Global Journal of Pharmacology 10 (1): 31-34, 2016
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2016.10.01.1126

Bacteriological Examination of Local Made Zobo Drinks Sold in Abakaliki Metropolis

[1]O. Nworie, [2]J.O. Orji, [2]U.O. Ekuma, [2]M.V. Agah, [3]C.S. Okoli and [4]N.N. Ukpai

[1]Department of Microbiology, Federal University Ndufu-Alike Ikwo, Ebonyi State, Nigeria
[2]Department of Applied Microbiology, Ebonyi State University, Abakaliki, Nigeria
[3]Department of Applied Microbiology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
[4]Health Department Ezza South Local Government Area, Onueke, Ebonyi State, Nigeria

Abstract: This research was conducted to evaluate the bacteriological quality of zobo drink produced locally. Three zobo samples were randomly purchased from Abakaliki metropolis, which include Presco campus, Meat market and Democracy market. They were analyzed microbiologically according to standard methods. Bacteria isolated from the zobo drink samples include *staphylococcus aureus and Escherichia coli*. 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 count of *Escherichia coli* and *Staphylococcus aureus*. The microbial load ranged from 1.0 x 55 to 55 x 99cfu/g and 1.0 x 69 to 1.0 x 102cfu/g. The present findings revealed that zobo drinks retailed and sold in these market are potential vehicles for transmitting food borne illness, thus the need to develop good manufacturing process (GMP) and post production preservation and packing techniques.

Key words: Bacteria · Zobo · Food and Microbial load

INTRODUCTION

Zobo drink is a non alcoholic local beverage made from different varieties of dried petals, acids succulent calyces of the flower *Hibiscus subdariffa* by boiling and filtration [1].

Zobo flower is an annual erect, bushy herbaceous smooth sub shrub with smooth or nearly smooth, cylindrical and typically red stem. This flower is highly cultivated in the northern part of this country probably because of climate. It is gaining wide acceptance, being consumed by several millions of people from different. Scio-economic classes and background in the West Africa sub region especially amongst the youth, who see zobo drink as an alternative source of cheap andrelaxing non alcoholic drink in social gathering.

The calyces of hibiscus sabdariffa have been found to be rich in vitamins, natural carbohydrate protein and vitamin C and other antioxidants [2] and also mineral [3] which constitute the major reasons for consuming soft drink and fruit juiced. The zobo drink if well prepared and packaged will complete favourably with most of the important non alcoholic beverage available in the country considering the increasing acceptance socio-economic vitamin C and other minerals, there is need to know the variety that can produces for zoborodo, however vary from one locality to another thereby leaching variation in the quality attributes especially the nutrients and appearance of the product. This study therefore sought to determine the variety that will not only give the desired colour but will so retain most of the nutrient after boiling for 10 minutes or 15 minutes. The aim of this study was to investigate, detect and evaluate the presence of microorganism.

MATERIALS AND METHODS

Sample Collection: Three samples from different market sellers at different location was purchased with a sterile container and taken to laboratory for analysis. The samples collected were labelled with an alphabet for easy identification such as sample A (Presco campus), B (Meat market) and C (Democracy market). The samples were then stored in the refrigerator till the time of use which did not exceed two hours.

Corresponding Author: O. Nworie, Department of Microbiology, Federal University Ndufu-Alike Ikwo, Ebonyi State, Nigeria.

Media Preparation

Eosin Methylene Blue (EMB) Preparation: This was done by suspending 36grams of the powder in 100ml of distilled water. The suspended was mixed very well and heated with frequent agitation to dissolve the powder completely and avoid over heat.The suspend was sterilized in autoclaving at 121°C and 15ps, for 20minutes.Cool to 50°C and shake the medium to oxidize the methylene blue and to suspend the flocculent precipitate.Pour into sterilize petridishes.Six petridish plates plate used for EMB and waslabelled as a sample A1 and A_2, B1 and B2 and C1 and C2.

Total Viable Count: Pour plate method was used. Each sample was serially dilution using sterile distilled water as diluents. Five tubes were used for each sample. Examples: serial dilution of 1 ml of the sample to 9 ml of water. The same serial dilution was carried out in sample B and C. after serial dilution 1 ml with new syringe is used to pipette the sample from the last inoculums and was poured on the plate aseptically until the entire sample finished. A new syringe was used in each sample, about 20ml of the media was poured aseptically on the samples and gently to mix and incubate inverted for 24 hours at 37°C. Bacterial Enumeration and isolation: Total bacterial count was determined by pour plate technique using standard methods. Nutrient agar medium was used for enumeration of bacteria in the sample. The total bacteria count was obtained by incubation aerobically at 37°C for 24 hours in the plate *Escherichia coli*.

Blood Agar: Blood agar is prepared using mammalian blood (horse) at a concentration of 5-10% or human blood. It is general purpose enriched medium often used to grow fastidious organisms and to differentiate bacteria based on their hemolytic properties. Blood agar was prepared accordingly using manufactures instructions and 6 plate were used serial dilution was also carried as did in Eson methylene blue (EMB). After the inoculation and incubation, the organisms seen are *Escherichia coli* and *Staphylococcus aureus.Escherichia coli* appears as medium sized, smooth round, grayish, white colonies on blood agar.*Staphylococcusaureus* appear as medium sized yellowish, white, hemolytic colonies on blood agar or they appear as a large creamy white.After the primary culture is gotten sub culturing was done to get a pure culture using streak plate method and nutrient agar is the media.

Preparetion of Nutrient Agar: Dissolve 28.08 in 100 ml distilled water gently heat to dissolve the medium completely sterilize by autoclaving at 15psi (121°C) for 15minutes.The media were autoclaved and allow cooling before pouring 15ml each on the plate and allowed to gel, after setting the microorganism isolated and streaked aseptically using a wire loop and inoculated for 24hours.After the inoculation, the growth was observed for pure culture.Pure culture is gotten and then stored for further examination such as gram staining.

Biochemical Test

Identification of Isolates of Microorganisms: Morphological features and biochemical reactions patterns were used for the identification of bacterial isolates.

Gram Staining: The method used was that described by [4] smear of the isolates was prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with a gentle running tap water. The slides were flooded with dilute gram iodine solution. This was washed out with a gentle running tap water. The slides were flooded with dilute gram iodine solution. This was washed off with water and smear were decolorized with 95% alcohol till the blue colour no more dripped out (about 30 seconds) the smear were then counter stained with saffranin solution for about 10 seconds finally the slides were washed with gentle running tap water, air dried and observed under oil immersion objective.

Catalase Test: This test was used to demonstrate which of isolated could produce the enzymes that release from hydrogen peroxide. It is also used as an aid to different staphylococci from streptococci and to differentiate other catalase positive organism from catalase negative. A loopful of pure colony was transferred into a plane; clean glass slide the sample was then mixed a drop of 3% v/v hydrogen peroxide. The reaction was observed immediately. The gas production indicated by the production of gas bubbles confirmed the presence of catalase.

Oxidase Test: It is used in the identification of organisms such as pseudomonas, Neisseria, Vibrobrucecla and pasteurella species, Bacillus, mycobacterium and other

gram-negative cocci. All organisms that are oxidase positive produce the enzyme cytochrome oxidase negative organisms such as staphylococcus Escherichia coli and klebsiell species etc. A freshly prepared reagent 0.2g of tetra-methyl-phenylene-diamine hydrochloride in 20ml of distilled water was poured over inoculated agar plate 24hrs incubation and the excess immediately poured off positive colonies turn bluish purple.

Indole Test: This test was used to determine which of the isolated has the ability to split indole from tryptophone present in buffered peptone water. This test is used for identification of Enterobacteria such as Escherichia coli, Bacilli species and gram negative [5]. The tube of peptone water were inoculated with young culture of the isolated, the tube were incubated at 37°C for 48hours about 4drop of koval reagent autoclaving at 121°C for 15minute one percent of solution of the sugar was prepared and sterilized separately at 115°C for minutes. This was aseptically dispensed in 5ml aliguot volume into the tube containing the peptone water and indicator. The tubes were inoculated with young culture of the isolated and incubated at 37°C. Acid and gas production were observed after 24hours of incubation. Add 0.5ml of kovals reagents shake gently and examine for colour the surface layer within 10 minutes. Red surface layer show positive indole test. Absence of red surface layer show Negative indole tests.

Coagulase Test: The coagulase test identifies whether an organism produces the exoenzyme coagulase which cause the fibrin of blood plasma to clot. Organism that produce catalase can form protective barriers of fibrin around themselves, making themselves highly resistants to phagocytosis, other immune responses and some other antimicrobial agent. The coagulase slide test is used to identify the presence of bound coagulase or clumping factor, which is attached to the cell walls of the bacteria. The coagulase test is useful for differentiating potentially form a pure culture is transferred aseptically to the serum indicative of a positive test.Positive coagulase test indicate the presence of *staphylococcus areus*because of the clumping within 10seconds. Negative coagulase test indicate *Eschericha coli.*

Motility Test: Is a test used to determine whether microbial species is motile or not. It was done by placing a clean depression slid and cover glass.Shake the culture tube and transfer two loopful of the culture onto the cover glass and place on a phase contrast microscope utilizing high dry magnification. The organism is motile, you see them moving about and if a bright field microscope is used, it is necessary to reduce the lighting to a bare minimum to the cover glass and swing the oil immersion lens into position. The advantage of the wet mount is that it is the quickest means for determining motility and enable us to see the organisms in their natural state.

RESULT AND DISCUSSION

The bacteria load was high which indicates a high level of contamination. This is very dangerous to the public as these contaminants has the ability to cause varying level of disease ranging from food borne illness and food poisoning due to *Staphylococcus aureus*.Presence *E. coli* can be responsible for prevalence of diarrhea, fever, nausea and cramps in children and adult exposed to contaminated drinks and beverages should be regulated in Nigeria by NAFDAC and food regulatory bodies as drinks of low and minimum safety standard is injurious to health on acute or chronic basis.

Consequently education of the manufactures and provision of basic facilities will greatly improve Zobodrink quality and safety. To be effect, regulation, effective monitoring and enforcement of the existing punitive measures is therefore recommended.

Table 1: The physiochemical properties of bacteria isolated from the Zobo drink sample.

Isolates	Gram reaction	Catalase test	Oxidase test	Indole test	Coagulase test	Motility test	Probable organisms
A1	-Rod, dark red	-	+	-	-	M	E.coli
A2	+ rod, dark red	+	+	-	-	M	E. coli
B1	+cocci, clusters golden yellow	+	+	-	+	NM	Staphylococcus auerus
B2	+ rod dark red	-	+	+	-	M	E. coli
C1	+ cocci golden yellow	-	-	+	-	NM	Staphylococcus auerus
C2	-cocci golden yellow	-	+	-	+	NM	Staphylococcus auerus
Keyword	+ positive -Negative M=motility NM = Non motility						

Table 2:Bacteria isolated from different zobo brands

	Zobo Brands					
Bacteria isolated	A1	A2	B1	B2	C1	C2
Escherichia coli	-	+	+	-	-	-
Escherichia coli	+	+	+	+	-	+
Staphylococcus auerus	-	-	-	+	+	-
Staphylococcus auerus	-	-	+	-	-	+

KEY: + = Positive, - = Negative.

Table 3: Cultural characteristics of organisms on EMB.

Sample Code	Shape	Colour	Bacterial Cell
Sample A1	Circular rod	Creamy dark blue	55 colonies
Sample A2	Circular rod	Creamy pinkish	99 colonies
Sample B1	Circular rod	Creamy blue	70 colonies
Sample B2	Circular rod	Creamy pinkish	71 colonies
Sample C1	Circular rod	Creamy blue	30 colonies
Sample C2	Circular rod	Creamy blue	51 colonies

Table 4: Cultural characteristics of organisms on blood ager.

Sample Codr	Shape	Colour	Colonies
Sample A1	Round clustered	Milkish creamy	
	circular rod	whitish	1
Sample A2	Circular rod	Creamy whitish	2
Sample B1	Circular	Greenish	2
	Circular	Yellowish	4
Sample B2	Circular	Whitish	19
	Circular	Creamy	14
Sample C1	Circular	Creamy grayish	69
	Circular	Creamy	102
Sample	Circular	Creamy	4
	Circular	Grayish	102

However, street drinks and food safety has remained a major public health concern globally and more importantly in Nigeria here the regulation of this critical sector is virtually non-existent or inadequate, making street food and drinks hazardous source of nutrition [6] food frequently serves as vehicle for spreading of several organism some of which are pathogenic.

Many picnic suppers and banquate home come to a disastrous and which home prepared foods and drinks serves not only as food and drinks for quest but also the vehicle for transmitting staphylococcus food poisoning [7]. In view of the facts, that zobo is new subjected to any form of post production reduce the bacteria load in the cooling and subsequent dispensing of the drink into containers also represent potential source of health hazard some gastrointestinal illness characterized by diarrhea, abdominal cramps and vomiting which may be assumed as been of unknown aetiolog may arise from drinking drinks contaminated with microorganisms.

CONCLUSION

It is important that both the grower and marketer of Hibiscus sabdariffa take necessary precaution in preventing contamination of the calyces with fungi to reduce possible contamination and hence reduce risk of aflatoxin and other mycotoxins that are deleterious to health.

REFERENCES

1. Oboh, G. and C.A. Elusiyan, 2004. Nutrient composition and antimicrobial activity of sorrel drink (soborodo). Journal of Medicine Food, 3: 340-342.
2. Wong, P., S. Yusof, H.M. Ghazah and Y.E. Cheman, 2002. Physico-chemical characteristicsof Roselle (Hibiscus sabdariffa L.), Nutrition and Food Science, 32: 68-73.
3. Babalola, S.O., A.O. Babalola and O.C. Aworh, 2001. Compositional attributes of Roselle (*Hibicussabdarriffa* L), Journal of Food Technology. Africa, 6: 133-134.
4. Cheesbrough, M., 2006. District Laboratory Practice in Tropical Countries Cambridge University Press, United State of America Part2. pp: 38-39, 64-67, 137-138.
5. Bukar, A., M. Yushau and E.M. Adikwu, 2009. Incidence and identification of potential pathogens on hands of some personnel in some small –scale food industries in Kano Metropolis. Nigeria. Biol. Environ. Sci. J. Trop., 6:4.
6. Oyeyi, T.I. and M.E.F. Lum-Nwi, 2008. Bacteriological quality of some street vended foods in Bayero University campuses, Kan, Nigeria. Niger. Biol. Environ. Sci. J. Trop., 5(4): 239-243.
7. Chomvarin, C., Y. Chantarasuk, S. Srigulbutr, S. Chareonsudjai and K. Chaicumpar, 2006. Enteropathogenic bacteria and enterotoxin producing *Staphylococcus aureus*isolated from ready to eat foods in KhonKaen, Thailand. Southeast Asian J. Trop. Med. Pub. Health., 37(5): 983-990.