BACTERIOLOGICAL QUALITY ASSESSMENT OF ZOBO DRINK SOLD IN AUCHI METROPOLIS, EDO STATE, NIGERIA.

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CERTIFICATION

We certify that this project titled "**BACTERIOLOGICAL QUALITY ASSESSMENT OF ZOBO DRINK SOLD IN AUCHI METROPOLIS, EDO STATE, NIGERIA**" was carried out by **OKEILE TITILAYO ESTHER** with **AST/2382070223** and **OKOJIE OMONO BECKY** with **AST/2382050032** in the department of Biology/Microbiology, Biological Science Laboratory Technology, Auchi Polytechnic, Edo State.

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DEDICATION

This project work is dedicated to God Almighty.

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We are grateful to Almighty God for the strength, knowledge and understanding to complete this project. His love has been more than sufficient to keep and sustain us.

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Abstract

Bacterial contamination of ready-to-eat locally packages food such as zobo drink (Roselle juice) sold by street vendors has become a major health problem as it is associated with pathogenic microorganism resulting from poor hygienic practices. This study investigated the bacteriological quality standard of zobo drinks sold in Auchi metropolis, Edo State, Nigeria. The zobo drinks were obtained from three different markets. Markets A, B, and C. A total of four samples, one from each market point and a prepared control sample were analyzed for bacterial isolation using media (Nutrients and MacConkey agar). The bacterial isolates obtained were suggestive of E.coli, Staphylococcus aureus, Salmonella species, Pseudomonas species and Shigella species based on their cultural morphology and biochemical characteristics. The tatal average number of viable colony counts ranged from 3.5 x 10^{-1} cfu1ml, 2.4 x 10^{-3}, and 1.3 x 10^{-5} cfu1ml respectively using the different dilution factors. This study shows a high level contamination of zobo drink beyond the acceptable limit by the Health standard and the route of contamination is traceable to the source of water used, raw materials and lack of personal and environmental hygiene.

CHAPTER ONE

1.0 Introduction

1.1 Background of Study

Food is essential resource needed for the maintenance and functioning of the various system of the body. Foods are classified based on the readiness to consumption including fully, minimally processed and ready-to-eat food. Zobo drink is one of the ready-to-eat nutritional drinks consumed by several families in Nigeria irrespective of the socioeconomic status. The impact of zobo drink (Roselle juice) is seen as one of the most vital nutrient drink for the promotion of human health and prevention of disease. Food is regarded as one of human primary needs besides clothing and housing. Due to the important role of food in human existence, it is imperious to maintain high level of food safety in order to ensure that human being is safe from disease or other related health hazard associated with food. Conditions of food safety include efforts to avoid contamination from biological, chemical and other substance that can endanger human health (Adolf and Azis, 2012).

Zobo drink (Roselle juice) which is also regarded as one of the ready-to-eat nutritional drink (RTE) can be defined as food/drink prepared and or sold by vendors on the street and in other public places for immediate consumption, or at a later time without further processing or preparation. Disease that result from these drink are one of the major health problems in developing countries, Razavilar, (2010). The traditional processing methods that are used in preparation of zobo drinks, inappropriate holding temperature and poor personal hygiene of handler are some of the main causes of contamination of street-vended food/drink. Consumers who depend on such food are more interested in its convenience and usually pay little attention to its safety, quality hygiene (Mensah *et al*., 2002; Barro *et al*., 2006).

Zobo drinks contamination with antibiotic resistant bacteria can also be a major threat to public health, since the antibiotic resistance determinants can be transferred to other pathogenic bacteria potentially comprising the treatment of severe bacterial infection (Oladipo and Adejumobi, 2010; Threfall *et al.,* 2000). It is expected that consumption of Zobo drink is of more health benefits. However, the recent increase in food infections and poisoning around the world affected the derivatives of these benefits (Balakarami *et al.,* 2016). In spite of nutritional challenges of Zobo drinks, it is also often contaminated with diverse group of microorganisms which could be harmful to the individual who consumed it. Drinks such as zobo are frequently linked with gastrointestinal disease such as diarrhea and typhoid fever due to improper handling and serving practice (Nkere *et al.,* 2022; Tambekar *et al.,* 2011). Read to eat food such as zobo drinks are frequently associated with diarrhea disease which occur due to improper use of additive, the presence of pathogenic bacteria, environmental contaminants and disregard of good manufacturing practices (GMPs) and good hygiene practices (GHPs). Mishandling and disregard to hygiene measures on the part of the food vendors have introduce contaminants and pathogens that survive and multiply in sufficient numbers to cause illness in the consumer (Greig *et al*., 2007). Symptoms of food borne illness include: diarrhea, vomiting, abdominal cramp and nausea, Nweze, (2010); most of which are caused by *Staphylococcus aureus, Salmonella species, Clostridium perfringens, Clostridium botulinum*, Campylobacter sp, Vibrio parahaemolyticus, *Bacillus cereus* and Entropathogenic *Escherichia coli*. Food safefy depends on conditions necessary during the production, processing, storage, and preparation of food to ensure that it is safe, sound, wholesome, and fit for human consumption, FAO/WHO, (1990). The diverse group of pathogenic microorganism are transmitted by facial oral route, often by the ingestion of contaminated foods, drinks and water. The isolation of unacceptable level of *S. aureus* , an Enteroxins producer responsible for *staphylococcal* food poisoning in zobo

drinks. Samples evidently revealed that zobo drinks can be source of bio toxins that may make food injurious to health on an acute or chromic basis (Achi and Madubuike, 2007). The isolation of S.*aureus* in zobo drinks is a pointer to largely poor hygiene, improper storage facilities use low quality raw materials and unhygienic environment.

In Nigeria, the unhygienic handling of foodstuff, health status of food vendors, poor sanitary conditions of markets, absence waste disposal facilities, use of contaminated water and kitchen equipment and poor storage affect food safety (WHO, 2007). The consciousness of the impact of certain contaminated food such as zobo drinks is critical for proper risk assessment, remediation and management. However, because there is limited information on the bacteriological assessment standards of some ready-to-eat food in Auchi, this study will provide up to date information on the bacteriological quality standards and safety of hawked zobo drink (Roselle juice) sold in Auchi metropolis, Edo State. Nigeria.

1.2 Statement of Research Problem

Due to socio-economic changes characterized by increasing by increased mobility, resulting in more ready-to-eat nutritional drinks such as zobo taken outside the home, food vendors services are on the increase and responsibility for the food safety have been transferred from individuals/families to the food vendors who rarely enforce good manufacturing practice. Ready-to-eat nutritional drinks have played a role in the human health and that is why today they are highly purchased in shops, markets and streets as well. Some of these ready-to-eat nutritional drinks are sold in a packaged way that the buyer or consumer does not need other preparation before eating or drinking and most of these vendors are in-experienced based on personal hygiene. During processing, those ready-to-eat nutritional drinks are exposed to contaminated air, unclean utensils and unclean

environment. In view of facts, Zobo drink is never subjected to any form of post-production treatment that can eliminate or at least reduce the bacteria load in the drink; it could be a potential source of health hazard. Also the activities involved in the cooling and subsequent dispensing of the drink into containers also represent potential sources of health hazard.

1.3 Aim and Objectives of Study

This study was aimed to ascertain the bacteriology quality assessment of ready-to-eat food (Zobo drink) sold in Auchi, Edo State, Nigeria.

The specific objectives were;

1. To isolate bacteria associated with zobo drink food.
2. To identify using cultural characteristics and Gram's reaction
3. To characterize isolates using biochemical test
4. To make adequate recommendation on the research findings.

1.4 Justification of the Study

Attribute to the increase cases of food and drinks infection and intoxication to human and inadequate sanitary condition observed in processing of many locally made foods and drinks. In Nigeria, hawking of ready-to-eat nutritional drinks in streets and markets even along the road for travelers is very common. These vendors enjoy huge patronage from different societal classes. Unfortunately, none of these hawkers or vendors is licensed or monitored by relevant agencies saddled with the responsibility of ensuring the safety of our foods and drinks. Thus, owing to the manner and condition these vendors operate, there is possibility that some of the ready-to-eat nutritional drinks and food may be contaminated with micro organisms. Many picnic suppers and

banquets have come to disastrous end 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

1.5 Scope of Study

This research study entails investigation of bacteriological quality assessment of Zobo drink (Roselle juice) sold in three randomly popular markets in Auchi metropolis, Edo State. All sample sources will be analyzed using Nutrition and MacConkey agar medium for the enumeration of bacteria in the samples while Morphological features and biochemical reactions patterns will be used for the identification of bacteria isolates.

1.6 Definition of Terms

Some terms expressions or technical work used in the process of the research write up is clearly defined and their meaning was well explained. Some of these terms include;

i. **Bacteria**: they are ubiquitous, mostly free-living organisms often consisting of one biological cell which also constitute a large domain of prokaryotic micro organisms…
ii. **Assessment:** is the systematic basis for making inferences about the learning and development of someone or something
iii. **Quality:** it is an attribute or a property characteristic of an object in philosophy
iv. **Production:** it is the process of combining various material input in order to make something for consumption.
v. **Roselle Plant (Hibiscus sabdariffa):** also known rosella Jamaican sorrel or java jute, Plant of the hibiscus or mallow family (*Malvaceae)* and its fibre, one of the bast fibre groups. The plant is very sensitive to frost.

vi. **Juice:** it is a drink made from the extraction or pressing of the natural liquid contained in fruit and vegetables.

vii. **Zobo Drink (Roselle juice):** it is a drink made from the extraction of Roselle plant which helps to boost immunity levels in human.

CHAPTER TWO

2.0 Literature Review

Zobo drinks are traditional non-alcoholic beverage which is consumed in most part of Nigeria, mostly in northern part of Nigeria (Osuntogun, 2004). The economic and religious situation in Nigeria has made the zobo drink gain wide acceptance in different occasions. It's used as refreshment, entertainment in parties or as appetizers before the main dish is served and it is also sold in market to various consumers (Onuorah *et al.,* 1987). The zobo drink is a red liquid drink and taste like fruit punch, served as a fair source of vitamin A, riboflavin, niacin ,calcium and iron Qi *el al.*, (2005), and is low in sugar content. This drink also contains anthocyanins and vitamins C, among others it is used in curing minor stomach ailments, sore throat and strengthening the heart among others uses (Olawale, 2001).

Zobo drink is extracted from the dried reddish purple calyces of the plant *Hibiscus sabdariffa*, (Scott, 2003). The calyces are used to produce herbal tea and other food products. The juice drink which is usually obtained by extraction of the calyx of Hibiscus contains about 1% solid. At present, the production processes in neither mechanized nor standardized. Consequently, the shelf life of the drink is less than two days. Furthermore, the mode of packaging or dispensing of the juice in nylon or plastic container before retailing, that is taken as Zobo and the largely unregulated nature of the trade, and poor hygienic practices as well as lack of running water, toilet, proper storage and waste disposal facilities at preparation and services point has resulted in exposure to potential contaminants and an increased risk to public health (Omemu and Aderoju, 2008).

Consequently, street drinks and foods safety has remained a major public health concern globally, and more importantly in Nigeria were the regulation of this critical sector is virtually non-

existent or inadequate, making streets foods and drinks hazardous source of nutrition (Oyeyi and Lum-nwi,2008; *Wada et al.,* 2009). Foods frequently serve as vehicle for spreading of several organisms some of which are pathogenic. Many picnic suppers and banquets have come to a disastrous end which home prepared foods and drinks serves not only as food and drinks for guests, but also as the vehicle for transmitting staphylococcus food poisoning (Alabi *et al.,*1996). In view of the facts, that Zobo is never subjected to any form of post-production treatment that can eliminate or at least reduce the bacteria load in the drink, it could be a potential source of health hazard. Also the subsequent dispensing of the drinks into containers also represent potential source of health hazard and have been reported that some gastro intestinal illness characterized by diarrhea, abdominal cramps, and vomiting which may be assumed as been of unknown etiology may arise from consuming drinks contaminated with microorganisms.

In this study, retailed Zobo drinks were purchased from Auchi environs, they were analyzed for their bacteriological quality as indicated by the number (counts) and kinds of bacteria they harbor Zobo is a non-alcoholic local beverage made from dried petals, acid-succulent calyxes of *Hibiscus sabdariffa by boiling and* filtration (Ogiehor *et al.,* 2008*;* Kolawole and Maduenyi, 2004). *Hibiscus sabdariffa* is an annual erect, herbaceous shrub with smooth or almost smooth, cylindrical and typically red stem. The flower is mostly cultivated in northern Nigeria and zobo is now popular in West African Sub Region, especially among the youths who see zobo as an alternative, cheap, relaxing non-alcoholic drink in social gathering (Ogiehor and Nwafor, 2004).

Zobo is a red coloured non-alcoholic local beverage made from different varieties of dried, succulent aqueons acid extract of Roselle carlyx. The beverage has soured taste but often sweetened. The name zobo is derived from zoborodo in Hausa, goneura in Hindi, krajeab in Thailand, bissap in Senegal and sorrel in Caribbean (Okereke *et al.,* 2015). Zobo as non-alcoholic beverage is quite

popular in northern part of Nigeria (Egbere et al., 2007). Religious and health campaigns against alcoholic beverages in Nigeria and the subsequent decrease in intake of alcoholic beverages in some areas have made zobo drink an alternative to alcoholic beverages. Zobo is known to be rich in carbohydrate, protein, calcium, vitamin, minerals, iron and antioxidants. Aside this, it was used in folk medicine as antidiuretic, mild laxative, treatment for cardiac and nerve diseases and management of cancer. It was reported that zobo is a good traditional medicine for the treatment of diseases such as hypertension, UTI etc. (Bamishaiye, *et al.,* 2011). The danger of food infections and food borne illness that may be associated with zobo outweighs the benefits derived. Bacteria isolated from zobo drink include, S. aureus, Bacillus sp, Lactobacillus sp, Escherichia coli, Pseudomonas sp, Enterobacter *sp,* (Nwachukwuet *et al.,* 2007). Other researchers isolated *S. aureus, Bacillus sp, micrococcus, Proteus sp, E. coli* (Ibitoye *et al.,* 2017). It was noted that the consumption of zobo might serve as vehicle for food borne disease agents (Amusa *et al.,* 2005; Ayandele, 2015).

In a study conducted on the microbiology quality of zobo in Auchi environs. The bacteria isolated were *Staphylococcus sp, E. coli, Lactobacilus, Bacillus sp and Pseudomonas sp*. The level of contamination was attributed to lack of personal and environment hygiene in the processing, packaging and preservation (Ezeigboet *et al.,* 2015). Looking at the nutritional, sensory and microbiological assessment of zobo, the following bacteria were isolated, *E coli. Klebsiella sp and Bacillus sp.* Which was linked to contamination from vendors and materials used for production (Adenji, 2017). In assessing the microbial quality of zobo sold Auchienvirons the bacterial isolated were *E. coli. S. aureus, Enterobacter sp. Micrococcus and Proteus sp.* (Makele *et al*., 2015). The poor bacteriological quality was attributed to poor handling, and poor quality materials used for production, unhygienic processing and vendors. Other researchers also isolated similar organisms

(Seiyaboh *et al.*, 2013; Ayandele *et al.*, 2015). It was noted that lack of proper hygiene and sanitary measures in processing and packaging of bottled zobo drinks were responsible for contamination.

Locally made drinks are regarded as one of the most popular indigenous drinks produced and have varying economic importance because of its high nutritional quality. It is consumed almost by all people because it is relatively cheap and nutritious when compared to carbonated drinks. These drinks are mostly prepared in condition lacking quality control measures in Nigeria and also lack information on the microbiological safety of ready to eat beverages or drinks and their implications.

2.1 Roselle Plant*(Hibiscus sabdariffa)*

Roselle (*Hibiscus sabdariffa)* is a species of Hibiscus native to Africa, most likely West Africa. It was in the 16^{th} and early 17^{th} century that it spread to the West Indies and Asia, respectively. It is used for the production of best fibre and as an infusion, in which it may be known as carcade (Malekian,2005).

2.1.1 Description

It is an annual or perennial herb or woody-based subshrub, growing to 2-2.5 m (7-8 ft) tall. The leaves are deeply three- to five-lobed, 8-15 cm (3-6 in) long, arranged alternately on the stems. The flowers are 8-10 cm (3-4 in) diameter, white to pale yellow with a dark red spot at the base of each petal, and have a stout fleshy calyx at the base, 1-2 cm (0.39-0.79 in) wide, enlarging to 3-3.5 cm (1.2-1.4 in), fleshy and bright red as the fruit matures. They take about six months to mature.

2.1.2 Botanical Classification of *Hibiscus sabdariffa*

Among numerous varieties of Hibiscus altisasima and Hibiscus sabdariffa are the commonest and better introduced. Hibiscus altissima is branchless plant with yellow flowers red or green

coloured calyces. Though this species is not used for food, this plant is more economically important than Hibiscus sabdariffa because of its high fiber content. The other distinct type Hibiscus sabdariffa or “Roselle” grows in a bush with many branches. The flowers of Roselle are auxiliaries or in terminal racemes, the petals are white with reddish center at the base of the stamina column and this species is widely used as food (Abu-Tarboush *et al*., 1997).

2.1.3 Composition of *Hibiscus sabdariffa*

Roselle is mainly cultivated for it carlyx, which is of three types: green, red and dark red. The red calyxes are the most used and characterized by their concentration of anthocyanin Delphindin -3-Sambubioside and Cyanidin3-Sambubioside are the major anthocyanin. Roselle is also rich in organic acids, minerals, amino acids, carotene, vitamin C and total sugar in its calyx, leaves and seeds at variable levels depending on the variety and geographical area (Mady *et al*., 2009). According to Manita-mishra (1999), a number of compounds have also been isolated and characterized from Reselle including flavonoids, anthocyaninins, triterpernoids, steroids and alkaloids.

Table 2.1: Nutritional Value of Roselle Plant

Nutrient	Calyces	Seeds	Leaves
Protein (g)	2	28.9	3.5
Carbohydrates (g)	10.2	25.5	8.7
Fat (g)	0.1	21.4	0.3
Vitamin A (I.E)	-	-	1000
Thiamine (mg)	0.05	0.1	0.2
Riboflavin	0.07	0.15	0.4
Niacin (mg)	0.06	1.5	1.4
Vitamin C (mg)	17	9	2.3
Calcium (mg)	130	350	240
Iron (mg)	3	9	5

Source: Natureland (2004)

2.1.4 Uses/Application of Roselle Plant

In india, the plant is primarily cultivated for the production of bast fibre used in cordage, made from its stem. The fibre may be used as a substitute for jute in making burlap (Duke, 1998) Hibiscus, specifically roselle, has been used in folk medicine as a diuretic and mild kaxative. The red calyces of the plant are increasingly exported to the United States and Europe, particularly Germany, where they are used as food colourings. It can be found in markets (as flowers or syrup) in places, such as France, where there are Senegalese immigrant communities (Peter, 2007). The green leaves are used like a spicy version of spinach. They give flavor to the Senegalese fish and rice dish thieboudinne. Proper records are not kept, but the Senegalese government estimates national

production and consumption at 700 t (770 short tons) per year (Peter, 2007). In Myanmar their green leaves are the main ingredient in chin baungkyaw curry (Sula, 2003). Brazilians attribute stomachic, emollient, and resolutive properties to the bitter roots.

i. Vegetable: Roselle is called khatefule by local tribal language. The khatefule leaves are mixed with green chillies, salt, some garlic to prepare a chutney and bhaji which is served with jowar (sorghum) or bajra (millet) made bakho (a flat bread). This is eaten by tribals as breakfast to start their day. A dry dish or sukhibajji is prepared with khatefule leaves (vanitascor.com). In Andhra cuisine, roselle is called gongura and is extensively used. The leaves are steamed with lentils and cooked with dal. Another unique dish is prepared by mixing fried leaves with spices and made into a gongurapacchadi, the most famous dish of Andhra and Telangana often described as king of all Andhra foods. In Manipuri, it is called Sougri and it is used as a vegetables. It is generally cooked without oil by boiling with some other herbs and dried fish and is a favourite of the Manipuri people. Almost every household has this plant in their homes.

ii. Bevarages: A drink is made from the Roselle Fruit (The Calyces with the seed pods removed). It is prepared by boiling fresh or dried reselle fruit in water for 8 to 10 minutes (or until the water turns red), then adding sugar, Bay leaves and cloves may also be added during boiling (sweetentmagazine.com)

iii. Jam and Preserves: in Nigeria, roselle jam has been made since colonial times and is still sold regularly at community fetes and charity stalls. It is similar in flavor to plum jam, although more acidic. It differs from other jams in that the pectin is obtained from boiling the interior buds of the rosella flowers. It is thus possible to make rosella jam with nothing but rosella buds and sugar (garden drum.com).

2.1.5 Production of Roselle Plant (*Hibiscus Sabdariffa)*

China and Thailand are the largest producers and control much of the world supply. The world's best roselle comes from Sudan and Nigeria, Mexico, Egypt, Senegal, Tanzania, Mali and Jamaica are also important suppliers but production is mostly used domestically (FAO.org). In the Indian subcontinent (especially in the Ganges Delta region), roselle is cultivated for vegetable fibres. Roselle is called mesta (or meshta, the s indicating an sh sound) in the region. Most of its fibres are locally consumed. However, the fibre(as well as cutting or butts) from the roselle plant has great demand in natural fibres using industries.

Roselle is a relatively new crop to create an industry in Malaysia. It was introduced in the early 1990s and it commercial was first promoted in 1993 by the Department of Agriculture in Terengganu. The planted acreage was 12.8ha (30 acres) in 1993 and steadily increased to peak at 506 ha (1,000 acres) by 2,000. The planted area is now less than 150 ha (400 acres) annually, planted with two main varieties, (Serban *et al.,* 2015). Terengganu State use to be the first and the lagest producer, but now the production has spread more to other States. Despite the dwindling hectare over the past decade or so, roselle is becoming increasingly known to the general population as an important pro-health drink. To the extent, the calyces are also processed into sweet pickle, jelly and jam.

2.2 Photochemical

The Hibiscus leaves are a good source of polyphenolic compounds. The major identified compounds include neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeoylshikimic acid and flavonoid compounds such as quercetin, kaempferol and their derivatives (Zhen *et al.*, 2016). The flowers are rich in anthocyanins, as well as protocatechuic acid. The dried calyces contain the flavonoids gossy petin, hibiscetine and sabdaretine. The major pigment is not

daphniphyline. Small amounts of myrtillin (delphinidin 3- monogucoside), chrysanthenin (cyaniding 3-monoglucoside), and delphinidin are present. Roselle seeds are a good source of lipid-soluble antioxidants, particularly gamma-tocopherol (Mohammed *et al.,* 2007)

2.3 Nutritional and Medical Importance of Roselle Plant (Hibiscus Sabradiffa

Roselle, the safe medicinal plant, Abbas *et al.,* (2017) having various medically important compounds called phytochemicals is known for delicacy and also for its nutritional and medical properties, Arvind *et al.,* (2011). The application of the plant is managing different medical problems including cancer, inflammatory disease different cardiovascular problems has been well investigated by different scholars in different settings (Okereke *et al.,* 2015).

2.4 Domestic Applications

Even though, the uses if different parts of Roselle are many and varied both in food and in traditional medicine, all parts of Roselle including seeds, leaves, fruits and roots are used as a food in different parts of the world. Fleshy red calyxes of Roselle are commonly used for the production of soft drinks and tonic without alcohol like wine, juice, jam, jelly, syrup and also dried and brewed into tea and spice. These are rich in carotene, riboflavin, anthocyanins, ascorbic acid, niacin, calcium, iron and vitamin C. They young leaves and tender stems of Roselle are consumed raw as green vegetable. The Roselle seed are good source of protein, fat, total sugars and are widely used in the diet in many African countries (Yodong *et al.,* 2005).

2.5 Herbal Medicine Applications

Roselle is used in many folk medicines. It is valued for its mild effect, ability to increase urination, relief during hot weather and treatment for cracks in the feet, bilious, scores and

wounds. Traditionally in Sudan, Roselle has been used for relief of sour throat and healing wounds (Aziz *et al.,* 2007). In African folk medicine, Roselle leaves are used for their, antimicrobial, emollient, antipyretic, diuretic, anti-helmentic, sedative properties as strong as a soothing cough remedy, whereas in India, leaves are poultice on abscesses.

a. **Hypo-Lipidemic Effects**: According to a study conducted among hype-cholesterolemic patients, two capsules of Roselle extract (1g), given three times a day (for a total of 3g/day) , significantly lowered serum cholesterol (Lin-Tzu *et al.,* 2007). Another scientific study also confirmed that ethanolic extract from the leaves of Roselle significantly exhibit hypo-lipidemic effect. Roselle ectract was also studied among subject, some with and some without metabolic syndrome. Subjects is with metabolic syndrome receiving ethanolic extract of Roselle had significantly reduced glucose, total cholesterol and low density lipoprotein, while increasing high density lipoprotein.
b. **Anti-Diabetic Activity:** was extracted the polyohenolic components of Roselle and their effect were demonstrated in type II diabetic rat model studied their effect in a type II diabetic rat model (high fat diet model). Studied revealed anti-insulin resistance properties of extract at a dose level of 200mg/kg, and reduction in hyper glycaemia and hyper insulinemia. The extract was found effective in lowering serum cholesterol, triacylgcerol, the ratio of low density lipoprotein/high-density protein (LDL/HDL), and also (AGE) formation and lipid per oxidation. Intestinal *a*-glycosidase and pancreatic *a*-amylase help in digestion of complex carbohydrates present in the food into bioavailable monosaccharide and plays an important role in postprandial hyperglycaemia; therefore inhibition of these enzymes has been reported as an effective mechanism for the control of postprandial hyperglycaemia. Hibiscus acid (hibiscus type (2S,3R)-hydroxycitric acid lactone) have been shown as a potent inhibitor of

pancreatic *a*-amylase and intestinal a-glucosidase and pancreatic a-amylase activity, (Yamada *et al.,* 2007). In another study Adisakwattana *et al.* (2012) conducted an *invitro* study and reported Roselle extracts as an effective inhibitor of pancreatic *a*-amylase.

c. **Anti-helminthes and anti-microbial effects:** Roselle is known for its antibacterial, antifungal and anti-parasite actions. Oil extraction from seeds of Roselle has been shown to have an in *vitro* inhibitory effects on *Bacilus anthracis and Staphylococus albus.* Aqueous and ethanol extracts were also found to be effective against *Schistosomamansoni* and other microorganisms. Afolabi *et al.* (2008) demonstrated the antibacterial effect of hibiscus extract on *Streptococcus mutans,* a bacterium from oral cavity. In a similar study, antibacterial potential of hibiscus was also observed on *Campylobacter* species (Yin *et al.,* 2008) An ethanol extract of the dried leaves of Roselle reduce aflatoxin formation and have in *vitro* inhibitory effect against some fungi (Guerin and Reveillere, 1984)

d. **Anti-oxidant Effect:** protective property of a compound t inhibit the oxidative mechanisms by scavenging reactive oxygen and free radicals is known as antioxidative activity. It protects lining organelles from premature cell damage and reduces ageing. A large number of invitro and invivo studies have shown that Roselle calyces contain potent antioxidant. According to Augustine *et al.* 2011) both the whole aqueous and anthocyanin-rich extracts of Roselle are effective antioxidant. Studies have also highlighted that poly-phenolic acid, flavonoids and anthocyanins which are found in Roselle are potent antioxidants (Crawford *et al.,* 1998).

e. **Other Pharmacological Effects:** Roselle has been reported to possess a lactogenic activity. Okasha *et al.* (2014) observed enhancement in the serum prolactin level of lactating female *Albin* Rats on administration of seed extract of Roselle. Bako, *et al.* (2014) studied the lactogenic effect of ethyl acetate fraction of *Hibiscus sabdariffa,* from 3-17days of lactation.

The results showed an increase in serum prolactin level and milk production in lactating female albino rats, which confirms the lactogenic property of *Hibiscus sabdariffa.* Studies have shown that Roselle tea contains an enzyme inhibitor which blocks production of amylase and it is possible that drinking a cup of hibiscus tea after meal can reduce the absorption of dietary that are also known to have to have effect on inflammatory disease and cancer (Da-Costa-Rocha *et al.,* 2014).

2.6 Nutritional Value of Locally Made Zobo Drink

Locally made zobo drinks ranks first in the relative contribution to human when compared to other drinks and carbonated drinks. It also have valuable vitamins such as vitamin A and C and also contain fibers, it also contains carbohydrates and very rich in mineral content (sodium, calcium, potassium, magnesium, zinc). It contains vitamin A, Riboflavin, niacin, calcium, and iron (Qi *et al., 2005*).

2.7 Preparation of Zobo Drink

Zobo drink (Roselle juice) is prepared by boiling the dry calyces of *Hibiscus sabdariffain* water for 10-15 minutes to extract the pigments. After extraction, the filtrate is spiced and packaged. The sharp sour taste of the raw extract may besweetened with sugarcane, granulated sugar, pineapple, orange or both fruits, depending on choice.

Figure 2.1: Zobo Drink (Roselle Juice)

2.8 Uses and health Benefit of Locally Made Zobo Drink

Locally made drinks have some health benefit which you can gain after consuming it. The nutrient contained in Zobo drink can even be used to prevent and cure certain diseases and illness. They are contains anti-bacteria properties, water soluble antioxidants, anti-hypertensive properties and many more healthy properties. Below are several health benefit which you can get when you consume these locally made drinks.

i. **It is Nutritious:** Zobo drink is a very nutritious drink and it is cheap. Regular intake of this drink can fight nutritional deficiencies and prevent malnutrition. Some important and vital nutrients provided by zobo drink are carotenes, vitamin C, thiamine, calcium, fat, iron, fiber, phosphorous, riboflavin, and niacin. All these nutrients boost your health and improve the functions of various tissues, organs, and systems in the body. This drink strengthens the immune system and improve your health (Amusa 2005).

ii. **It boosts Healthy Eyes:** Carotene is present in zobo leaves, the body coverts this nutrient into vitamin A. vitamin A keeps the eye heathy, it boosts clear and healthy visions and prevents eye problems like night blindness. A combination of vitamin A and C strengthens immunity and guards against eye problems and also prevents eye problems caused by aging (Bello 2014).

iii. **It Guards against Kidney Problems:** Doctors recommend zobo drink for patients with kidney disease and other kidney problems. It contains vital organic acids like critic acid. Acetic acids, tartaric acids and maleic acid; all these acids help the kidneys function properly. They help the kidney eliminate uric acid and oxalic acid, these are the two main causes of kidney stones and this, in turn, can lead to kidney damage and failure if not

treated properly. It also eliminates other wastes from the kidneys and body. Don't add sugar or artificial sweeteners when taking zobo for the health of your kidneys.

iv. **It Boosts the Levels of your Energy:** Vitamin B2 or riboflavin present in zobo leaves increase the levels of energy and fight fatigue and mild cases of chronic fatigue syndrome. This vitamin plays a role in energy metabolism and thereby increase the levels of energy in the body.

v. **It Boost Appetite:** Fiber, carotene, iron and calcium and other important nutrients found in hibiscus flower increase appetite; it fights the loss of appetite and other eating disorders. It boosts digestion and prevents digestive issues that can affect appetite. It fights worms and bacteria in the gut and digestive system because it has antibacterial properties (Bamishaiye 2011).

vi. **It Boosts a Heathy Nervous System:** Thiamine or vitamin B1 present in zobo drink helps build and maintain a healthy nervous system. It also supports the muscles of the heart and improves cardiovascular functions. It protects the nervous system and promotes healthy hairs, mouth, liver, and eyes (Karemaker 2017).

vii. **It Prevents Cancer:** Regular consumption of zobo drink increase the levels of antioxidants in body thereby preventing and treating cancer. These antioxidants fight free radicals which cause oxidative damage and stress which later lead to cancer. These antioxidants also boost the immune system and prevent cancer and other chronic and life-threatening diseases. Don't add sugar or artificial sweeteners because they feed cancer cells (Armitage 2003).

viii. **It Improves Brain Function:** Niacin or vitamin B3 present in zobo leaves supports healthy functions of the brain and fights brain problems and mood disorder like depression (Aderson,2002).

ix. **It Fights High Blood Pressure:** Zobo drink is very effective against pre-hypertension and mild hypertension. It contains enzyme inhibitors that block the production of amylase and this helps to lower blood pressure because this enzyme breaks down starches and complex sugars (Lauer 2009).

x. **It Boosts the Immune System:** Zobo drink is rich in vitamin C, this vitamin fortifies the immune system and fights all kinds of diseases and infections. Regular intake of zobo drink will increase the levels of vitamin C in your body and thus empower your immune system. Due to the high content of vitamin C in zobo drink, it can fight colds and coughs. It fights inflammation and kills bacteria and this is helpful in treating colds and coughs. It has a cooling effect that can help reduce high body temperature and relieve fever (Arbert, 2002).

xi. **It Strengthens the Bones:** Calcium is found in large amount in zobo leaves, this helps to strengthen the bones and teeth and guard against osteoporosis and other bone problems. Calcium also benefits the heart and muscles; it helps them function properly. It prevents diseases of the teeth and bones, (Margart, 2017).

xii. **It Fights Diabetes**: Unsweetened zobo drink brings down high levels of sugar in the bloodstream and it guards against and treats diabetes. Diabetic patients are free to take this drink without any fear or reservations as long as there is no sugar or any type of artificial sweeteners (Ayandele, 2015).

xiii. **Health Implication of Locally Made Zobo Drink**: Poor hygiene and preparation practices also introduce microbial pathogens in foods and have been implicated in causing food-borne illness. The consumption of locally made drinks by both old and young people including market women and children at home pose serious health threats because these products are subject to microbial contamination as producers do not adhere to standard methods of preparation. Food borne illness are diseases, usually infections or toxic in nature, cause by agents that enter the body systems through ingestion which can lead to typhoid fever, dysentery, gastrointestinal disease, coronary heart disease, etc.

CHAPTER THREE

3.0 Materials and Methods

3.1 Study Area

The study was conducted in Auchi, Etsako West LGA, Edo State, Nigeria. Auchi is located in the northern part of Edo State within the coordinates of latitude 07^0 04^1N and longitude 06^0 16^1E. It is situated in the south-south geographical zone of Nigeria with a population of over 500,000 people according to the 2015 population census. It is approximately one hundred and thirty kilometer (130km) away from Benin City, the capital of Edo State. Auchi is the headquarters of Etsako West Local Government and has witnessed territorial development owing to rural-urban migration. It is bounded to the north by Jattu, to the south by Aviele, to the east by Iyakpi and to the west by Owan Local Government Area. It is also the seat of the Federal polytechnic, Auchi.

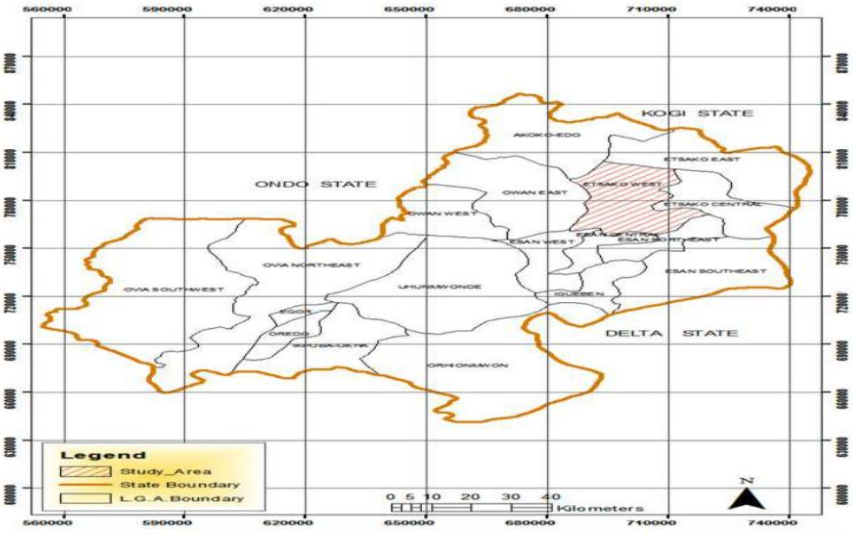

Figure 1. Edo State showing Etsako West LGA (Source: Produced from Arcmap10.1, 2013).

Figure 3.1: Map of Edo State Showing Etsako West LGA (Source: Produced from Aremap10.1.2013

3.2 Sample Collection

Three samples of zobo drink each was purchased from three popular markets in Auchi (Market sample coded A-C). The sample were held at 4^0C. using an iced cooler and immediately transported to Auchi Polytechnic Microbiology Laboratory for analysis as describe by Ayandele (2015). Dried calyces of zobo (Roselle) were purchased from a local market at Agbede (Market sample coded Z) 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.

3.3 List of Apparatus and Experimental Reagents

The material that were used include:

Nutrient agar (general purpose medium), MacConky agar (without salt), Test-tube, Distilled water, Hand glove, Conical flask, Agar plate, Wire loop, Autoclave, Glass-slide, Colony counter. Microscope, Syringe, Cotton wool, Hydrogen, Safranin (secondary stain), Acetone (discolourize), Iodine (modant) and Crystal violet (primary stain).

3.4 Sterilization of Material

`All materials that were used in the course of this project such as glassware's were properly washed with detergent and water to remove dirty and contaminations and dried property. The wash glassware's was sterilized in a portable laboratory autoclave at a temperature of 121^0C for 15 minutes as described by Bukar, *et al* (2015). All media used was also sterilized in the autoclave at a temperature of 121^0C for 15 minutes according to Nwachukwu and Osuocha (2014).

3.5 Preparation of Culture Media

a. **Nutrient agar (NA):** Nutrient Agar was prepared by dissolving 14g of nutrient agar powder in 500 ml of distilled water in a clean flask. The mouth of the flask was plugged with non-absorbent cotton wool wrapped with aluminum foil paper that was extended up to the neck of the flask as described by (Syvester *et al.* (2015). The flask was placed on a Bunsen flame and allows to boil and mix completely. It was sterilized in an autoclave at 121^0C for 15 minutes and allowed to cool to 45^0C and aseptically dispensed into Petri dishes. Nutrient agar was used for the total bacterial aerobic plate count (Roseline *et al*, 2006)

    a. **MacConkey Agar (MA):** this agar was prepared by dissolving bile salt, then 24_g of the powder was dissolved in 500 ml of distilled water. The pH was adjusted to 7.8. It was autoclave at 121^0C for 15 minutes and allowed to cool to a temperature of 45-50^0C before poring into plates. This was used to determine coliform as described by Cheesebrough (2004). This is a selective and differential media designed to isolate and differentiate organism based on their ability to ferment lactose as described by Sebastia *et al.*, (2012)

3.6 Sample Extraction

1ml of zobo drink were measures into 9ml of sterile 0.1% peptone water as diluents to make a 1:10 dilution, further tenfold serial dilution were made and examined by means of the pour plate method. The plates were marked for easy identification and 1ml of the dilution used for the inoculation and incubation (Yeboah-mennuu *et al*., 2010).

3.7 Bacteriology Analysis of Samples

3.7.1 Bacterial Enumeration and Isolation:

Total bacteria count was determined by pour plate technique using standard methods. Nutrient agar medium was used for the enumeration of bacterial in the samples. The total bacteria count was obtained by incubation aerobically at 37^0C for 24 hours.

3.7.2 Viable cell count:

The representative Petri dish incubated was visualized under a colony counting machine and was used to count the total bacteria count (labtech, India) and result was expressed as colony forming unit per milliliter (CFU/ml) at the end of the count as reported by Olayemi *et al*. (2011).

3.7.3 Identification of bacteria isolates:

Identification of the bacteria isolate was performed using classical methods based on their morphological and biochemical characteristic with reference to systematic manual of bacteriology described by (Cheesbrough, 2004).

3.7.4 Gram staining technique:

Gram staining reaction has the wide application that is capable of distinguishing virtually all bacteria into one of two large group-gram positive or gram negative as described by Dr Hans Christian Gram (1884). Smear of each isolate was made on the slide and heat fixed. Primary stain (crystal violet) was added in drops. Lugols iodine was added for 60 seconds decolorized with acetone and washed with water, then you add saffranin for 45 seconds and wash. It was then air dried examined at X100 under oil immersion as described by Bello *et al*. (2014). Positive gram staining appears purple and negative grams staining appeared pink.

3.8 Bio chemical Test

3.8.1 Catalase test:

Catalase test was carried out using a drop of hydrogen peroxide. 2 ml of 3% hydrogen peroxide (H_202) was placed in a clean test tube. A sterile wire loop was used to pick colony of the test organism and mixed with 2 ml of 3% hydrogen peroxide (H_20_2) in the test tube and observed for the production of gas bubbles which indicates a positive reaction. The test was used to identify *Staphylococcus aureus* (James, 2001).

3.8.2 Oxidase test:

A few drops of kovac's reagent were added to piece of filter on a Petri dish. The bacteria isolates were then smeared on the filter paper with a glass rod. The paper was observed. Positive result gave a dark purple color while negative result showed no color change. This test was used to identify *coliforms* as reported by James, (2001).

3.8.3 Coagulase test:

The use of blood plasma is being introduced in coagulase test. A loop full of human plasma was added to culture isolate on a slide. Positive isolate gave agglutination reage reaction with plasma. Test was also carried out at 37^0C for 24 hours positive tubes showed coagulation of the plasma in the tube. This test was used to identify *Staphylococcus aureus,* (James, 2001).

CHAPTER FOUR

4.0 Results and Discussion

4.1 Result

The result of the bacteriology analysis of the zobo drink sold in Auchi metropolis is tabulated below. Table 4.1 represent the pHvalue of the zobo samples, table 4.2 represent cultural, morphological and biochemical characteristics of the bacterial isolates, table 4.3 represent percentage occurrence of isolates and table 4.4 represent percentage occurrence of isolates in each sample.

Table 4.1: pH value of zobo drinks sample

Samples	pH
A	2.9
B	3.0
C	2.9
CTR	2.9

Key: A= Igbe market, B= Uchi market, C= Ughele market, CTR=Control

Table 4.2: Cultural, morphological and biochemical characteristics of the bacterial isolates

Cultural Characteristics	Gram Reaction	Catalase Test	Oxidase Test	Coagulase Test	Isolate
Smoothly oily lactose farmenter	-Rod	+	-	-	E. coli
Smooth cream colony	+cocci	+	-	+	Staphyloccocus aureus
Large grey white mucold colonies	-Rod	+	+	-	Salmonella Spp
Grey white irregular colonies	-Rod	+	+	-	Pseudomina Spp
Pale colour non-lactose fermenting colonies	-Rod	+	-	+	Shigella

Note: + = positive, - = negative

Table 4.3: Total number of viable colony count of the sample isolates

SAMPLE	DILUTION FACTOR	NUMBER OF COLONIES ON AGAR PLATES	TOTAL NUMBERS OF COLONIES FORMING UNIT (CFL/ML)
A	10^{-1}	85	8.5×10^{-1}
	10^{-3}	65	6.5×10^{-3}
	10^{-6}	54	5.4×10^{-6}
B	10^{-1}	76	7.6×10^{-1}
	10^{-3}	80	8.0×10^{-3}
	10^{-6}	85	$8.5x\ 10^{-6}$
C	10^{-1}	90	9.0×10^{-1}
	10^{-3}	97	9.7×10^{-3}
	10^{-6}	99	9.9×10^{-6}
CTR	10^{-1}	21	2.1×10^{-3}
	10^{-3}	17	1.7×10^{-3}
	10^{-6}	6	0.6×10^{-6}

Key: A= Igbemarket, B= Uchi market, C= Ughele market, CTR= Control

Table 4.4: Percentage occurrence of isolates in each sample

Sample	*E.coli*	*Staphylococcus Aureus.*	*Salmonella spp.*	*Pseudomonas sp*	*Shigella spp.*
A	+	+	-	-	+
B	+	-	+	-	-
C	+	+	+	+	+
CTR	-	-	-	-	-
Total %	75	50	25	50	50

KEY: - = Negative and + = Positive.

4.2 Discussion

Pathogenic micro organisms remain the major contaminants of ready to eat food such as zobo drinks. In this study, the bacteria isolate obtained were suggestive of E.coli, Staphylococcus aureus, Salmonella spp, Pseudomonas spp and Shigella spp. The total average number of viable aerobic count ranged from 3.5×10^{-1} CFU/ml, 2.3×10^{-3} CFU/ml, and 1.3×10^{-5} CFU/ml respectively using the different dilution factor. Each of these results represents the value which correspond with the result obtained by Egbere *et al.,* (2007). All samples indicated significant level of contamination and the presence of bacteria may be due to both pre and post contamination. Although all the samples were contaminated with varying levels of bacterial counts that can be classified as satisfactory as repoted by Zumbes *et al.,* (2014). It is possible that the occurrence of these pathogens occurred during processing, which was reported as the major source of contamination of locally made drinks by Fowoyo (2012). Necessary precautions might have been neglected and as such contamination could be inevitable as reported by Musa and Hamza (2013).

The percentage occurrence of bacterial isolates in table 4.3 shows that E.coli spp. has the highest occurrence of isolates with 100%, followed by Staphylococcus aureus with 75%. Pseudomonas spp and Shigella spp. has 50% occurrence respectively while Salmonella spp. has the least occurrence of 25%. The occurrence of these micro-organisms is largely due to their presence in nature (Bristone *et al.*, 2018). Their association with foods such as commercial zobo drinks may be as result of poor hygiene or poor sanitary condition as reported by Raima, (2013).

According to Omemu *et al*. (2006), Staphylococcus is a common contaminant of foods and other similar preparations if good hygienic practices are not employed. Staphylococci aureus as normal flora of human inhabit the nostrils, hands, skin, mouth and dresses etc. They might easily gain access to zobo without good sanitary practices. Those who prepare zobo locally do not consider the use of good water as a means of reducing contamination therefore water may also serve as source of contamination introducing Staphylococcus aureus and other bacterial into the zobo drinks, especially water used for washing and rinsing of recycled containers for packaging. Salmonella Spp. might be present as a result of the handling process from contaminated hands and water.

Zobo drinks are always been sold in recycled bottles of water and soft drinks like coke, Fanta, sprite etc. These bottles could have only been washed or rinsed without any proper sterilization process. This process might bring about the contamination of zobo drinks. The percentage occurrence of bacteria isolated from zobo is an indication of poor hygienic handling.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 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 micro organisms can cause varying levels of diseases from food poisoning. 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.

5.2 Recommendation

Ready to eat food such as zobo drink is consumed by all and sundry and 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 see zobo as an alternative source of cheap non-alcoholic drink. It is therefore recommended that

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

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