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**RESEARCH ARTICLE**

# EVALUATION OF "OGI" LIQUORS FROM THREE GUINEA CORN VARIETIES FOR ANTIMICROBIAL ACTIVITIES AGAINST SELECTED BACTERIAL AND FUNGAL ISOLATES.

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## *Abstract*

The inhibitory activities of raw ogi liquors from three varieties of guinea corn grains (red, yellow and white) were investigated using agar diffusion assay against selected pathogens: *Staphylococcus aureus, Escherichia coli, Shigella dysenteriae, Aspergillus niger* and *Rhizopus nigricans.* The red guinea corn hot water steep liquor had higher inhibition (9.8mm) against *E. coli* than the cold water liquor. Also, the hot water steep liquors of the white and yellow guinea corns had higher antibacterial activities (8.5mm against *Shigella dysenteriae* and 9mm against *E. coli*) than their cold water liquors. Hot water steep liquor of the red grain gave the lowest MIC (50%) against *E. coli*. However, only the cold steep liquors gave inhibition against *S. aureus*. The various liquors from hot and cold water steeped grains had no inhibitory activity on the test fungi. *Lactobacillus* species were the predominant bacteria that survived the fermentation process after 24hours. The differences in the mean inhibitory activities of both hot and cold water steeped liquors of the red, white and yellow guinea corns were not significant (P <0.05). Ogi liquor could be administered to people with diarrhoea especially people found in rural areas where immediate antibiotic therapy may be lacking or not readily available during diarrhoeal outbreaks.



## Introduction

Cereals are seeds of cultivated grasses that belong to the monocotyledon family Gramineae. The major cereal crops are maize, sorghum and millet which belong to different genera with different structure and chemical composition. These seeds have three parts; namely the outer covering, the endosperm and the germ (Marero *et al.*, 2008). Cereals are the most important staple foods being the major sources of carbohydrates. Compositionally cereals consist of 12 – 14% water, 65 – 75% carbohydrates, 2 – 6% lipids and 7 – 12% protein on dry weight basis (Dada and Muller, 1970).

Ogi is a porridge prepared from fermented maize, sorghum or millet in West Africa (Odunfa and Oyewole, 1998). Ogi has been recognized as the most popular traditional health sustaining fermented food in Western Nigeria. It is prepared from cereals: White Maize (Zea mays), yellow maize (Zea mays, yellow variety), red guinea corn (Sorghum vulgare), white guinea corn (Sorghum bicolor), yellow guinea corn and millet but not from rice and wheat (Adebolu *et al.*, 2007). Ogi is often marketed as a wet cake wrapped; in leaves or transparent polythene bags, it is diluted in water and boiled into a pap or cooked and turned into a stiff gel called 'agidi' or 'eko' prior to consumption (Akinrele, 2000).

In Nigeria, the first complementary food given to infants is usually a light cereal porridge (Ogi). It provides 20-20 kcal/ kg per day and Nigerian infants have an average energy density of 0.26 kcal/g (Brown *et al*, 1998). Ogi

is easy to get and cheap in comparison with tinned baby food. It is given to old people and convalescent patients due to its extremely light nature (Akinrele, 2000).

Traditionally, ogi can be preserved by the continual changing of water. Ogi can also be preserved by refrigeration (Ernst and Pittler, 2001). Locally, in some communities in South Western Nigeria, uncooked ogi is normally administered to people having running stomach (diarrhoea) to reduce the frequency of stooling (Adereiye and Laleye, 2004). Diarrhoea is an illness characterized by an increase in frequency and fluidity of stools is one of the most common diseases causing infant death in developing countries (Walderman, 1998; Cheesbrough, 1994). Some pathogens that cause this condition include; *Escherichia coli, Salmonella* species, *Shigella* species, *Staphlococcus aureus, Clostridium difficile* and *Campylobacter jejuni* (Prescott *et al.,* 2005). It is of great need to find several ways for treating this disease especially for the benefit of those living in rural areas where there are no health workers, health facilities and health care centres. The research work carried out in 2013 was aimed at:

i. investigating the antimicrobial activities of ogi liquors;
ii. determining any possible effects of steeping water types on the possible antimicrobial activities of the "ogi" liquors against the test pathogens.

## MATERIALS AND METHODS

### COLLECTION OF SAMPLES

The species of the guinea corn cereals used in the work: red guinea corn: Sorghum vulgare; white guinea corn: Sorghum bicolour and yellow varieties were purchased from the Gyel Market in Jos, Plateau State.

### TEST ISOLATES USED.

The bacterial (*Staphylococcus aureus, Shigella dysenteriae, Escherichia coli*) and fungal (*Aspergillus niger, Rhizophus nigricans*) test isolates were recovered from stool and urine specimens collected from Federal Medical Centre (FMC), Umuahia, Abia State.

### PREPARATION OF "OGI"

This was carried out according to the method of Odunfa and Adeleye (1985) with some slight modifications. The grains used were sorted to remove pebbles, mouldy and deformed grains followed by washing in clean tap water to remove dirt and surface contaminants, after which two hundred grains of each cereal grain was weighed and placed into different sterile plastic buckets with cover containing 300ml sterile distilled water and left at room temperature for 72 hours. The grains were then washed in three changes of clean water and wet milled using a local grinding machine. The resulting pastes were sieved using different clean muslin clothes to remove bran, hulls and germs. The pomace was retained in the sieve and was later discarded as animal feed while the filtrate was collected into clean plastic buckets with cover. The filtrate was allowed to settle and ferment for 72 hours by the natural flora of the grains. The liquid on top after the filtrate has settled is referred to as the liquor while the sediment is referred to as the slurry. The liquors of the fermented ogi slurries were then decanted; their pH was measured and recorded before they were tested for antibacterial and antifungal activity against the test organisms.

## ANTIBACTERIAL ANALYSIS

### PREPARATION OF INOCULA OF TEST ISOLATES

The inoculums size of all bacterial isolates tested was standardized by the use of overnight broth cultures prepared by inoculating isolated colonies of the different test bacteria in 10ml of nutrient broth which was then incubated at 37$^{o}$C for 24 hours. Loopfulls of the overnight broth culture was diluted in 4ml of sterile physiological saline (0.8w/v) such that its turbidity marched with that of 0.5 Macfarland Standard (Barium sulphate standard) against a white paper background.

### DETERMINATION OF ANTIBACTERIAL ACTIVITY OF THE DIFFERENT LIQUORS

One ml of 18hr old broth culture of each of the test organisms was taken using a sterile pipette and placed into sterile Petri-dishes (different organism per plate). Each plate was then overlaid with 20ml nutrient agar, carefully swirled to allow even distribution of the organisms within the agar and were allowed to gel before seven wells (8mm in diameter) were bored in the agar with the aid of a sterile cork borer. The ogi liquors from the grains were put into the wells; 0.1ml (100µl) per well and different type for different well, sterile distilled water was used as control. The plates were incubated at 37$^{o}$C for 24 hours. The diameter zone of inhibition around the wells containing the liquors was determined and recorded (Adebolu *et al.,* 2007; Abdus-Salaam *et al.,* 2014).

**ANTIBIOTIC SENSITIVITY PATTERN/TESTING OF THE TEST ORGANISMS**

This was carried out as described above, except that instead of making wells on the already seeded plates and pouring in the prepared liquors, standard commercial antibiotics discs was placed on the seeded agar plates and the plates were then incubated for 24 hours at 37$^{o}$C after which the zones were observed, measured and recorded (Adebolu *et al.*, 2007).

**DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERIOCIDAL CONCENTRATION (MBC) OF THE PREPARED OGI LIQUORS**

The three liquors were serially diluted by placing 2ml of sterile Mueller Hinton broth in 4 different tubes which were labelled in a ratio of decreasing concentration as 2:2, 2:4, 2:8 and 2:16 and in percentages as 100, 50, 25 and 12.5% after which 2ml of the liquor were placed in the first test tube and then serially diluted. 1ml of the test isolates was then dispensed in all the tubes, then covered with aluminium foil and incubated at 37$^{o}$C for 24 hours. Thereafter, the concentration with no visible bacterial growth on the solid Nutrient agar medium was noted as the MIC and was recorded.

The MBC was determined by collecting a loop full of broth from the clear tubes used for the MIC determination and inoculating same on sterile Nutrient agar plates. The plates were incubated at 37$^{o}$C for 24 hours and the lowest concentration that had no microbial growth was recorded as the MBC (Oluwafemi and Adetunji, 2011)

**DETERMINATION OF THE MIC AND MINIMUM FUNGICIDAL CONCENTRATION (MFC) OF OGI LIQUORS**

Two ml of sterile Sabourand dextrose broth were dispensed in 4 different test tubes after which 2ml of the liquor were dispensed in the first tube and ten-fold serial dilution was carried out to the last tube. The tubes were labelled in a ratio of decreasing concentration as 2:2, 2:4, 2:8 and 2:16 (100, 50, 25, 12.5%). 0.5ml of the inoculums was then dispensed in all the tubes and then incubated at 22$^{o}$C for 96 hours after which the MIC values were observed and recorded as the lowest concentration of the liquor that completely inhibited the fungi.

The MFC was also determined by collecting a loop full of broth from the clear tubes used for MIC determination, and inoculating same on sterile SDA plates. The plates were incubated at 22$^{o}$C for 72 hours after which the result was observed and recorded. The concentration with no fungal growth on the SDA was noted as the MFC (Oluwafemi and Adetunji, 2011).

**ISOLATION AND IDENTIFICATION OF MICROORGANISMS PRESENT IN THE DIFFERENT LIQUORS**

The liquors obtained from different grains were streaked on different Nutrient agar plates, De Man Rogosa Sharpe and SDA plates after 0, 24 and 72hours of fermentation. The plates were incubated at 37$^{o}$C for bacterial growth and at 22$^{o}$C for fungal growth for 24 and 72hours. Different biochemical tests were then carried out for proper identification of the test isolates. (Adebolu *et al.*, 2007).

## RESULTS AND DISCUSSION

The antimicrobial activities of the ogi liquors from red, white and yellow guinea corn varieties were investigated. It was found that the liquor from red guinea corn showed antibacterial activity for both hot and cold water steeping methods with a higher antibacterial effect (9.8mm) on *Escherichia coli* from hot water steep than the cold water steep which had a maximum inhibitory zone of 7mm against *E. coli* too. Incidentally, the hot water steep liquor did not inhibit *S. aureus*. This finding is in agreement with the report of Adebolu *et al.*, (2007) and is so possibly because of the phytochemical components of the red guinea corn grain which were extracted by the cold water but most probably were negatively affected by the hot water. Thus, the absence of the inhibition recorded. Neither the hot nor cold water steep liquor of red guinea corn had any inhibitory effect on any of the fungal isolates. The MIC for the hot water steep against *E. coli* was 50% compared with 100% MIC recorded against *Shigella dysenteriae* and 100 recorded against *S. aureus*. The differences in the mean inhibitory activities of the hot and cold steep liquors of red guinea corn were not significant ($P < 0.05$).

Antibacterial activity of the ogi liquors from white guinea corn was higher (8.5mm) against *Shigella dysenteriae* (for hot water steep) and lower (7.00mm) against *E. coli*. The hot water steeped liquor again had no inhibition on *S. aureus* possible due to same reason adduced above in the case of hot water steeped liquor of red guinea corn as the cold water steeped liquor inhibited *S. aureus*. This finding is also agreement with the report of Adebolu *et al.*, (2007). Again, both the hot and cold water steeped liquors of the white guinea corn had no inhibitory

effect on any of the fungal isolates. The MIC of *S. dysenteriae* was 100 but was greater than 100 for *S. aureus*. The inhibitory effects of the white guinea corn steeped liquor (hot and cold) on the three bacterial pathogens confirms the traditional claims where such liquors (especially the cold one) are used to treat diarrhoea in many localities in Nigeria. The difference in the mean inhibitory activities of both hot and cold water steeped liquor of white guinea corn was not significant ($P < 0.05$).

The hot water steeped liquor of yellow guinea corn had no inhibition on *S. aureus*, but inhibited *E. coli* more (9mm) than *S. dysenteriae* (7mm). Also, the MIC of the two inhibited pathogens was 100% respectively. However, the cold water steeped liquor had inhibitory effect against *S. aureus* (7mm). This is in agreement with the report of Adebolu *et al.,* (2007). However, Abdus-Salaam *et al.,* (2014) reported a much higher inhibition by a three, four and five-day old corn steep liquors against *E. coli* and *S. aureus*. Also, no observable inhibition was noted against any of the two fungal isolates. The difference in the mean inhibitory activities of both hot and cold water steeped liquors from yellow guinea corn was not significant ($P < 0.05$).

From the above results it can be seen that ogi liquor of the three varieties of guinea corn investigated in this work irrespective of the mode of steeping had inhibitory activities on the three test bacterial isolates. However, only the cold water steeped liquor inhibited *S. aureus* in the three cases examined. This shows that cold water steeped liquor of guinea corn grains will be effective in handling food borne health issues caused by *S. aureus* while the hot water steeped liquors will be more effective in handling similar health challenges caused by enteric pathogens. Although Gentamycin had much inhibition against the bacterial isolates, the hot water steep liquor of white guinea corn had greater inhibition against *S. dysenteriae* than Gentamycin (Table 6). During the fermentation processes, different bacteria: *E. coli, S. aureus, Proteus vulgaris, Salmonella* species, *Bacillus* species, *Lactobacillus fermentum* and *L. plantarium* were recovered from the various liquors (Table 4). However, at the end of the fermentation, only the Lactic acid bacteria were recovered from the ogi. This shows that raw ogi liquor has bioactive components responsible for the inhibitions recorded in the work against the test isolates. Such bioactive components could be bacteriocins, $CO_2$, lactic acids and hydrogen peroxides. (Tagg *et al.,*1976; Ogunbanwo *et al.,* 2003). There is a great possibility to this because Lactobacilli which were reported by Olukoya *et al.,* (1994) to produce bioactive metabolites such as lactic acid, hydrogen peroxide and bacteriocin were isolated from the different liquors irrespective of the mode of steeping. *Lactobacillus* species associated with ogi have been found to be safe for human use and are termed probiotics. These are live and non-pathogenic organisms which are used to inhibit the growth of potential pathogens either directly through the colonization of the host's body or indirectly through the production of secondary metabolites that are harmful to the pathogens which when administered in adequate amounts confer a health benefit to the host. (Onarhein and Raa, 1990).

The pH of the different liquors could also be responsible for the steeped liquors' antibacterial activity as seen above. The pH of the red guinea corn was 5.32 and 5.30 (the lowest for hot and cold water steeped liquors respectively; Table 8) and it was found that red guinea corn had the greatest inhibition (9.8mm; Table 5) against the enteric bacterial pathogens tested . This is attributed to the fact that most bacteria cannot grow at low pH except for few such as Lactobacillus species (Adebolu *et al.,* 2007).

**Table 1: Cultural, morphological and biochemical characteristics of bacterial Isolates from fermenting guinea corns**

| S/No. | Growth Features | Grain Staining | Motility | Citrate | Catalase | Coagulase | Glucose | Lactose | Sucrose | Fructose | Maltose | Isolate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Creamy, dull, translucent colonies | - rods in clusters | + | + | - | - | A | - | A | - | - | *Proteus* species |
| 2. | Yellow, elevated, small, smooth colonies | + cocci in clusters | - | - | + | + | A | A | A | A | A | *Staphylococcus aureus* |
| 3. | Creamy, rough, circular colonies | - rods in chain | + | - | - | - | A | A | A | A | A | *Escherichia coli* |
| 4. | Small, creamy, raised, dull colonies | - cocci in pairs | + | + | + | - | A | A | - | - | - | *Enterobacter* Species |
| 5. | Creamy, smooth, circular colonies with dark spots (hydrogen sulphide) | - rods | + | - | - | - | A | A | A | - | - | *Salmonella typhimurim* |
| 6. | Creamy, smooth colonies | - rods | + | - | - | - | A | - | - | - | - | *Shigella dysenteriae* |
| 7. | Creamy, smooth colonies | - rods | + | - | - | - | A | - | - | - | - | *Pseudomonas aeruginosa* |

KEY: A= acid production; G=gas production

**Table 2: Identification characteristics of fungal test isolates**

| S/No. | Surface | Elevation | Spore Colour | Type of Mycelium | Reproduction | Septation | Appearance of special Structure | Suspected Organism |
|---|---|---|---|---|---|---|---|---|
| 1. | Cottony | Raised | Black | Sporangiospore | Asexual | Non-Septate | Rhizold | *Rhizopus nigricans* |
| 2. | Powdery | Raised | Black | Condiospore | Sexual | Septate | Foot cell | *Aspergillus niger* |

**Table 3: Cultural, morphological and biochemical characteristics of bacteria isolated from the "ogi" liquor at 0hr of fermentation**

| S/No. | Growth Features | Gram Stain | Motility | Citrate | Coagulase | Oxidase | Glucose | Sucrose | Lactose | Maltose | Fructose | Isolate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Circular, Opaque, Convex, Cream, Smooth Colonies | + rods in chain | _ | - | - | - | A/G | A | A/G | A/G | A/G | *Lactobacillus plantarium* |
| 2. | Circular, Opaque, Flat, Creamy, Smooth Colonies | + rods in chains | _ | - | - | - | A/G | A | A/G | A/G | A/G | *Lactobacillus brevis* |
| 3. | Irregular, Dull, Flat, Creamy Rough Colonies | + rods in clusters | + | - | - | + | A | A | - | - | - | *Bacillus* species |
| 4. | Yellow, Elevated, Small Smooth Colonies | + cocci in clusters | _ | - | + | - | A | A | A/G | A | A | *Staphylococus aureus* |
| 5. | Creamy, Swarming, Fat, Dull, Translucent Colonies | - rods in clusters | + | + | - | - | A | - | - | - | - | *Proteus* species |
| 6. | Circular, Opaque, Convex Creamy Rough Colonies | - rods in chain | + | - | - | - | A | A | - | - | - | *Escherichia coli* |
| 7. | Circular, Translucent, Convex, Creamy, Smooth Colonies | + rods in chains | _ | - | - | - | A | A/G | A/G | A/G | A/G | *Lactobacillus fermentum* |

KEY: A= acid production; G=gas production

**Table 4: Cultural, morphological and biochemical characteristics of bacteria isolated from the "ogi" liquor after 24 hours of fermentation**

| S/No. | Growth Features | Gram Stain | Motility | Citrate | Coagulase | Oxidase | Glucose | Sucrose | Lactose | Maltose | Fructose | Isolate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Circular, Opaque, Convex, Cream, Smooth Colonies | + rods in chain | - | - | - | - | AG/ | A/G | A | A | A | *Lactobacillus plantarium* |
| 2. | Circular, Opaque, Flat, Creamy, Smooth Colonies | + rods in chains | - | - | - | - | A/G | A/G | A/G | A/G | A/G | *Lactobacillus brevis* |
| 3. | Yellow, Elevated, Small Smooth Colonies | + cocci in clusters | - | - | + | - | A | A | A/G | A | A | *Staphylococus aureus* |
| 4. | Circular, Opaque, Convex Creamy Rough Colonies | - rods in chain | - | - | - | - | A | A | - | - | - | *Escherichia coli* |
| 5. | Circular, Translucent, Convex, Creamy, Smooth Colonies | + rods in chains | - | - | - | - | A/G | A/G | A/G | A/G | A/G | *Lactobacillus fermentum* |

KEY: A= acid production; G=gas production

**Table 5: Inhibitory and steeping parameters of ogi liquor from red guinea corn on test isolates (mm)**

| Steeping method/ antibiotics | Test bacteria | | | Test fungi | |
|---|---|---|---|---|---|
| | *Staphylococcus aureus* | *Escherichia coli* | *Shigella dysenteriae* | *Aspergillus niger* | *Rhizopus nigricans* |
| HOT | 0.0±0.0[a] | 9.8±0.0[a] | 4.5±0.7[a] | NT | NT |
| MIC (%) | 100 | 50 | 100 | 100 | 100 |
| MBC/MFC (%) | 100 | >50 | 100 | 100 | 100 |
| COLD | 4.0±0.0[a] | 7.0±0.0[a] | 3.0±0.0[a] | NT | NT |
| MIC (%) | 100 | 100 | 100 | 100 | 100 |
| MBC/MFC (%) | 100 | 100 | 100 | 100 | 100 |
| *Gentamycin (30µg) | 18 | 14 | 8 | NT | NT |
| *Ketoconazole (30µg) | NT | NT | NT | Inhibited | Inhibited |

*Gentamycin and Ketoconazole were control antibiotics.

NT = Not Tested.

**Table 6: Inhibitory and steeping parameters of ogi liquor from white guinea corn on test isolates (mm)**

| Steeping method/antibiotics | Test bacteria | | | Test fungi | |
|---|---|---|---|---|---|
| | *Staphylococcus aureus* | *Escherichia coli* | *Shigella dysenteriae* | *Aspergillus niger* | *Rhizopus nigricans* |
| **HOT** | $0.0\pm0.0^{b}$ | $7.0\pm0.0^{b}$ | $8.5\pm0.7^{b}$ | NT | NT |
| MIC (%) | 100 | 100 | 100 | 100 | 100 |
| MBC/MFC (%) | 100 | 100 | 100 | 100 | 100 |
| **COLD** | $6.0\pm0.0^{b}$ | $4.0\pm0.0^{b}$ | $4.0\pm0.0^{b}$ | NT | NT |
| MIC (%) | 100 | 100 | 100 | 100 | 100 |
| MBC/MFC (%) | 100 | 100 | 100 | 100 | 100 |
| *Gentamycin (30µg) | 18 | 14 | 8 | NT | NT |
| *Ketoconazole (30µg) | NT | NT | NT | Inhibited | Inhibited |

*Gentamycin and Ketoconazole were control antibiotics.

NT = Not Tested.

Table 7: Inhibitory and steeping parameters of ogi liquor obtained from yellow guinea corn on test isolates (mm)

| Steeping method/antibiotics | Test bacteria | | | Test fungi | |
|---|---|---|---|---|---|
| | *Staphylococcus aureus* | *Escherichia coli* | *Shigella dysenteriae* | *Aspergillus niger* | *Rhizopus* species |
| **HOT** | $0.0\pm0.0^{c}$ | $9.0\pm0.0^{c}$ | $7.0\pm0.0^{c}$ | NT | NT |
| MIC (%) | 100 | 100 | 100 | 100 | 100 |
| MBC/MFC (%) | 100 | 100 | 100 | 100 | 100 |
| **COLD** | $7.0\pm0.0^{c}$ | $3.0\pm0.0^{c}$ | $5.0\pm0.0^{c}$ | NT | NT |
| MIC (%) | 100 | 100 | 100 | 100 | 100 |
| MBC/MFC (%) | 100 | 100 | 100 | 100 | 100 |
| *Gentamycin (30µg) | 18 | 14 | 8 | NT | NT |
| *Ketoconazole (30µg) | NT | NT | NT | Inhibited | Inhibited |

*Gentamycin and Ketoconazole were control antibiotics.
NT = Not Tested.

Table 8: pH of different "ogi" liquors used

| Grains | Method of Steeping | pH |
|---|---|---|
| White Guinea Corn | Hot | 5.45 |
| | Cold | 5.40 |
| Red Guinea Corn | Hot | 5.32 |
| | Cold | 5.30 |
| Yellow Guinea Corn | Hot | 5.40 |
| | Cold | 5.42 |

## CONCLUSION AND RECOMENDATION.

It can be concluded that the various growth inhibitions exerted by the different liquors on the test bacteria has proved that such liquors have antibacterial effects in vitro due to the possible actions of the different metabolites like organic acid, bacteriocin and hydrogen peroxide produced by the Lactobacilli, present in the ogi liquor and also by the phytochemical components of the guinea corn grains. Ogi liquor could be administered to people with diarrhoea especially in rural areas where antibiotic therapy may not be readily available in cases of diarrhoeal outbreaks.

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