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Full Length Research Paper

In vitro effect of steep water and slurry from fermented sorghum varieties on bacteria that causes diarrhea

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The effect of steep water and ogi slurry from two varieties of sorghum (red and white) on some important bacteria causing diarrhea (*Salmonella typhimurium*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Escherichia coli* and *Enterobacter* spp.) were investigated. Both the steep water and slurry were found to be effective against all test organisms except for *Enterobacter* which was only inhibited by the steep water from white sorghum variety. The slurry from white and red sorghum showed a maximum inhibition potential on *Escherichia coli* and *S. dysenteriae* with 10.6 and 6.00 mm diameter, respectively while the steep water from red and white varieties showed a maximum inhibition of 4.00 and 5.00 mm on *S. dysenteriae*. This study therefore suggest that raw ogi slurry from sorghum could be exploited in the treatment of bacterial that causes diarrhea.

Key words: Sorghum, steep water, slurry, bacterial, diarrheal.

INTRODUCTION

Each year in developing countries, one out of five children dies of diarrhea before the age of 5 years. Indeed, most of the 5 million individuals who die of diarrhea each year are infants (Schiller, 2007). Diarrhea can be copious and watery with infections of the small intestine, or in smaller amounts, containing mucus, pus and sometimes blood when the large intestine is involved (Nester et al., 2004). The problem of malnutrition, lack of pipe borne water and good sanitary conditions has contributed to this problem.

Lactobacilli are known to be involved in the fermentation of locally fermented food. They have been isolated from cassava products, fermented cereal grain and milk curd (Oyewole and Odunfa, 1988; Nduka, 2004). A great number of strains of lactic acid bacterial (LAB) produces metabolites such as bacteriocins (ribosomally synthesized peptides), lactic acid, acetic acid, hydroperoxide, lactoperoxidase system with lysozyme, reuterin, diacetyl and fatty acid that exhibit antagonistic activity against closely related species (Tagg et al., 1996; Holzapfel et al.,1995). These compounds have received increasing attention since they have the potential to inhibit food pathogens (Hammes and Hertal, 1998). Antimicrobials of *Lactobacilli* have been successfully used to prevent the formation of biogenic amines (Jooten and Nune, 1996), may inhibit pathogens causing mastitis (Niku et al., 1999) and may inhibit enteropathogens in the small intestine of animals. Although, there is little information on the antibacterial activity of ogi from sorghum, this study was aimed at investigating the antibacterial activity of steep water and slurry from sorghum varieties on bacterial that causes diarrhea.

MATERIALS AND METHODS

Collection of sample

The two varieties of sorghum (red and white varieties) used were obtained from Lagos street market, Ring road, Benin city. Bacterial isolates (*Staphylococcus aureus* and *Escherichia coli*) where collected from State Hospital Akure, while *Salmonella typhimurium*, *Shigella dysenteriae* and *Enterobacter* spp. were obtained from University of Benin Teaching Hospital, Benin.

Preparation of ogi

Three hundred grams of each carefully selected sorghum varieties were cleaned and steeped in 500 ml of cold water for 72 h at 28 ± 2°C. After the water was removed, the grains were washed and wet milled using a sterile blender. Muslim cloth was then used to sieve

Table 1. Mean inhibitory effects of sorghum ogi slurry and steep water on bacterial causing diarrhea.

Parameter	*S. aureus* Mean ± S.D (mm)	*S. dysenteriae* Mean ± S.D (mm)	*S. typhi* Mean ± S.D (mm)	*E. coli* Mean ± S.D (mm)	*Enterobacter* species Mean ± S.D (mm)
SL_W	$4.00^a \pm 2.00$	$4.00^a \pm 2.65$	$4.00^a \pm 1.00$	$10.67^b \pm 2.31$	$0.00^a \pm 0.00$
SL_R	$5.00^a \pm 2.00$	$6.00^a \pm 1.73$	$3.00^a \pm 1.00$	$4.00^a \pm 1.00$	$0.00^a \pm 0.00$
ST_R	$3.00^a \pm 0.00$	$4.00^a \pm 1.00$	$2.00^a \pm 1.00$	$3.00^a \pm 1.00$	$0.00^a \pm 0.00$
ST_W	$2.00^a \pm 1.00$	$5.00^a \pm 1.00$	$2.00^a \pm 1.00$	$3.00^a \pm 1.73$	$2.00^b \pm 1.00$

Mean for group having the same superscript are of homogenous subset (similar). SL_W, Sorghum slurry white; SL_R, sorghum slurry red; ST_R, steep water red; ST_w, steep water white.

into sterile containers and the filtrate was allowed to ferment for 72 h to produce ogi slurry.

The steeping process of the grains is known as primary fermentation, while the latter fermentation of slurry is known as secondary fermentation. The pH for both stages was determined using Jenway pH meter that has been standardized with appropiate buffers.

Isolation of microorganisms from steep water and slurry

One milliliter of the steep water and slurry was serially diluted separately using sterile peptone water up to 10^5. A loopfull from dilutions 10^3 and 10^5 was taken and introduced into separate sterile Petri dishes and streaked appropriately. Nutrient agar was used for the isolation of bacteria, PDA for fungi and MRS for lacto-bacillus. The nutrient agar was incubated at 37°C for 24 h, PDA 27°C for 48 to 72 h, while MRS, which is selective for lactobacilli, was incubated anaerobically at 25°C for 24 h. Isolates were identified according to Buchanan and Gibbons (1974), Schillinger and Lucke (1987), Collins et al.(1989), Wood and Holzapfel (1995) and Holt et al. (1994).

Determination of antibacterial activity of steep water and ogi slurry against test bacteria isolates

One milliliter of 24 h broth culture of the test organisms was introduced into separate sterile Petri dishes, different organism per plate. Each plate was then overlaid with 20 ml of nutrient agar already cooled to about 45°C, carefully swirled to allow even distribution of the organisms within the agar and allowed to gel. With the aid of a sterile cork borer (10 mm), five wells were bored on the agar surface containing the seeded bacteria. 0.3 ml of the steep water from the primary fermentation and that of the slurry mixed with ogi liquor were introduced into the wells. Distilled water was also introduced into one well to serve as the control. The wells were all coded as follows: Steep water red (St_R), steep water white (St_W), slurry red (SL_R) and slurry white (SL_w). The plates were incubated at 37°C for 24 h and zones of inhibition determined. Diameter of zones of inhibition were subtracted from 10 mm (diameter of cork borer). The experiment was repeated three times for accuracy. The results obtained were recorded.

Antibiotic sensitivity test

Agar diffusion method was used. 1 ml of each test bacterial was introduced into the surface of the solidified nutrient agar. They were swab appropriately and left for few minutes before placing the antibiotics disc. The plates were incubated at 37°C for 24 h. The diameter of zones of inhibition was measured and recorded.

RESULTS

The result from this study revealed that ogi slurry and steep water from both sorghum varieties had antibacterial effect against the entire test organisms except, *Enterobacter* spp. which was only inhibited by the steep water from the white variety (Table 1).

The zones of inhibition ranged from 4.0 to 10.6 mm and 2.0 to 5.0 mm for slurry and steep water from the white sorghum variety, and 3.0 to 6.0 mm and 2.0 to 4.0 mm for slurry and steep water from the red sorghum variety. Slurry from white sample gave the highest zone of inhibition on *E. coli* with 10.6 mm diameter, while the slurry from red sample gave the lowest inhibition zone on *S. typhi* with 3.0 mm diameter. The steep water on the other hand had the highest zone on *S. dysenteriae* (5.0 mm) and was lowest on *S. aureus* and *S. dysenteriae* with 2.0 mm each.

The conventional antibiotics sensitivity assay showed that not all the selected antibiotics had antimicrobial activity against the test bacteria, only gentamycin was found to be effective against all the test bacterial with zones of inhibition greater than those produced by the steep water and slurry from both sorghum varieties (Table 2). The microorganisms isolated from the steep water and slurry at peak of each fermentation are *Lactobacillus plantarum*, *Leuconostoc mesenteroides, Corynebacterium* spp.*, Staphylococcus aureus and Sacharomyces cerevisiae*. The average total microbial population is shown in Figure 1. The pH of the steep water (white and red sorghum varieties) were 4.6 and 4.7, while that of the ogi slurry (white and red sorghum varieties) were 4.0 and 4.2, respectively.

DISCUSSION

The steep water and ogi slurry from both sorghum varieties had growth inhibitory activity on the test organisms used at varying capacities. The inhibitory effects may be largely due to the production of antibacterial substance by the lactic acid bacterial present. Lactic acid bacterial such as *Lactobacilli* species are known to be present in many locally fermented food like cereal grains and milk curd and has been reported to produce antimicrobial

Table 2. Antibiotics sensitivity partern of the bacteria causing diarrhea.

Bacteria	GEN	ERY	CHL	TET	CXC	AUG	AMO	AMP	CPK	NIT
S. aureus	10.0	9.0	13.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0
Enterobacter	3.0	0.0	8.0	2.0	0.0	0.0	12.0	0.0	12.0	0.0
S. dysenteriae	10.0	0.0	5.0	8.0	0.0	0.0	0.0	0.0	10.0	0.0
S. typhi	16.0	0.0	0.0	12.0	0.0	15.0	13.0	0.0	7.0	0.0
E. coli	22.0	0.0	0.0	12.0	0.0	15.0	13.0	0.0	7.0	0.0

GEN, Gentamycin; ERY, erythromycin; CHL, chloromphenicol; Tet, tetracycline; CXC, cloxacillin; Aug, augumetin; Amo, amoxicillin; Amp, ampicillin; CPX, ciprofloxacin; NIT, nitronidazole.

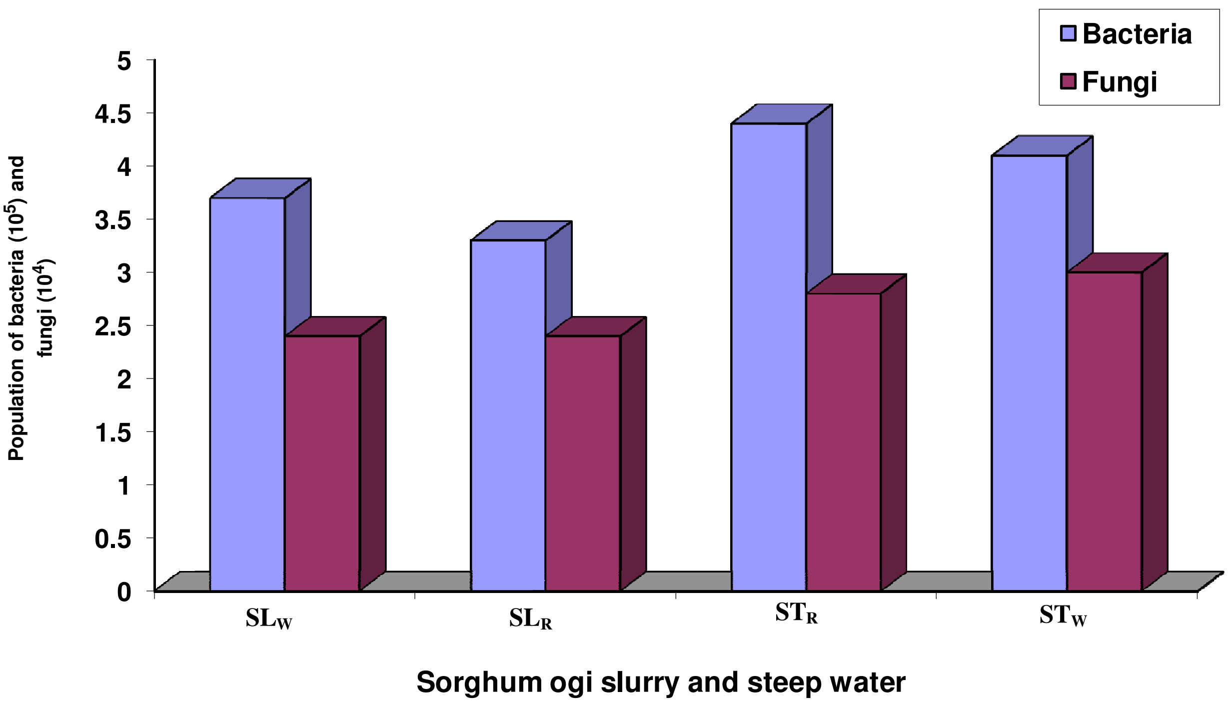

Figure 1. Average total microbial population during primary and secondary fermentation. SL_W, Sorghum slurry white; SL_R, sorghum slurry red; ST_R, steep water red; ST_w, steep water white.

substance (such as organic acids, bacteriocins and hydrogen peroxide) employed successfully to inhibit entheropathogens causing diarrhea in the intestine of animals (Benneth et al., 1997). The reduction in pH from 5.2 to 4.0 and 5.6 to 4.2 for white and red sorghum, respectively shows that acidic compounds were released to the medium during fermentation which made the pH to drop. Acidification and pH reduction is necessary to avoid problems with contaminating spoilage or pathogenic organisms which might counteract the fermentation process, especially in early stages.

The antibacterial activity of the sorghum ogi slurry and steep water could have been affected by the resistant of the test microorganisms, the variety of grains used and the amount of lactic acid bacteria present to release antimicrobial substance. Banigo and Muller (1972) reported that not all the fermenting organisms are present in all fermentation. The non inhibition of *Enterobacter* spp. except by the steep water from white sorghum may be due to the fact that they posses inducible beta-lactamase (ESBL) which is also responsible for their resistant to some conventional antibiotics (Ehrhardt and Sanders, 1993). The slurry from both sorghum varieties gave higher growth inhibition than the steep water samples. This could be as a result of the presence of more lactic acid bacteria during the secondary fermentation. It is therefore recommended that raw ogi slurry from sorghum be exploited in the treatment of diarrhea

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