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Short Communication

Effect of kunnu-zaki on clinical bacteria isolates

J. O. Olaitan[1]*, O. E. Adeleke[2] and Alabi O. S.[2]

[1]Department of Biological Sciences, College of Science, Engineering and Technology, Osun State University, P.M. B.4494, Osogbo, Nigeria.
[2]Department of Pharmaceutical Microbiology, University of Ibadan, Oyo State, Nigeria.

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Ten clinical isolates of Gram-negative bacteria were exposed in agar-cup diffusion sensitivity test to Kunun-zaki, a non-alcoholic fermented beverage, taken from eighteen samples. The undiluted samples of the beverage appreciably inhibited the growth of all the bacteria tested including, remarkably, *Pseudomonas aeruginosa, Vibro cholerae, Salmonella enterica* and *Staphylococcus aureus*. The bacteria however varied in their sensitivities to the fractional dilutions of the samples. This antimicrobial effect represents another property for Kunun-zaki and obviates the possibility of public health risk in its consumption.

Key words: Kunnu-zaki, clinical isolates, antibiotic sensitivity.

INTRODUCTION

Kunnu-zaki is a cereal food drink that has become a popular refreshing non-alcoholic beverage in Nigeria, particularly, in the North and South West. Millet, sorgum and maize grains are the three principal cereals from which Kunun-zaki can be produced (Adeleke et al., 2004; Inatimi et al., 1988). It is usually flavoured with such spices as ginger, black pepper and tamarind for improvement in its taste and aroma, and also to serve as purgative and cure for flatulent conditions (Omakwu, 1980). The final product is claimed to enhance lactation in nursing mothers (Efiuvwevwere and Akoma, 1997). Like other cereal foods, Kunun-zaki also protects the body against cholesterol and bile acid metabolism related diseases such as gallstones and certain forms of heart diseases (Bangert, 1989). It was observed that Kunun-zaki elevates lymphocyte counts obtained in the blood samples of animals fed with Kunun-zaki which is indicative of its medicinal attributes, a concept widely believed by its numerous consumers (Akoma et al., 2006)

Kunun-zaki is supposed to be self-sanitizing considering the generation of such fermentation products as organic acids, hydrogen peroxide, antibiotic-like substances and lowered oxidation-reduction potential (Bangert, 1989). Also, the three spices (ginger, pepper and tamarind) have been shown to possess varying levels of antimicrobial property (Bankole et al., 1999), contrary to a much earlier report that these harboured bacteria and toxigenic fungi (Christensen et al., 1967). Utensils, water source, product handling and non-regulated production and dispensing methods are factors that can encourage microbial contamination of Kunun-zaki with its attendant objection-able degradative changes. (Nkanga and Uriah, 1981; Onuorah et al., 1987; Peters and Odeyemi, 1990)

Fungi, Gram-positive and Gram-negative bacteria had been isolated from sampled Kunun-zaki (Adeleke et al., 2004; Edema and Anetor, 2008). It had been discovered that microorganisms especially *E. coli* can grow and survive during the storage of Kunun-zaki at different temperature (Oshoma et al., 2009). It is possible that the high counts of spoilage and pathogenic microorganisms in Kunun-zaki could be reduced if starter cultures are employed in its fermentation process as done in the developed world (Agarry *et al.*, 2010).

The foregoing contradictory assumed self-sanitizing property of Kunun-zaki and its potential for microbial contamination vis-à vis its nutritional and health importance have necessitated this study to ascertain the effect of Kunun-zaki on bacteria from different clinical sources.

*Corresponding author. E-mail: bukolaitan@yahoo.com.

Table 1. The bacterial clinical isolate.

Isolate	Hospital number	Clinical source
Vibrio cholerae	1181	Stool
Proteus vulgaris	1136	Stool
Proteus mirabilis	1231	Wound swab
Pseudomonas aeruginosa	1213	Wound swab
Klebsiella pneumoniae	1244	Sputum
Staphylococcus aureus	1202	Conjuctival swab
Escherichia coli	1296	Vaginal swab
Salmonella enterica	1343	Blood
Salmonella paratyphi	1320	Blood
Staphylococcus albus	1391	Blood

Table 2. Determination of antimicrobial activity of Kunnu- zaki samples.

Clinical isolate	Zone of inhibition by Kunnu-zaki (mm)											
	K_1				K_2				K_3			
	0	1:2	1:4	1:6	0	1:2	1:4	1:6	0	1:2	1:4	1:6
V. cholerae	10	8.0	7.5	-	16.5	9.0	-	-	11	9.0	7.5	-
P. vulgaris	8.0	-	-	-	14	9.5	8.5	-	14	8.0	-	-
P. mirabilis	10	8.0	7.5	-	16.5	11.5	7.5	-	18	9.5	8.5	-
P. aeruginosa	12.5	8.5	6.5	-	15	14.5	10	-	10	7.5	-	-
K. pneumoniae	8.5	-	-	-	12.5	-	-	-	14.5	8.0	-	-
S. aureus	14	-	-	-	12.5	-	-	-	11.0	9.5	-	-
E. coli	8.0	7.5	-	-	13.5	12.5	9.5	-	12.5	-	-	-
S. enterica	14	9.5	-	-	10.5	-	-	-	9.6	-	-	-
S. paratyphi	13.5	11.5	10.0	-	12	11.5	-	-	10.5	7.5	-	-
S. albus	10.5	9.5	-	-	11.5	-	-	-	10.5	7.5	-	-

K_1 - K_3 = Kunnu-zaki. 0 = undiluted Kunnu-zaki. - = no zone of inhibition (resistance). 1:2 – 1:6 = Fractional dilutions of Kunnu-zaki.

MATERIALS AND METHODS

Organisms

The bacteria were collected as pure clinical isolates on culture and sensitivity plates from the Routine Section of the Department of Medical Microbiology and Parasitology, University College Hospital (U. C. H), Ibadan (Table 1). Some confirmatory conventional biochemical tests (Cowan, 1994) were done to confirm their identities. The isolates were preserved as slant cultures on Nutrient agar (OXOID) at 4°C.

Kunnu-zaki

Samples of freshly prepared Kunun-zaki were collected from three retailers in Abeokuta in the South West, Nigeria, selected for producing Kunun-zaki under strict hygienic conditions. The samples were packaged in 35 ml plastic bottles and immediately transferred to the laboratory for the experiment. Samples were collected for six consecutive fortnights, giving a total of 18 samples. K_1, K_2 and K_3 (Table 2) represent samples collected from three retailers.

Agar-cup sensitivity test

Fractional dilutions 1:2, 1:4, 1:6, 1:8 and 1:10 were prepared in sterile distilled water for each Kunun-zaki sample. A 10^{-2} dilution of overnight broth culture of each organism equivalent to 10^7 cells/ml was obtained and seeded (0.1ml) into Nutrient agar pour-plate. Wells were then cut with flame-sterilized size 3 cork-borer, followed by drying of the plates for about 20 min at 37°C in an incubator. The wells were then filled in turns with undiluted sample and the fractional dilutions of Kunun-zaki. The culture plates were allowed to remain on the laboratory bench for a pre-incubation diffusion period of 2 h followed by incubation at 37°C for 24 h. Thereafter, every zone of growth inhibition observed on the plates was measured and recorded in millimeters indicating the sensitivity of a particular organism. Lack of clear zone of growth inhibition showed resistance (Table 2).

RESULTS

All the bacterial isolates had their respective growth inhibited by every undiluted Kunun-zaki sample as evident in clear zones of growth inhibition that ranged from 8.0 mm for *Proteus vulgaris* to 16.5 mm for *Vibrio cholerae* and 18.0 mm for *Proteus mirabilis*. With increased fractional dilutions of the samples, the bacteria varied in their level of sensitivity. Samples K_3 and K_2

showed a higher level of growth inhibition than K_1. The zones of growth inhibition for K1 ranged from 8.0 mm for *Proteus vulgaris* to 14.0 mm for *Staphylococcus enterica*; for K_2, the range was from10.5 for *Staphylococcus enterica* to 16.5 for *Vibrio cholerae* while K3 had a range of 9.6 mm for *S. enterica* to 18.0 mm for *Proteus vulgaris* (Figure 1). The susceptibility of Kunun-zaki was lower in the Gram-negative bacteria than the Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus albus*). *Pseudomonas aeruginosa* and *Salmonella paratyphi* were particularly outstanding in the sensitivity episode among the Gram negatives. *Proteus mirabilis* was most sensitive to the three samples of Kunun-zaki (Figure 1).

DISCUSSION

Kunun-zaki is a fermented cereal beverage produced by various indigenous fermentation methods. The consumers believed that the drink is nutritious and medicinal and the consumption rate has relatively increased in Nigeria because it is cheap and readily available. The concern for a public health risk in the consumption of Kunun-zaki in Nigeria has been obviated in this study due to the encouraging inhibitory effect of the drink on all the bacteria tested. This however is subject to the hygienic preparation of the drink because it had been observed that application of corrective measures such as hand-washing practices, washing of processing areas and utensils, hygienic handling of materials during processing and pasteurization of final product proved effective in significantly reducing microbiological hazards associated with Kunun-zaki (Edema and Anetor, 2008). The non-inhibitory effect of its higher fractional dilutions is negligible because the drink is not normally diluted before consumption. Thus, Kunun-zaki in its ready-to-drink form is effective in minimizing the risk of bacterial infection or intoxication. This apparently substantiated self-sanitizing role of Kunun-zaki is attributed to its acid pH 3.9 to 5.6 (Adeyemi and Umar, 1994), various fermentation products (Bangert, 1989) with antimicrobial activity, the volatile oils and constituent acids of the spices –tamarind, ginger and black pepper (Frazier and Westhoff, 1986). The little difference in the effect of the K_1, K_2 and K_3 samples on the clinical isolates could be attributed to the method of production in terms of dilution and ingredients which also is indicative of the microbiological status (Nkama et al., 2010; Edema and Anetor, 2008)

A moderated consumption of Kunun-zaki is advocated such that the general public would take advantage of its antimicrobial effect in addition to its acclaimed physiological roles–lactation in nursing mothers, purgative effect and cure for flatulence as well as nutrient composition (Omakwu, 1980; Efiuvwevwere and Akoma, 1997; Hulse et al., 1980; Sopade and Kassum, 1992). The peculiar sweet-tainted sour taste of Kunun-zaki is noteworthy. The results obtained showed that Kunun-zaki has antimicrobial properties and further supports the claim that the drink is medicinal.

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