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Research Article
Antimicrobial Activity of *Lactobacillus* from Zobo Drink Against Various Pathogens

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Abstract

Background and Objective: The bio-preservation of food products using bacteriocin-producing lactic acid bacteria (BPLAB) isolated directly from fermented foods is an innovative approach in ensuring food safety for human health sustainably. This study was designed to isolate and identify BPLAB from zobo drinks and evaluate their antimicrobial effects on selected spoilage and pathogenic microorganisms *in vitro*. **Materials and Methods:** Lactic acid bacteria were isolated using bacteriological media, while agar diffusion bioassay was employed to screen bacteriocin or bacteriocin-like substances and their antimicrobial effect was tested on selected pathogens, namely *Salmonella pullorum*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* as indicator organisms. **Results:** A high pH value of 5.5 was recorded from the control sample (LAB) while high temperature and total titratable acid of 28°C and 0.62% were obtained from the ETF sample, respectively. Out of 14 lactic acid bacterial isolates, *Lactobacillus minor* and *L. buchneri* exhibited total inhibition while *L. bifermentans* and *L. fructivorans* exhibited no inhibition to the tested microorganisms. Varied antimicrobial susceptibility profiling for the indicator isolates was recorded. **Conclusions:** The potential of BPLAB to inhibit some pathogens suggest their potential use as bio-preservatives in foods. Hence, this study was performed to test for the antimicrobial ability of *Lactobacillus* isolated from zobo drinks against some selected pathogens.

Key words: Antibiotics, bacteriocin, beverages, food preservation, pathogenic bacteria

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In recent times, food processing and production for human consumption with multiple health benefits formed a fundamental aspect of human culture[1,2]. Production of local beverages sourced from simple and cost-effective food crops is known but less explored, however, previous findings concerning their maximum utilization cannot be overemphasized[3]. Different beverages produced are known to satisfy human thirst as a source of drinks for body homeostasis[4-6]. The common liquid drinks include milk, soft drinks, juice, coffee, hot chocolate and simple drinking water[7].

For more than 8 decades, the use of alcoholic beverages or drinks, such as liquor, beer, spirit and wine have been progressively subscribed both for cultural and ceremonial purposes[8,9]. Relative to the types of non-alcoholic drinks which can be processed locally compared to alcoholic drinks which are costly, however, there is a need to adequately proven theoretically the health benefits of consuming non-alcoholic beverages with high therapeutically potential, such as zobo drinks, which are not popularly known in many countries[10].

Zobo drinks are often sourced from *Hibiscus* plants as a potential local beverage in many parts of the world[11]. The drink can be extracted from the dried reddish-purple calyces of the plant, *Hibiscus sabdariffa*, which have been implicated in the production of herbal teas and other food products. In Nigeria, the religious and economic value of zobo drink has received a boost with more credence to its acceptability on many occasions as a refreshment, appetizer and source of income to the processors[7]. Descriptively, zobo drink is a liquid drink, red in color and taste like fruit punch, which contain vitamins and minerals[12]. Zobo drinks usually contain low sugar and have been used as a curative agent to stomach disorderliness, improper heart functioning and sore throat[13].

Inefficient hygiene practices along with production processes of zobo drink from sorting of plant material, washing, heat treatment, packaging to supplying can largely expose this product to potential contaminants which may pose a health risk to the public/consumers[14]. To this premise, food safety and the consumption of street drinks remain a major public health concern globally, more importantly, in Nigeria where strict regulations are less enforced in monitoring locally produced drinks supply to the consumers, thus increasing possible hazards of street drinks for human nutrition. These hazards can be linked to some pathogenic microorganisms capable of causing food spoilage as zobo drinks can serve as a vehicle for pathogen transmission[15]. Hence, care must be taken to avoid food contamination during zobo production and if possible, zobo drinks can be subjected to heat treatment after production to reduce the microbial load that may likely pose a health hazard[16]. Post-contamination after production of zobo drink may serve as a potential source of pathogenic microorganisms. Risiquat[14] reported that post-treatment contamination of foods with fecal coliforms might serve as a source of pathogens microbes, thus causing food contamination and spoilage.

Despite the quest and demand for soft drinks and other local beverages, the greatest limitation for large-scale production of zobo drinks is the rapid deterioration and short shelf-life. The shelf-ability of zobo drinks after production is usually approximately 24 hours if not refrigerated[13]. Therefore, a combination of effective preservation strategies to inhibit the growth of spoilage microorganisms become essential. The bio-preservative potential of lactic acid bacteria in foods has been reported with promises as probiotics due to their acidification in the production of lactic and acetic acids, bacteriocin and other inhibitory compounds against pathogenic microorganisms[17,18]. Some examples of potent genera include *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*[19]. Due to the bioprospecting of lactic acid bacteria in the fermentation processes for desirable food production, many of their species have been explored as starter cultures in the industry for the production of both alcoholic and non-alcoholic drinks[20]. For example, *Lactobacillus* species as starter cultures in the production of wine, yogurt, cheese, pickles, beer, cider, kimchi, kefir and other fermented drinks have been reported[4,21].

The bacteriocins produced by lactic acid bacteria, such as nisin, lactococcin and pediocin are regarded as potent antimicrobial peptides and synthetic metabolites possessing broad-spectrum inhibition against some Gram-positive and Gram-negative are commonly known as foodborne bacterial pathogens[22]. The limitation surrounding the use of bacteriocins is that they may inhibit closely related organisms, i.e., the desirable starter cultures and less effectiveness against Gram-negative causing food spoilage. Nevertheless, the antimicrobial properties of the inhibitory compounds may be desirable when lactic acid bacteria are used specifically against the targeted pathogenic microorganisms[23].

Consumption of fermented foods by lactic acid bacteria with therapeutic effects in promoting human health has been reported in maintaining the gastrointestinal tract by suppressing the growth of pathogens on the mucosal surfaces, thus stimulating mucosal immunity[24]. Aside from other factors necessitating probiotic selection, the systemic adherence ability of lactic acid bacteria to the intestinal mucosa is a major factor determining their selection as probiotics, because adhesion to the intestinal mucosa is

considered to be a prerequisite for their colonization[25]. Amraii *et al.*[26] evaluated the probiotic potential of lactic acid bacteria and bifidobacteria as adjunct cultures in various types of food products for therapeutic preparations. Lactic acid bacteria influence nutritional profiling and sensory properties of fermented foods and are also involved in the conversion of lactose and citrate (glycolysis and pyruvate metabolism), fat (lipolysis) and proteins (proteolysis, peptidolysis and amino acids catabolism)[27]. The involvement of *Lactobacillus* species in the food fermentation process and control of food-borne pathogens necessitates and their probiotic potential has been studied[26]. Hence, this study was performed to investigate the inhibitory effect of bacteriocin extracted from lactic acid bacteria isolated from zobo drinks.

MATERIALS AND METHODS

Sample collection: This study was carried out at the Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria in March, 2020. The zobo drinks were sampled from four different locations in Akungba-Akoko with proper labeling before being taken to the microbiological laboratory for further analysis. Isolation of microorganisms was performed by serial dilution up to the appropriate dilutions and then pour plating on different bacteriological media (De ManRogosa and Sharpe agar, nutrient agar and Muller-Hinton agar) after sterilization at 121°C for 15 min. Briefly, 1 mL of the stock sample was introduced into 9 ml sterile distilled water in test tubes and serially diluted with sequential homogenization of the diluents for two minutes and further diluted until 10^7 dilutions were reached. From the dilutions, 10^4 and 10^6, 0.1 mL was pipetted and aseptically dispensed into sterile Petri dishes and pour plated with molten sterilized media. The inoculated plates were allowed to cool and solidify and then incubated at 37°C for 24 hrs. The number of colonies formed on the plates was counted and expressed as colony-forming units per milliliters (CFU mL^{-1}) for each plate. The colonies were continually streaked on the fresh media until pure cultures were obtained. The bacterial isolates were identified following gram staining, biochemical, cultural and morphological methods[28].

Physicochemical properties of the zobo samples: The physicochemical properties of the zobo samples were expressed in their different units of measurement, which include temperature, pH and total titratable acid (TTA). Potentiometric measurements of samples were done upon collection using a pin electrode of a pH meter. The temperature of each sample was determined using a mercury-bulb thermometer. The TTA was measured by titrating a 10 mL *zobo* sample in a conical flask, to which 1 mL of 0.5% phenolphthalein solution was added as an indicator. The mixture was then titrated against 0.1 M sodium hydroxide (NaOH) solution until the development of faint pink coloration[29]. TTA was expressed as a percentage of lactic acid. The experiments were performed in triplicates and readings were recorded appropriately.

Antimicrobial activity of BPLAB isolated from zobo drink: The agar diffusion bioassay was used to screen for the bacteriocin-producing ability of lactic acid bacteria isolated from zobo drink[30]. Some pathogenic bacteria, namely *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella pullorum*, *Pseudomonas aeruginosa* and *Escherichia coli* were used as indicator organisms. One mL of each indicator organism (5×10^5 CFU mL^{-1}) was inoculated into 15 mL of semisolid MRS broth maintained at 50°C and then poured into a petri dish. After solidification, five wells (5 mm diameter) were designed and 35 µL of cell-free supernatant (CFS) from each lactic acid bacterial isolates was appropriately added to each well. The CFS was prepared by culturing 1 mL frozen lactic acid bacterial isolates in 20 mL MRS broth and incubated overnight, then, 1 mL culture from the overnight was sub-cultured into fresh 20 mL MRS broth and incubated at 37°C for 24 hrs. Lactic acid bacteria cells were harvested by centrifuging at 14,000 g for 5 minutes to obtain a supernatant. The supernatant was filtered through a sterile 0.22 µm syringe and 35 µL of the unadjusted aliquot of CFS was added to each well of the plated agar. The MRS plates were incubated at 37°C aerobically for 24 hrs. Inhibition zones were measured using a sterile transparent ruler[30].

Antibiotic susceptibility test: An antibiotic susceptibility test was performed following Kirby-Bauer's disc diffusion method on Mueller-Hinton agar[31]. A 24-hour broth culture of the bacteria isolate was diluted with normal saline to 0.5 M McFarland standard. An aliquot of 1 mL of the standardized broth was mixed with 19 mL of molten Muller-Hinton agar in sterile universal bottles and then poured on sterile Petri dishes and allowed to solidify. The antibiotic discs were placed (at least 20 mm apart) on the surface of the agar plate. The plates were incubated at 37°C for 24 hours. After incubation, the observed zones of inhibition were measured in millimeters and interpreted as susceptible (S), intermediate (I) and resistant (R)[32]. Choice of antibiotics was based on commonly prescribed antibiotics[33].

Statistical analysis: All procedures were carried out in triplicates and data obtained from the experiment were analyzed using Excel Microsoft 2013.

RESULTS

Total bacterial counts: The total lactic acid bacteria counts of the zobo samples were presented in Table 1. A high lactic acid bacteria count of 1.93×10^7 CFU mL^{-1} was recorded from the samples collected at the big gate (BIG), followed by Aluta market (ALT) of 1.85×10^7 CFU mL^{-1} and the least count of 1.19×10^7 CFU mL^{-1} from the small gate (SMG) with 1.19×10^7 CFU mL^{-1}.

Identification and occurrence of the bacterial counts: The identifiable lactic acid bacteria from zobo drinks include *Lactobacillus acidophilus*, *L. minor*, *L. fermentum*, *L. plantarum*, *L. fructosus*, *L. fructivorans*, *L. yamancishiensis*, *L. buchneri*, *L. delbrueckii*, *L. fruetosus*, *L. tolerans*, *L. crispatus*, *L. brevis* and *L. bifermentans* (Table 2). *L. bifermentans* and *L. fermentum* were the most dominant strains in the control sample (LAB) and samples collected from ALT and SMG, while *L. acidophilus* was dominant in the samples collected from ALT, BIG and EFT (Table 3).

The pH, temperature and TTA: zobo samples from ALT had the lowest pH of 4.2 while the highest value of 5.5 was observed in the control sample (LAB) (Fig. 1). Samples from BIG, SMG and ETF had the same pH of 4.4 (Fig. 1).

In Fig. 2, the highest temperature of 28°C and lowest temperature of 23°C were recorded from the zobo samples collected from ETF and BIG, respectively compared to the control sample (LAB). Samples from ALT and LAB had the same temperature value of 26°C. From the samples, the highest TTA of 0.62 was obtained from ETF, followed by BIG with a TTA value of 0.52%, while the least TTA value of 0.37% was obtained from ALT (Fig. 3). Table 4 showed the antimicrobial assay of lactic acid bacteria culture against test organisms.

Antibiotic susceptibility test: High susceptibility tests of *Escherichia coli* and *K. pneumoniae* with 28 mm zone of inhibition to *L. acidophilus* and *L. fructosus* were recorded. *P. aeruginosa* was susceptible to the lactic acid bacteria isolates except for *L. plantarum*, *L. fructosus*, *L. bifermentans*, *L. crispatus* and *L. fructivorans* that showed resistance. All the bacterial isolates were resistant to *L. bifermentans* and *L. fructivorans.* Table 5 showed the results of the antibiotic susceptibility test of the clinical isolates (gram-positive and gram-negative) using antibiotic discs. *S. aureus* exhibited resistance to Amoxicillin

Table 1: The lactic acid bacterial counts of the zobo samples

Sample codes	(CFU mL^{-1})
ALT	1.85×10^7
BIG	1.93×10^7
LAB	1.54×10^7
ETF	1.43×10^7
SMG	1.19×10^7

ALT: Aluta market, BIG: Big gate, LAB: Laboratory, ETF: ETF sample and SMG: Small gate

Table 2: The biochemical characteristics of the bacterial isolates of zobo samples

Isolate codes	Casein hydrolysis	Catalase	Methyl red	Indole	Starch hydrolysis	Galactose	Sucrose	Fructose	Maltose	Glucose	Gram stain	Probable organism
A1	+	+	-	-	-	+	+	+	+	+	+ve/cocci	*L. acidophilus*
A2	+	+	-	-	+	+	+	+	+	+	+ve/rod	*L. plantarum*
A3	+	+	-	-	+	-	-	+	-	+	+ve/rod	*L. fructusus*
A4	+	-	-	-	+	-	+	+	+	+	+ve/rod	*L. yamancishiensis*
A5	+	+	-	-	+	+	+	+	-	+	+ve/cocci	*L. kandleri*
A6	+	-	-	-	+	+	-	+	+	+	+ve/cocci	*L. fermentum*
A7	+	-	-	-	+	+	+	+	+	+	+ve/rod	*L. beuteii*
A8	-	-	-	-	+	+	+	+	+	-	+ve/rod	*L. delbrueckii*
A9	-	-	-	+	+	-	+	+	-	-	+ve/rod	*L. minor*
A10	+	-	-	-	+	-	+	+	+	+	+ve/rod	*L. yamancishiensis*
A11	+	-	-	-	+	+	+	+	-	-	+ve/rod	*L. fruetosus*
A12	+	+	-	-	+	+	+	+	+	+	+ve/cocci	*L. tolerans*
A13	+	-	-	-	+	+	-	+	-	-	+ve/rod	*L. bifermentans*
A14	+	+	-	-	+	+	-	+	+	+	+ve/rod	*L. crispatus*
A15	-	+	-	-	+	+	+	+	+	+	+ve/rod	*L. bifermentans*
A16	-	+	-	+	+	-	+	+	+	+	+ve/cocci	*L. brevis*

+: Positive and -: Negative

Table 3: Frequency of occurrence of LAB isolates from *zobo* samples

Sample codes	*L. acidophilus*	*L. plantarum*	*L. fructusus*	*L. fructivorans*	*L. yamancishiensis*	*L. buchneri*	*L. fermentum*	*L. reuteri*	*L. delbrueckii*	*L. minor*	*L. tolerans*	*L. crispatus*	*L. bifermentans*	*L. brevis*
ALT	+	-	+	-	+	+	+	-	-	-	-	-	+	-
SMG	-	-	-	-	-	-	+	+	+	+	-	-	+	-
BIG	+	+	+	-	-	-	-	+	-	-	-	-	-	-
ETF	+	-	-	-	+	-	-	-	-	-	+	-	-	-
LAB	-	-	-	-	-	-	+	-	-	-	+	+	+	+

ALT: Aluta market, BIG: Big gate, LAB: Laboratory, ETF: ETF sample, SMG: Small gate, +: Present and -: Absent

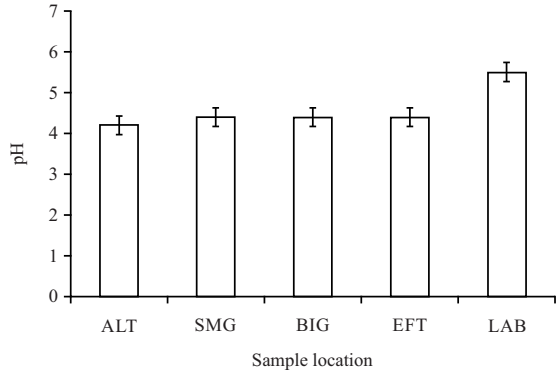

Fig. 1: The pH of the zobo samples

ALT: Aluta market, BIG: Big gate, LAB: Laboratory, ETF: ETF sample, SMG: Small gate, +: Present and -: Absent

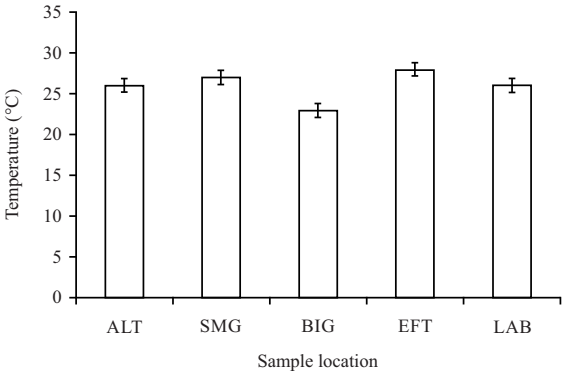

Fig. 2: Temperature of the zobo samples

ALT: Aluta market, BIG: Big gate, LAB: Laboratory, ETF: ETF sample, SMG: Small gate, +: Present and -: Absent

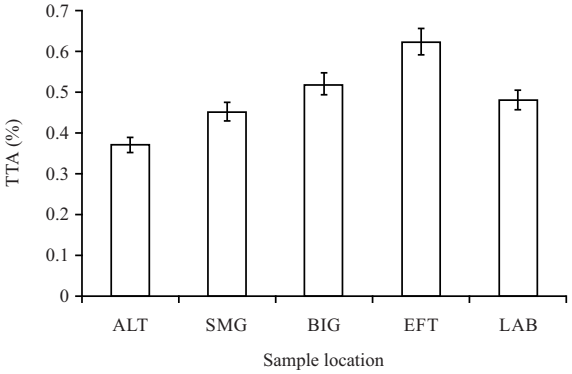

Fig. 3: Total titratable acidity of the zobo samples

ALT: Aluta market, BIG: Big gate, LAB: Laboratory, ETF: ETF sample, SMG: Small gate, +: Present and -: Absent

and Ampiclox. *K. pullorum* was susceptible to Septrin, Pefloxacin and Gentamicin but resistant to Ciprofloxacin, Augmentin, Streptomycin, Sparfloxacin and Ofloxacin. *Salm. pneumoniae* was susceptible to Augmentin,

Table 4: Antimicrobial assay of LAB culture against test organisms

	Diameter zone of inhibition				
Isolates	*E. coli*	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*	*Salm. pullorum*
L. acidophilus	28	23	24	0	24
L. plantarum	18	0	0	20	0
L. fructosus	0	20	0	28	20
L yamancishiensis	15	23	15	20	0
L. buchneri	26	25	23	26	22
L. fermentum	15	0	25	0	0
L. reuteri	15	15	22	20	0
L. delbrueckii	0	0	25	0	0
L. minor	15	20	20	10	26
L. fruetosus	0	26	25	24	0
L. tolerans	0	24	25	0	15
L. bifermentans	0	0	0	0	0
L. crispatus	10	0	0	10	0
L. fructivorans	0	0	0	0	0

Table 5 Antimicrobial susceptibility profile for indicator isolates

	Antibiotic susceptibility test for Gram-positive bacteria									
Isolates	R	AMX	S	ND	CH	CPX	E	LEV	CN	APX
S.a	19(I)	0(R)	20(I)	20(I)	15(I)	20(I)	20(I)	20(I)	17(I)	0(R)
	Antibiotic susceptibility test for Gram-negative bacteria									
Isolates	AU	CPX	SXT	S	PN	SP	OFX	Z	PEF	CN
K.p	0(R)	0(R)	14(I)	0(R)	13(I)	0(R)	0(R)	14(I)	13 (I)	15(I)
Salm.	20(S)	20(S)	5(R)	0(R)	0(R)	0(R)	17(S)	20(S)	20(S)	20(S)
P.a	15(I)	12(I)	20(S)	20(S)	14(1)	0(R)	0(R)	0(R)	20(S)	20(S)
E.c	13(R)	20(S)	20(S)	0(R)	0(R)	0(R)	20(S)	17(S)	20(S)	20(S)

Salm.: *Salm. pullorum*, K.p: *K. pneumoniae*, S.a: *S. aureus*, P.a: *P. aeruginosa*, E.c: *E. coli*, CPX: Ciprofloxacin, PEF: Pefloxacin, AU: Augmentin, AMX: Amoxicillin, CH: Chloramphenicol, APX: Ampiclox, S: Streptomycin, R: Rocephin, OFX: Ofloxacin. SXT: Septrin, SP: Sparfloxacin, CN: Gentamicin, Z: Zinacef, E: Erythromycin, PEF: Pefloxacin, LEV: Levofloxacin, PN: Penicillin, R: Resistance (<12), I: Intermediate (13-15) and S: Sensitivity (>16)

Ciprofloxacin, Ofloxacin, Septrin, Zinacef, Pefloxacin and Gentamicin, respectively but resistant to Streptomycin, Streptomycin and Penicillin. *P. aeruginosa* showed resistance to Sparfloxacin, Ofloxacin and Zinacef. *E. coli* was sensitive to the antibiotic used except resistance to the streptomycin, penicillin and streptomycin.

DISCUSSION

A total of 14 lactic acid bacterial isolates was characterized from 5 zobo drinks sampled within the University environment. Assumptions have been stipulated that zobo drinks can be a source of pathogenic bacteria capable of causing food spoilage[15], hence, evaluating the quality control check of zobo drinks for safety and nutrition becomes imperative. The bacterial counts recorded indicated their presence in the sample. zobo collected at BIG (big gate) had the highest counts of 1.93×10^7 CFU mL^{-1}, while the least counts of 1.19×10^7 CFU mL^{-1} were recorded from zobo drink collected from SMG (small gate). The relative bacterial counts observed in all the samples collected can be an indication that the zobo drinks may share similar quality based on the fact that the processors might have adopted similar processing techniques[6].

The high bacteria count recorded from BIG (big gate) samples might be attributed to the metabolic potential, adaptive mechanisms and secretion of organic acids, which favors their growth compared to other sample sources, while the low bacteria count in some samples may be due to the low metabolic rate of the bacterial isolates, high sterility level and proper packaging[34]. The varied bacterial counts observed might be due to the processing techniques, different sample sources and substrate itself[11]. The permeability of gases, such as carbon(iv)oxide into the package foods has been reported to influence microbial growth and survival[19]. For example, the presence of carbon(iv)oxide in plastic bottles may aid the growth of identifiable lactic acid bacteria in this study. Furthermore, the presence and the high number of these bacteria genera can be explained by the fact that these bacteria can withstand adverse processing conditions of the zobo samples.

Nwafo and Ikenebomeh[34] reported the effect of different packaging materials on the microbiological, physiochemical and organoleptic quality of zobo drinks stored at room temperature. In their study, authors inferred that the presence of some microorganisms in zobo drink after one month of storage could be an indication of partial treatments at the initial production process. Identification of *L. plantarum* from zobo drinks stored at room temperature has been reported[34]. The bacteria genera identified in this study have been implicated in some locally fermented beverages[5,8].

pH is one of the determining factors needed for microbial growth[35]. Some microorganisms can survive at high or low pH, depending on their nature and types. The pH of a growth medium can be adjusted under a controlled experiment to favor the growth of targeted microorganisms for a desirable end product[3,16]. The pH values obtained in this study were higher compared to the report of Nwafor and Ikenebomeh[34] on the pH value of 3.50 from the fresh and pH values of 3.50, 3.00 and 2.75, respectively from the packaged zobo drinks. Varied pH values in the zobo drinks treated with lactic acid bacteria compared to the zobo drinks from different sources can be linked to the lactic acid contents in the samples. Reports on the high pH values of some local beverages, such as Kunu Zaki, orange juice and zobo products have been documented[2,36]. The temperature of the samples ranged from 23°C to 28°C, which most likely favor the growth of lactic acid bacteria. Pasteurization of zobo drinks has been reported to influence its shelf life[37]. The high metabolic rate of lactic acid bacteria may underline the release of potent metabolite compounds, such as organic acids, which increase the total titratable acidity (TTA) by lowering the pH. The results obtained corroborate the findings of Nwafo and Ikenebomeh[34] who reported a TTA value of 0.80% from the zobo stored in the polythene sachet.

Lactic acid bacteria have been studied to exhibit antimicrobial activity against pathogenic and spoilage microorganisms[38]. All the test organisms exhibited varied inhibitions by the lactic acid bacteria except *L. bifermentans* and *L. fructivorans,* which showed no inhibitory effect against the entire test organisms and this might be due to their spectra of action. The antibacterial activity of the lactic acid bacteria showed they are highly effective against bacteria pathogens[24]. The activities of lactic acid bacteria can vary due to the type of metabolizable product and pathogens[39]. This study showed zones of inhibition of different supernatant obtained against the test organisms. These results corroborate with the work of Omemu and Faniran[24] and Hernandez *et al.*[18], who reported antimicrobial activity of some lactic acid bacteria from Kunu-Zaki and cheese against some contaminating spoilage bacteria and *Enterobacteriaceae* due to bacteriocin-producing potential.

The choice of antibiotic use in treating human diseases has been threatened due to the prevalence and the presence of drug resistance genes in pathogenic isolates, which is worrisome in developing countries where antibiotic use is abused[40]. The test microorganisms, such as *S. aureus*, *K. pneumoniae*, *Salm. pullorum*, *P. aeruginosa* and *E. coli* were also subjected to antimicrobial susceptibility tests using antibiotic-disk methods. From this study, high levels of the antibiotics susceptibility test revealed that most pathogens were found to be resistant to all the antibiotics except streptomycin which is sensitive. Also, the result revealed that *Salm. pullarum*, *P. aeruginosa* and *E. coli* exhibited resistance to three different antibiotics, while *K. pneumoniae* was resistant to only two antibiotics. The reason for the resistance of these bacteria to the antibiotics could be that the bacteria were isolated from patients who might abuse the use of antibiotics. The results obtained correlate with the previous work of Cardonha *et al.*[41] who reported the isolation of resistant *E. coli* strains to more than one antibiotic from water samples. Also, the test bacterium*, K. pneumoniae* in this study was slightly sensitive to gentamicin which correlates with the previous work of Makinde *et al.*[42].

CONCLUSION

In conclusion, this study has proven that zobo drink is a good source of lactic acid bacteria, which can exhibit diverse antimicrobial properties in fermented foods due to the production of organic acids and related compounds, such as bacteriocins, diacetyl and ethanol. However, the metabolite compounds produced by lactic acid bacteria, which cause inhibition on some pathogenic and food spoilage bacteria, may be explored as probiotics and bio-preservatives, especially in acid food fermentation. The promising results of this study underline the important role that BPLAB may play in the food industry as starter cultures to improve food quality and safety.

SIGNIFICANCE STATEMENT

The use of *Hibiscus sabdariffa* in the production of local beverages needs to be maximally explored based on its nutritional, social and economic importance, but less explored and their microbiological analysis is understudied. To this premise, the authors investigated the antimicrobial properties

of LAB from zobo drink sourced from *H. sabdariffa*, with a prospect in producing quality non-alcoholic drinks for the rural and urban dwellers. Many foods and foods products can be contaminated by spoilage microorganisms, which may pose potential health risks to the consumers. These microbes in foods can be inhibited by natural compounds, such as bacteriocin produced by some fermenting bacteria. Varied antimicrobial activities of LAB against pathogenic bacteria were evident in this study, thus suggesting their possible use as starters in the fermentation processes to ensure food safety. Interestingly, adequate processing of *H. sabdariffa* and consumption of foods fermented with BPLAB with therapeutic potential can help control foodborne pathogens, maintain intestinal flora and sustain human health. Hence, understanding the medicinal importance of *H. sabdariffa* and exploration of associated LAB as a source of bacteriocin can underline their future use in the synthesis of narrow-spectrum antimicrobial agents and their role in food safety.

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