-	+	-	+	+	-	+	<i>Staphylococcus sp</i>
F	R	-	+	-	+	-	-	+	-	-	-	-	A/G	-	A/G	-	<i>E. coli</i>

Key: A/G=Acid and Gas production; A= Acid production; R = Rod; C = Cocci

The percentage occurrence of the bacterial isolates (Table 3) in the various zobo samples showed that Sample B 100% occurrence of the isolates while Sample A and Sample C has 80% and 60% occurrence respectively. The fungal genera isolated

and identified (Table 4) from the zobo samples were from the genera *Aspergillus*, *Penicillium* and *Saccharomyces*.

**Table 3: Percentage Occurrence of Bacterial Isolates in each Sample**

Sample	<i>E. coli</i>	<i>Staph.sp</i>	<i>Pseu. Sp</i>	<i>Bacillus Sp</i>	<i>Lactsp</i>	Total (%)
A	+	+	-	+	+	80.0
B	+	+	+	+	+	100.0
C	+	+	-	+	-	60.0

Key: *E. coli* = *Escherichiacoli*, *Staph.sp* = *Staphylococcus* specie  
*Pseu. sp* = *Pseudomonas* specie, *Lact. sp* = *Lactobacillus* specie

**Table 4: Morphological and Cultural Characteristics of Fungi Isolates**

Isolates	Cultural characteristics	Morphological features	Microscopy	Identity
F1	Yellowish-green mycelium	Conidia in long chains, branched cells	Branched smooth conidiophores, brush-like conidia head	<i>Penicillium sp</i>
F2	Woolly white, black domains	Conidia in chains	Non-septate hyphae with many branches	<i>Aspergillus sp</i>
F3	Yellow fluffy colonies	Conidia in chains, columnar head	Non-septate hyphae with branches	<i>Aspergillus sp</i>
F4	Colourless	Nil	Egg shaped	<i>Saccharomyces</i> sp

## DISCUSSION

The moisture content was found to be very high with high carbohydrate content which maybe as a result of additives such as sugar added to improve taste. Ekanem, (2018) reported high moisture and carbohydrate contents from laboratory prepared zobo drink with values of 82.4% and 8.54% respectively. There was no crude fibre while the crude fat and ash contents were low (Table 1). Zobo is a good source of nutrient which can provide energy and nourishment apart from its obvious medicinal value. The group of bacteria isolated and identified from the three zobo drink samples include; *Lactobacillus sp*, *Bacillus sp*, *Pseudomonas sp*, *Staphylococcus sp* and *E. coli*. The fungal isolates are as presented in Table 4 and the genera include; *Penicillium sp*, *Aspergillus sp* and *Saccharomyces sp*.

The results obtained from this study was similar to the one reported by Ameh and Abubakar, (2002), who isolated *S. aureus*, *Proteus sp*, *Streptococcus sp*, *Bacillus sp*, *E. coli* and Yeast from freshly produced zobo drinks. The occurrence of *E. coli* in Zobo is an indication of faecal and environmental contamination and a signal for the presence of other enteric pathogens. Therefore, their presence may be linked to faecal, environmental and human contaminations (Ameh and Abubakar, 2002), which may occur probably through the use of water. *Bacillus sp*. has been implicated in food poisoning especially in cereals that have been cooked and stored at warm temperature (Wonang *et al.*, 2001). These *Bacillus* species can produce toxin that cause pneumonia and bronchopneumonia, and besides *Bacillus cereus* is known to produce heat-resistant spores that cannot be eliminated by boiling. The isolation of yeasts from these 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 (Umaru *et al.*, 2014). The microbes identified from this study has some similarity with the findings of other authors on zobo drinks sold in different locations in Nigeria including Kano metropolis (Bukar *et al.*, 2010), Aba

metropolis (Ezeigbo *et al.*, 2015a), and Jos metropolis (Zumbes *et al.*, 2014).

The highest number of bacteria was in sample B (100%) followed by sample A (80%) and sample C (60%) had the lowest. *E. coli*, *Staphylococcus sp* and *Bacillus sp* were the most frequent among the bacteria isolated.

## CONCLUSION

Typically, microorganisms invade food products from several perspectives ranging from exposure, handling and storage. The microbial diversity found in zobo drink is mainly bacteria and fungi/yeast. The microbes that have been widely isolated from zobo drink sold in public places in Ikot Ekpene belongs to the genera *Staphylococci*, *Escherichia*, *Streptococci*, *Pseudomonas*, *Aspergillus*, *Penicillium* and *Saccharomyces*. To a large extent the genera *Staphylococcus*, *Escherichia*, *Bacillus* and *Saccharomyces* were the predominant microbial isolates found in zobo drink consumed in Ikot Ekpene metropolis. Most of these microbes have invaded the products from the dried *Hibiscus sabadariffa* bought in the market and through water sources. The microbial load counts were high, which indicates a high level of contamination. This is very dangerous to the public, as these contaminants has ability to cause varying level of diseases, ranging from food borne illnesses and food poisoning due to *Staphylococcus aureus*. Presence of *E. coli* can be responsible for prevalence of diarrhea, fever, nausea, and cramps in children and adult exposed to contaminated drinks.

## RECOMMENDATIONS

I recommend that;

- i. Drinks and beverages should be regulated in Nigeria by NAFDAC and other food regulatory bodies, as drinks of low and below minimum safety acute or chronic basis.
- ii. The manufacturers should be educated and the provision of basic facilities will greatly improve zobo drink quality and safety.
- iii. To the effect, regulation, effective monitoring and enforcement of the existing puncture measure is therefore recommended.

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