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ISOLATION AND IDENTIFICATION OF MICROORGANISMS FROM LOCALLY PREPARED ZOBO DRINK IN KONDUGA TOWN, BORNO STATE, NIGERIA

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ABSTRACT

Isolation and identification of microorganisms from locally prepared *Zobo* drink in Konduga town were carried out using standard bacteriological analysis. The results obtained showed that, all the samples were contaminated with different microorganisms which include; *Klebsiella Species, E. coli, Proteus Species, Pseudomonas Species, Salmonella Species* and *Staphylococcus aureus*. Sample from Ajari (H) and Fulatari (I) contained six potentially pathogenic bacteria. Ajari (H) had the highest total heterotrophic count of $5.0x10^6$ while G.G.C low cost (F) had the lowest cfu/ml of $3.5x10^6$. *E. coli* was the most prevalent isolates from all the samples with the overall frequency of 22 (25.0 %). Therefore, there is a need to educate the producer of such products on the importance hygiene at various stages of processing and at the point of sale. This will greatly enhance and improve the quality of the products and it will reduce the incidence of food born poisoning caused by consumption of contaminated food products.

KEYWORDS

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INTRODUCTON

Zobo drink is a non-alcoholic local beverage made from different varieties of dried petals, acid succulent aqueous extract of the calyx of Roselle, *Hibiscus sabdariffa* which is an annual plantand is widely cultivated in India and some part of Africa (Lawton, 2004)[1]. It is a redliquid drink and tastes like fruit punch. Zobo is a name derived from "Zoborodo" which is a language (Hausa) in the Northern part of Nigeria name for *Hibiscussabdariffa*. The non-alcoholic drink or Zobo is quite popular, especially in the Northern part of Nigeria and is usually served chilled at

various social gatherings (Aliyu *et al.,* 2000)[2]. There is arapid increase in the demand of Zobo due to its low price, nutritional and medicinal properties. The economic situation in Nigeria has made Zobo drink gain more acceptance in different occasions (Oboh and Elusiyan, 2004)[3]. It is being consumed by millions of people from different socio-economic classes and backgrounds in the West African sub-region especially among the youths, who also see Zobo as an alternative source of cheap and relaxing non-alcoholic drink in social gatherings (Ogiehor and Nwafor, 2004). It is used for refreshment, entertainment at parties, weddings, and naming ceremonies as appetizers before the main dish is served and also sold in the markets to various consumers (Onuorah *et al.,* 1987)[4]. Zobo drink is prepared by boiling the dry calyces (sepals) of *Hibiscus sabdariffa* in water for about 15-20 minutes from which the pigments embedded in the flower are extracted. The extract may be taken hot as tea or taken as a refreshing drink when chilled. The sharp, sour taste of the raw extract is usually sweetened with sugar cane, granulated sugar, pineapple, orange or other fruits or flavors depending on choice (Osueke and Ehirim, 2004)[5]. 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, 2002)[6]. Zobo drink is extracted from thedried reddish purple calyces of the plant *Hibiscussabdariffa* (Scott, 2003). The calyces are used toproduce herbal teas and other food products (Akanya *et al.,*1997)[7]. The juice drink which is usually obtained by extraction of the calyx of *Hibiscus sabdariffa* contains about 1 % solid. The drink contains some microorganisms which can cause food spoilage (Omemu *et al.,* 2006)[8]. At present, the production processes in neither mechanized nor standardized. Consequently, the shelf life of the Zobo drink is less than two days (Samy 1980)[9]. 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 in preparation and service point has resulted in poor unsanitary conditions exposure to potential contamination and an increased risk to public health (Omemu and Aderoju, 2008). Consequently, street drinks and food safety have remained a major public health concern globally, and more important lyin Nigeria, were the regulation of this critical sector is virtually non-existent or inadequate, making street foods and drinks hazardous source of nutrition (Oyeyi and Lum-nwi, 2008, Wadakura *et al.,* 2009). A food frequently serves as a vehicle for spreading of several infections, some of which are pathogenic (Singleton, 1999)[10]. 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 guest, but also as the vehicle for transmitting staphylococcus food poisoning (Alabi and Akinsinyun, 1996)[11]. In view of the facts, that Zobo isnever 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 involvedin the cooling and subsequent dispensing of the drink into containers also represent a potential source of health hazard. Cruck and Shank (1984)[12] have reported that some gastrointestinal illness characterized by abdominal cramps, diarrhea and vomiting, which may be assumed has been of unknown etiology may arise from drinking products contaminated with microorganisms. In this study, retailed Zobo drinks were purchased from various locations within Konduga town, they were analyzed for their bacteriological quality as indicated by the number (counts) and kinds of bacteria they harbor illness characterized by abdominal cramps, diarrhea, and vomiting which may be assumed has been of unknown etiology may arise from drinking drinks contaminated with microorganisms.

MATERIAL AND METHODS

Study Area

The study was conducted in a Konduga local government area of Borno state, Nigeria. Konduga is located within the vicinity of Maiduguri, the State capital. The local government area shares boundary with Mafa to the East, Bama to the South, Damboa to the West, Nganzai and Maiduguri to the North. In terms of land mass, it covers a land mass of 5.825 square kilometers with a population of 125, 915 according to the 1991 provisional census. In this study, ten samples were randomly collected from

different part of the local government, these include; Low cost (A), Babban Gari (B), Mandarari (C), Hausari (D), Tashan Kifi (E),G.G.C Low cost (F), Bulamari (G), Ajari (H), Fulatari (I), and Gwazari (J). All samples were collected from the sampling site within the Konduga town and taken to the Microbiologylaboratory, University of Maiduguri for analysis.

COLLECTION OF SAMPLES

Retailed ready to drink Zobo juice were purchased from various locations in Konduga town, samples were collected using sterilized bottle, and transported in a cool container to the Microbiology laboratory, Faculty of Science, University of Maiduguri, for Microbiological analysis.

Heterotrophic Plate Count

Serial dilution of Zobo samples was prepared with sterile distilled water. The 10^6 dilution was used. The 1ml of the Zobo sample was aseptically transferred into the sterile petri dish containing the suitable media. The Zobo sample was spread uniformly using sterilized glass rod and the media was incubated at 37˚C for 24 hours. After the period of incubation, the colonies on the plates were counted and recorded as colony forming unit per milliliter (cfu/ml) as described by Harrigan and McCance (1976)[13]. Each of the bacterial colonies on the agar plates was sub-cultured and the pure culture obtained. Isolates were identified using Gram staining, and biochemical tests whichinclude; catalase, coagulase, oxidase, citrate utilization, indole, methyl red, urease and voges proskauer as described by Cheesbrough (2005)[14].

RESULTS

The morphological and biochemical characteristics of the bacterial isolates are shown in Table No.1. A total of six bacterial isolates were identified, which include *Salmonella* spp, *E. coli*, *Proteus*spp, *Staphylococcus aureus*, *Klebsiella* spp and *Pseudomonas* spp.

Table No.2 showed the total heterotrophic counts and the isolated organisms. Ajari (H) had the highest number of colony count of $5.0x10^6$cfu/ml while G.G.C Low cost (F) had the lowest colony count of $3.5x10^6$cfu/ml.

Table No.3 showed the distribution of the different pathogenic bacteria isolated from each sample. *E. coli* had the highest frequency of occurrence of 25%, followed by *Salmonella* and *Pseudomonas* spp which had the frequency of 11.4%. *Klebsiella* spp has the least frequency of occurrence of 10.2%.

DISCUSSION

Generally microorganisms are said to be ubiquitous in nature (Hobbs and Gilbert, 1978), and their isolation in locally prepared drinks sampled in this study is paramount, due to some of them are potentially pathogens. The fact that most of the sample from the various sources (local market) tested positive showed that the product could be hazardous to the community and this finding is similar to the ones reported earlier by Adesiyun (1986). Furthermore, the danger associated with the consumption of such products is further compounded by the fact some of the microorganism isolated in this study could be enter otoxigenic and could therefore be potentially a source of food borne outbreak of a greater magnitude. In addition, the fact that most of the hawkers of such products are young children of unknown health status, they could serve as a potential source of contamination at the point of sales, post processing apart from the contamination that could occur prior to and during processing. In an earlier study on similar products, production practices have been associated with contamination of such products (Atepapunam *et al.,* 1997). In fact, most of the ready to drink sampled in this study yielded a very high total plate count is significant from a public health point of view. The significance lies in the fact that none of the drink is safe and all are a potential source of food borne out break of great magnitude. The possibilities of such contaminations are coming from the producers or some of the ingredients used in preparation are high especially the water used as diluents. This is because previous records showed that contaminated water has been reported to contain viable microorganisms that are potentially of health significance (Aliya *et al.,*1995).

Table No.1: Morphological and Biochemical Characteristics of the Isolated organisms from *Zobo* drink

Morphological Characteristics		Gram Staining	Biochemical Characteristics							Suspected Organism
Colour	Shape		Catalase	Indole	Meth yl red	Voges Proskauer	Citrate Utilization	Oxidase	Urease	
Black spot	Circular mucoid, convex colony	-ve bacilli	-	-	-	-	+	-	-	*Salmonella spp*
Pinkish colony	Large smooth and glisterin g colony	-ve bacilli	-	+	+	-	-	-	-	*E. coli*
	Circular convex colony	-ve bacilli	-	-	-	-	+	-	+	*Proteus spp*
Golden colony	Colony arrange in cluster	+ve cocci	+	-	-	-	-	-	-	*S. aureus,*
Pinkish colony	Large circular mucoid colony	-ve bacilli	-	-	-	+	+	-	-	*Klebsiella spp*
Greenis h and Urine colony	Circular mucoid colony	-ve rod	+	-	-	-	-	-	+	*Pseudomo nas spp*

Table No.2: Total heterotrophic count and the isolated organisms from *Zobo* drink

S.No	Samples Area	Total heterotrophic count (cfu/ml)	Isolates
1	A	4.0 x 10^6	*Salmonella spp. E. coli, S. aureus, Klebsiella spp.* and *Pseudomonas spp*.
2	B	4.4 x 10^6	*E. coli, Proteus spp.* and *Pseudomonas spp*
3	C	3.6 x 10^6	*E. coli, Proteus spp. S. aureus, Klebsiella spp.* and *Pseudomonas spp*
4	D	4.9 x 10^6	*Salmonella spp. E. coli, Proteus spp. S. aureus, Klebsiella spp.* and *Pseudomonas spp*
5	E	3.8 x 10^6	*Salmonella spp. E. coli, Proteus spp. S. aureus, Klebsiella spp.*
6	F	3.5 x 10^6	*E. coli, Proteus spp. and Klebsiella spp.*
7	G	4.1 x 10^6	*Salmonella spp. E. coli, Proteus spp. S. aureus, and Klebsiella spp.*
8	H	5.0 x 10^6	*Salmonella spp. E. coli, Proteus spp. S. aureus, Klebsiella spp.* and *Pseudomonas spp*
9	I	4.9 x 10^6	*Salmonella spp. E. coli, Proteus spp. S. aureus, Klebsiella spp.* and *Pseudomonas spp*
10	J	3.9 x 10^6	*E. coli, Proteus spp. S. aureus, Klebsiella spp.* and *Pseudomonas spp*

Table No.3: Frequency of bacterial occurrence in *Zobo* drink

S.No	Isolated Organism	Frequency of occurrence	Frequency of occurrence (%)
1	*Salmonella spp*	10	11.4
2	*E. coli*	22	25.0
3	*Proteus spp*	17	19.3
4	*S. aureus,*	20	22.7
5	*Klebsiella spp*	9	10.2
6	*Shigella spp*	10	11.4
7	Total	88	100

CONCLUSION

The finding of this study showed that the locally prepared and marketed Zobo drink in Konduga town of Borno State, contaminated with a wide variety of potentially pathogenic microorganisms. Therefore, there is need to enlighten the general public on the danger associated with the consumption of such products. There is also need to educate the producer of such products on the importance hygieneat various stages of processing and at the point of sale. This will greatly enhance and improve the quality of the products and it will reduce the incidence of food born poisoning caused by consumption of contaminated food products.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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