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Antibacterial Activity of Sorghum "Ogi" on Diarrhoeagenic *Escherichia coli*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate and compare the antibacterial activity of sorghum "ogi" slurry, liquor and that of conventional antibiotics against diarrhoeagenic *Escherichia coli*.
Place and Duration of Study: This research work was carried out at the Department of Microbiology Laboratory, Federal University of Technology, Akure. Ondo state, Nigeria between June and September, 2017.
Methodology: The pH and Titratable acidity (TTA) of 'ogi' slurry and liquor were determined at the 0, 24, 48, 72 and 96 hours of fermentation. Microorganisms were isolated from both the slurry and liquor via a pour plate method. Identification and characterisation of various bacterial isolates including the stock culture (*Escherichia coli*) were based on Gram-staining technique and biochemical tests. The fungal isolates were identified by their morphological features and lactophenol cotton blue staining procedure. Antibacterial activity of 0.1 ml of 72 hours fermented liquor and slurry of Sorghum "ogi" including organisms isolated from the liquor and slurry were tested against *Escherichia coli* and were separately determined via agar well diffusion method. Antibacterial activity of conventional antibiotics such as Tetracycline, Amoxycylin and Ciprofloxacin were also determined. SPSS was used to analyse all the data in this research work.

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Results: Bacteria isolated from the slurry and liquor of sorghum 'ogi' include: *Bacillus* species, *Corynebacterium* species, *Lactobacillus plantarum*, *Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus* species. Fungi isolated include: *Mucor mucedo*, *Penicillium notatum*, *Rhizopus* species and *Saccharomyces cerevisiae*. *Corynebacterium* sp, *Staphylococcus epidermidis* and *Lactobacillus plantarum* exerted growth inhibitory activity on the diarrhoeagenic *E. coli* used. *Staphylococcus epidermidis* exerted the highest growth inhibitory effect. *Aspergillus niger* exerted higher growth inhibitory effect on most of the *E. coli* used than *Penicillium notatum*.
Conclusion: This study has shown that Sorghum "ogi" can be used as an alternative therapy to antibiotics to treat people who are suffering from diarrhoea caused by *Escherichia coli*.

Keywords: Diarrheal bacteria; antibacterial effects; sorghum; "ogi" liquor; slurry.

1. INTRODUCTION

Diarrhoea is one of the most common diseases that cause infant death in developing countries, it is an illness characterised by an increase in frequency and passage of loose (unformed) stools. It is usually accompanied by urgency, and occasionally incontinence [1]. It is the second leading killer of children under five years [2]. Diarrhoea is caused by both infectious and non-infectious agents [3]. Infectious diarrhoea is caused by the consumption of pathogens. These pathogens include *Escherichia coli*, *Salmonella* species*, Shigella* species, *Staphylococcus aureus* and so on [4]. While non-infectious diarrhoea may be due to bowel functional disorders. It is estimated that diarrhoea kills young children around the world more than malaria, acquired immunodeficiency syndrome (AIDS) and tuberculosis combined [5]. Diarrhoeagenic bacteria; *Escherichia coli*, *Shigella* species, *Salmonella* species cause diarrhoea when their live cells and/or toxins are consumed through contaminated food or water. The objective of any antidiarrhoeal treatment is to replace or minimise fluid and electrolyte loss, reduce stool frequency and any other symptoms such as abdominal pain, reduce faecal losses and ultimately reduce the severity and duration of illness [6]. Widely accepted treatment of diarrhoea include: use of oral rehydrated salt (ORS), Use of intravenous fluid therapy (IFT), use of zinc tablets, use of antibiotics, probiotics, Bovine colostrum, herbs, nutrition and lactase enzyme supplements. Diarrhoeagenic pathogens are becoming resistant to conventional antibiotics, continual usage of antibiotics on the vital organs of the body may lead to damaged tissues and organs and which may as well cause bacteria-induced diarrhea. Given that hospitals may not be well accessible in rural African communities, the need for alternative treatments such as the use of "ogi" becomes important. "Ogi" is a porridge prepared from fermented maize, sorghum or millet. 'Ogi' is known to be one of the common weaning foods in West Africa including Nigeria. It is called pap, 'akamu', 'ogi', or 'koko' and is made from maize (Zea mays), millet (*Pennisetum americanum*), or guinea corn (*Sorghum* spp.) [7]. The most predominant microorganism involved in the fermentation of sorghum during "ogi" production is *Lactobacillus plantarum* while other microorganisms are associated with it [8]. *Lactobacillus plantarum* is responsible for the production of Lactic acid which is the major component of "ogi" flavour and taste. Although diarrhoea is self-limiting, when it is as a result of bacterial infections, antibiotics therapy may be required. However, access to these antibiotics may be very difficult in rural areas as a result of lack of medical facilities in such places. Moreover, antibiotics have been reported [1] to cause antibiotics induced diarrhoea. Therefore, the search for alternative measures become important.

2. MATERIALS AND METHODS

2.1 Study Area Description

Akure is a city in south-western Nigeria and is the largest city and capital of Ondo state. It is situated in the tropic rainforest zone in Nigeria [9]. The city had a population of 484,798 as at 2006 population census, with a total land area of 991km^2. Akure lies about 7^{o}25' North of the equator and 5^{o}19' East of the meridian.

2.2 Sample Size and Collection of Samples

Sorghum grains used for 'ogi' were purchased from Oba's market, Akure, Ondo State, Nigeria. Stock culture of diarrhoeagenic bacteria used in this study (5 isolates of *Escherichia coli*) were collected from Don Bosco Health Centre Laboratory, Akure, Ondo State, Nigeria.

2.3 Preparation of "Ogi" from Sorghum Grains

This was prepared according to the method of Adebolu [10]. The sorghum grains used were sorted to remove pebbles, mouldy and deformed grains followed by washing in sterile distilled water to remove dirt and surface contaminants. Clean grains (1.084kg) were steeped in clean water that sufficiently covered the grains inside a clean plastic bucket with a cover and left at room temperature (30±2^{o}C) for 72 hours. The grains were washed in three changes of clean water and wet-milled using a local grinding machine. The resulting paste was sieved with a clean muslin cloth and the filtrate was collected into a clean plastic bucket with cover. The filtrate was allowed to settle at 30±2^{o}C for 5 hours before the first analysis was carried out. After the first analysis, the filtrate was left for natural fermentation to take place. The liquid on top after the filtrate has settled is referred to as the liquor while the sediment is referred to as the slurry.

2.4 Determination of pH and Titratable Acidity (TTA)

2.4.1 pH

The pH of 'ogi' slurry and liquor was determined at the 0, 24, 48,72 and 96 hours of fermentation. This was carried out by standardising the pH meter in buffers 4.0 and 6.0 and dipping the probe into the samples, until the meter read a stable value pH. [4].

2.4.2 Titratable acidity (TTA)

TTA of both slurry and liquor was determined at 0, 24, 48,72 and 96 hours of fermentation as described by Adebukunola et al. [11]. Ten milliliter (10ml) of the slurry and liquor was dispensed into separate conical flasks and 2 drops of phenolphthalein indicator was added. The content of the flask was thoroughly mixed in the flask and titrated against 0.1M NaOH. The appearance of a pink colour marked the end point of the reaction.

2.5 Isolation, Identification and Characterisation of Isolates

Culture media used include; nutrient agar, potato dextrose agar, nutrient broth, eosin methylene blue agar and mannitol Salt agar. Microorganisms were isolated via pour plate method as described by Fawole MO and Osho BA [12].

2.6 Antibacterial Activity of Sorghum "Ogi" on *Esherichia coli*

Agar well diffusion assay was used for this test. Mueller Hinton agar plate was prepared and 0.1ml of each of the *Escherichia coli* isolates was seeded on the plates with the aid of a sterile spreader and holes were bored on the plates using a sterile cork borer. 0.1 ml of the liquor and slurry of Sorghum "ogi" of 72 hours fermentation days was introduced into the holes accordingly. Distilled water was used as a control, and the plates were carefully incubated at 37°C for 18-24 hours. Zones of inhibition were carefully measured after 24 hours and were properly recorded as described by Adebolu et al. [10].

2.7 Antibacterial Activity of the Organisms Isolated from the Liquor and Slurry of Fermented Sorghum "Ogi" on *Escherichia coli*

Agar well diffusion assay was used for this test. Each of the organisms isolated from the liquor and slurry of fermented sorghum "ogi" was grown separately in nutrient broth at 37^{o}C for 24 hours. After inoculation, the broth cultures were centrifuged at 3000 rpm for 5 min. The resulting supernatants (cell free extracts) were decanted and used immediately against the test bacteria. Mueller Hinton agar plate was prepared and 0.1ml of each of the *Escherichia coli* isolates was seeded on the plates with the aid of a sterile spreader and holes were bored on the plates using a sterile cork borer. Then 0.1 ml of the supernatant of the microbial isolates was introduced into the holes accordingly. The plates were carefully incubated at 37°C for 18-24 hours. Zones of inhibition were measured with a ruler after 24 hours and were properly recorded as described by Adebolu et al. [10].

2.8 Antibiotic Sensitivity Assay

This test was carried out to determine the inhibitory effects of liquor, slurry and antibiotics against *Escherichia coli* and to compare the inhibition mediated by the liquor and slurry with that of the antibiotics. Tetracycline, Amoxycylin and Ciprofloxacin tablets were prepared in a solution according to their concentration on the antibiotics disc. Agar well diffusion assay was used in carrying out this test. Mueller Hinton agar plate was prepared and 0.1ml of each of the *Escherichia coli* isolates was seeded on the plates with the aid of a sterile spreader and holes were bored on the plates using a sterile cork

borer. Then 0.1 ml of "ogi" slurry, liquor and each of the antibiotics were introduced into the holes accordingly. The plates were carefully incubated at 37°C for 18- 24 hours. Zones of inhibition were measured with a ruler after 24 hours and were properly recorded as described by Adebolu et al. [10].

3. RESULTS

3.1 pH and TTA

The changes in pH and TTA of sorghum "ogi" slurry during fermentation ranged from 6.10mm from 0 hour to 3.5mm by 96 hours and 6.00 from 0 hour to 11.13mm by 96 hours respectively (Figs. 1 and 2). For the liquor, the pH ranged from 6.10mm by 0 hour to 3.4mm by 96 hours while the TTA ranged from 3.3mm by 0 hour to 4.85mm by 96 hours.

3.2 Microbial Loads

The total bacterial counts of sorghum 'ogi' liquor and slurry is presented in Table 1. Slurry of sorghum 'Ogi' had colony forming unit which ranged from 2.06 ± 0.00 to 2.33 ± 0.00 CFU/ml while for the liquor of sorghum "ogi" it ranged from 0.79 ± 0.06 to 1.95 ± 0.00 CFU/ml. The bacteria isolated from the slurry and liquor of sorghum 'ogi' investigated in this study are *Bacillus* species, *Corynebacterium* species, *Lactobacillus plantarum*, *Staphylococcus aureus* *Staphylococcus epidermidis* and *Streptococcus* species, while the fungi isolated are *Mucor mucedo, Penicillium notatum*, *Rhizopus* species and *Saccharomyces cerevisiae*.

3.3 Growth Inhibitory Effect of Sorghum "Ogi" on Diarrhoeagenic *Escherichia coli*

The sorghum "ogi" used exerted growth inhibitory activity on the *Escherichia coli* used. The liquor of the "ogi" was observed to exert higher growth inhibitory effect than the slurry (Table 3). The inhibition mediated by the liquor was also superior to that mediated by Amoxycillin, but was lower than that mediated by Ciprofloxacin.

3.4 Growth Inhibitory Effect of Microorganisms Isolated from Sorghum "Ogi" on *E. coli*

The bacteria isolated from sorghum "ogi" such as *Corynebacterium* sp, *Staphylococcus epidermidis* and *Lactobacillus plantarum* exerted growth inhibitory activity on the diarrhoeagenic *E. coli* used. The inhibition mediated by the bacteria ranged from 3.0mm to 27.0 mm (Table 4). *Staphylococcus epidermidis* exerted the highest growth inhibitory effect on the *E. coli* used. *Penicillium notatum* and *Aspergillus niger* exerted growth inhibitory activity on the diarrhoeagenic *E. coli* used. The inhibition mediated by the fungi ranged from 14.0mm to 25.0mm. *Aspergillus niger* exerted higher growth inhibitory effect on most of the *E. coli* used than *Penicillium notatum* (Table 5).

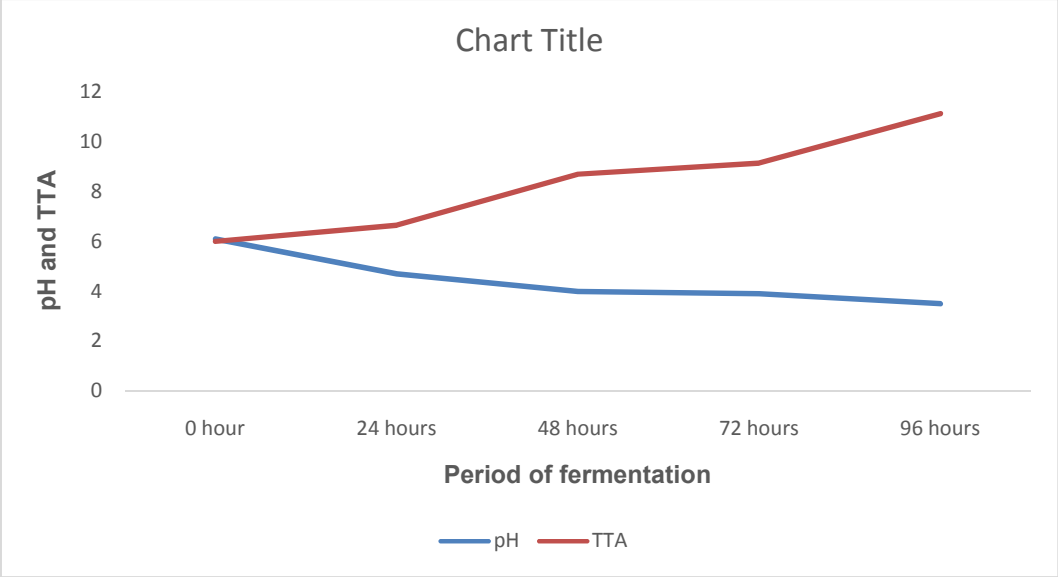

Fig. 1. Graph of pH against TTA of sorghum "ogi" slurry during fermentation

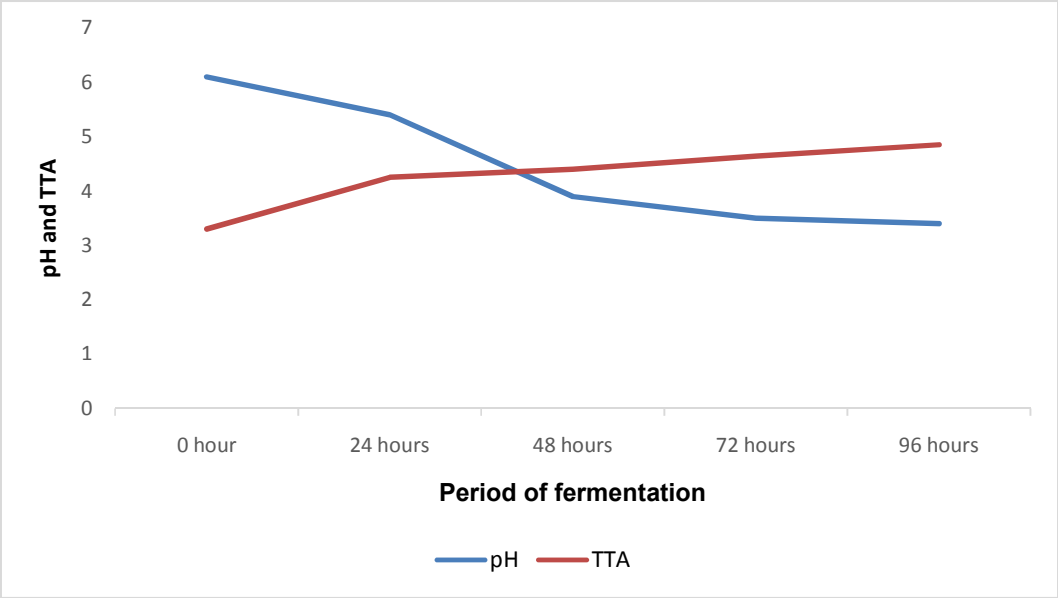

Fig. 2. Graph of pH against TTA of sorghum "ogi" liquor during fermentation

Table 1. Effects of fermentation duration on the total bacterial counts present in both slurry and liquor of sorghum "ogi"

Time (hour)	Mean log count(CFU/ml)* slurry	Mean log count(CFU/ml)* liquor
0	2.29 ± 0.00	1.95 ± 0.00
24	2.33 ± 0.00	1.42 ± 0.01
48	2.24 ± 0.00	1.15 ± 0.02
72	2.14 ± 0.00	0.79 ± 0.06
96	2.06 ± 0.01	1.12 ± 0.47

**values are for replicates of 3 ± S.E.M*

Table 2. Effects of fermentation duration on the total fungal counts present in both slurry and liquor of sorghum "ogi"

Time (hour)	Mean log fungal count(SFU/ml)* slurry	Mean log fungal count(SFU/ml)* liquor
0	1.34 ± 0.02	1.61 ± 0.01
24	1.48 ± 0.00	1.87 ± 0.01
48	1.70 ± 0.00	1.73 ± 0.01
72	1.59 ± 0.01	0.93 ± 0.04
96	1.22 ± 0.26	1.11 ± 0.19

Table 3. Growth inhibitory effect of sorghum "ogi" on diarrhoegenic bacteria

Treatment	Diameter zones of inhibition (mm)
Sorghum "ogi" slurry	4.67 ± 0.33*
Sorghum "ogi" liquor	11.33 ± 6.11*
Ciprofloxacin	20.00
Amoxicillin	0.00
Tetracycline	12.00
Distilled water	0.00

Table 4. Growth inhibitory effect of bacteria isolated from sorghum "ogi" on *Escherichia coli*

Escherichia coli	Bacterial isolates	Zones of inhibition
E_1	*Bacillus* species	0.00
	Corynebacterium species	22.00
	Lactobacillus plantarum	0.00
	Staphylococcus aureus	0.00
	Staphylococcus epidermidis	27.00
	Streptococcus species	0.00
E_2	*Bacillus* species	0.00
	Corynebacterium species	0.00
	Lactobacillus plantarum	0.00
	Staphylococcus aureus	0.00
	Staphylococcus epidermidis	0.00
	Streptococcus species	0.00
E_3	*Bacillus* species	0.00
	Corynebacterium species	9.00
	Lactobacillus plantarum	5.00
	Staphylococcus aureus	0.00
	Staphylococcus epidermidis	9.00
	Streptococcus species	0.00
E_4	*Bacillus* species	0.00
	Corynebacterium species	0.00
	Lactobacillus plantarum	3.00
	Staphylococcus aureus	0.00
	Staphylococcus epidermidis	14.70
	Streptococcus species	0.00
E_5	*Bacillus* species	0.00
	Corynebacterium species	7.00
	Lactobacillus plantarum	14.30
	Staphylococcus aureus	0.00
	Staphylococcus epidermidis	19.00
	Streptococcus species	0.00

Keys: E_1 = Diarrhoeagenic E. coli (DEC) isolate 1; E_2 = Diarrhoeagenic E. coli (DEC) isolate 2; E_3 = Diarrhoeagenic E. coli (DEC) isolate 3; E_4 = Diarrhoeagenic E. coli (DEC) isolate 4; E_5 = Diarrhoeagenic E. coli (DEC) isolate 5

4. DISCUSSION

The inhibition mediated by the "ogi" was as a result of the metabolites produced by microorganisms isolated from "ogi" which include *Corynebacterium* species, *Staphylococcus epidermidis*, *Lactobacillus plantarum*, *Aspergillus niger* and *Penicillium notatum*. The observed decrease in microbial population of sorghum "ogi" during fermentation after the initial increase by 24 hours for bacteria and 48 hours for fungi might be as a result of the decrease in the pH of the medium. As fermentation increased, microbial growth reduced. This is because of the increase acidity of the medium. Therefore, some organisms are not able to survive in acidic medium and as a result they die leaving only organisms that are able to survive i.e. acidophiles [13]. Earlier reports have shown that most bacteria cannot grow at low pH [14]. As fermentation progresses, the pH decreases as a result of the production of organic acids such as lactic acid leading to increase in TTA significantly from 6.00 to 11.13 for slurry and 3.30 to 4.85 for liquor. This agrees with the work of Shittu et al. [15] who indicated that lactic acid, which is the major metabolite of the *Lactobacilli* is responsible for significant pH changes, which is sufficient to discourage the growth of other microorganisms which might be spoilage microorganisms. The increase in the titratable acidity and consequent drop in pH during fermentation of sorghum "ogi" was likely due to the utilisation of free sugars by yeast (*Saccharomyces cerevisae*) and *Lactobacillus plantarum*. The growth inhibition mediated by *Aspergillus niger* on diarrhoeagenic *E. coli* used shows that this fungus produces potent metabolites that has growth inhibitory activity against *E. coli*. This agrees with the work of Adebolu [16] who observed superior growth

Table 5. Growth inhibitory effect of fungi isolated from sorghum "ogi" on *Escherichia coli*

E. coli isolate	Fungi	Zone of inhibition(mm)
E_1	*Aspergillus niger*	15.00
	Penicillium notatum	0.00
	Rhizopus sp	0.00
	Mucor mucedo	0.00
	S. cerevisiae	0.00
E_2	*Aspergillus niger*	14.00
	Penicillium notatum	15.0
	Rhizopus sp	0.00
	Mucor mucedo	0.00
	S. cerevisiae	0.00
E_3	*Aspergillus niger*	16.00
	Penicillium notatum	0.00
	Rhizopus sp	0.00
	Mucor mucedo	0.00
	S. cerevisiae	0.00
E_4	*Aspergillus niger*	25.0
	Penicillium notatum	0.00
	Rhizopus sp	0.00
	Mucor mucedo	0.00
	S. cerevisiae	0.00
E_5	*Aspergillus niger*	25.00
	Penicillium notatum	21.00
	Rhizopus sp	0.00
	Mucor mucedo	0.00
	S. cerevisiae	0.00

Keys: E_1 = Diarrhoeagenic . E. coli (DEC) isolate 1; E_2 = Diarrhoeagenic E. coli (DEC) isolate 2; E_3 = Diarrhoeagenic E. coli (DEC) isolate 3; E_4 = Diarrhoeagenic E. coli (DEC) isolate 4; E_5 = Diarrhoeagenic E. coli (DEC) isolate 5

inhibitory activity of maize "ogi" liquor than the slurry against the diarrhoeal bacteria they worked with therefore should be exploited in the treatment of diarrhoea caused by the test organisms.

5. CONCLUSION

This study has shown that sorghum "ogi" can be used as an alternative therapy to antibiotics to treat people who are suffering from diarrhoea caused by diarrhoeagenic *E. coli*, the predominant bacteria implicated in infantile diarrhoea especially in the South West, Nigeria. It can be used both in rural communities where there is no quick access to hospitals and pharmacies and in urban communities as an alternative treatment to conventional antibiotics. Moreover, the metabolites of microorganisms such as *Corynebacterium* sp., *Staphylococcus epidermidis*, *Aspergillus niger* isolated from sorghum "ogi" can also be exploited for the production of novel non-conventional drugs to treat cases of diarrhoea caused by this bacterium.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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