British Microbiology Research Journal
13(3): 1-11, 2016, Article no.BMRJ.24381
ISSN: 2231-0886, NLM ID: 101608140

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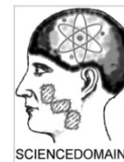

Food-borne Bacteria: Occurrences, Multidrug Resistant Patterns and Susceptibility to Aqueous Leaf Extracts of *Acalypha hispida* (*Linn*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author OJA designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors OJA, KOA and MUO managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/24381
Editor(s):
(1) Laleh Naraghi, Plant Disease Research Department, Iranian Research Institute of Plant Protection, Tehran, Iran.
Reviewers:
(1) Ashraf S. Hakim, National Research Centre, Giza, Egypt.
(2) Maria Margarita Canales Martinez, FES Iztacala, UNAM, Mexico.
Complete Peer review History: http://sciencedomain.org/review-history/13611

Original Research Article

Received 18th January 2016
Accepted 14th February 2016
Published 10th March 2016

ABSTRACT

The occurrence and susceptibility of multidrug resistant (MDR) bacteria obtained from food to aqueous leaf extracts of *Acalypha hispida* (ALEAH) were determined using standard bacteriological and disc diffusion methods. In rice, Total Heterotrophic Counts (THC), Total Coliform Counts (TCC) and Total Feacal Counts (TFC) ranged from $3.3x10^3$ to $1.9x10^4$ (CFU/g), $1.9x10^3$ to $3.6x10^3$ (CFU/g) and $1.0x10^2$ to $1.6x10^2$ (CFU/g), respectively. In 'garri', highest THC (2.0x 10^4 CFU/g), highest TCC (1.2x 10^4 CFU/g) were obtained in sample GA-03 and lowest TCC (2.0x 10^3 CFU/g) were obtained in samples GA-04 and GA-11. In 'fufu', the THC and TCC ranged from 7.3x 10^3 to 9.5x 10^3 (CFU/g) and 2.6x 10^3 to 4.6x 10^3 (CFU/g), respectively, while ≥ 70% 'fufu' had no TFC. Eight genera (*Escherichia, Staphylococcus, Enterobacter*, *Streptococcus, Klebsiella*, *Proteus, Pseudomonas* and *Salmonella* were obtained in the food samples. Growths of single bacterial isolate were obtained in 41.7% of rice, while co-contamination with two and three isolates were observed in 33.3% and 25.0% samples, respectively. Only 50.0%, 16.7% and 33.3% of 'garri' had growth of single, two and three bacterial isolates, respectively, while between 1 (8.3%) to 5 (41.7%) of 'fufu' and rice had growth of two and three bacterial isolates. The ALEAH contained alkaloids,

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cardiac glycoside, phenolics, saponin, tannin, flavonoids, deoxy- sugar, anthraquinones and phlobatanins. The results showed that between 45.5% to 66.7% bacterial isolates were multidrug resistant. Of 56.3% MDR *S. aureus* obtained from the boiled rice, fufu' and 'garri' only SAF4 and SAR3 had the same resistance patterns. The discs containing 20 mg/ml and 80 mg/ml ALEAH showed the lowest and highest activity of 6.7±1.8 mm and 13.6±1.0 mm, respectively. *Streptococcus* spp SPF1; *E. coli* ECG3, and *P. aeruginosa* PAF2 were resistant to 20 mg/ml and 40 mg/ml of the ALEAH. Conclusively, there is need to always evaluate the bacteriological quality of the food sold in the fast food centres / restaurants from time to time and train the food handlers on food safety practices.

Keywords: Acalypha hispida; antibiotics; phytochemicals; extracts; susceptibility; Uyo.

1. INTRODUCTION

There have been significant increases in the patronage of restaurants, cafeteria and fast food centres all over the world as a result of the busy nature of people [1]. Rice is one of the most widely consumed staple foods in Asia, Pacific, America and Africa. The nutritional value of rice is provided by its content in carbohydrate, sugar, fibre, energy, fat, protein, water, iron, calcium and zinc [2]. Among the fermented cassava products of cassava roots are "garri" and "fufu" [3,4]. Cassava root is normally processed before consumption so as to detoxify, preserve, modify and improve their overall organoleptic properties [5]. Fufu and 'garri' are staple foods of West, Central and Southern Africa. Fufu is made by steeping whole or cut peeled cassava roots in water to ferment for three to five days, depending on ambient temperature, while garri is processed by peeling the root, washing, grating, solid state fermentation, pulverizing and roasting [6]. Cooked garri or 'Eba' as it is called by the 'Yorubas' or 'Garri' by the 'Igbos', is stiff dough made by soaking 'garri' in hot water and kneading it with a flat wooden baton. Both cooked fufu and 'garri' are eaten by taking small ball of these food samples in one's fingers and then dipping into an accompanying soup. The partially unhygienic conditions in which these foods are prepared posses serious concerns to public health.

The global incidence of food borne diseases is difficult to estimate, but it has been reported that in the year 2005, 1.8 million people died from diarrheal diseases. A great proportion of these cases were attributable to the consumption of contaminated food and water. Food and Agricultural Organisation and World Health Organisation concluded that illness due to contaminated food was the most prevalent health problem in the world and also an important cause of reduced economic productivity [7]. The nutritional constituents of these foods serve as a rich medium for the growth of microorganisms that might contaminate them as the potential sources of contamination of these foods are soil, water, air, human beings, processing equipment, raw materials and packaging materials [1,8]. The continuous spread of multidrug-resistant pathogens has become a serious threat to public health and also a major concern to the clinicians [9,10]. One of the ways to prevent antibiotic resistance of pathogenic species is to use new compounds that are not based on existing antimicrobial agents [10-12]. Screening of compounds obtained from plants for their pharmacological activity has resulted in the isolation of innumerable therapeutic agents representing molecular diversity engineered by nature [10,12]. *Acalypha hispida* is an erect, dioecious flowering plant of 1.8 to 3.7 metres tall. It belongs to the family Euphorbiaceae, subfamily Acalyphinae and is commonly called Philippines Medusa, red hot cat's tail or fox tail. *A. hispida* originated in Oceania, but has become naturalized to multiple countries in America, Asia and Africa [13]. The leaves of *A. hispida* are astringent and used to treat leprosy and kidney ailments [14,15]. The decoction of the leaves and flowers is used as a laxative and diuretic in gonorrhea, and the bark acts as an expectorant in asthma and the flowers are used to treat diarrhoea and dysentery [14]. The aim of this study was to determine the occurrence of food pathogens in ready to eat foods sold in some restaurants and fast food centres in Uyo and also to evaluate the susceptibility of multidrug resistant bacteria obtained to aqueous leaf extracts of *Acalypha hispida.*

2. MATERIALS AND METHODS

2.1 Study Area

Uyo is a city in South-Southern Nigeria and is the capital of Akwa Ibom State. Akwa Ibom State shares boundaries with Abia, Cross River and Rivers States. The population in Uyo is estimated

to be about 451,128. Uyo is located between latitudes 5° 02' 37" North and longitudes 7º 54' 06" East. There are many restaurants, canteens and fast food centres in Uyo. The busy and cosmopolitan nature of this city necessitates that people eat out to satisfy the natural desire for nutritious diets.

2.2 Collection and Bacteriology of Food Samples

A total of 36 food samples consisting of boiled rice (N=12),'garri' (N=12) and 'fufu' (N=12) were purchased from twelve (12) fast-food centres / restaurants between May to June, 2013. The food samples were aseptically collected using sterile containers, carefully labelled to reflect the date, time and place of collection, and were transported to the Microbiology Laboratory immediately for bacteriological analysis. One gram of each of the food samples was added into 10 ml of peptone water and then shaken vigorously to dislodge adhered bacteria. Serial dilutions were made to obtain 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions. Zero point one (0.1) ml of the serially diluted sample was transferred onto plates of MacConkey Agar (MCA), Nutrient Agar (NA) and Eosine Methylene Blue (EMB) agar and incubated aerobically at 37℃ overnight. After incubation, the colonies on the positive plates were counted to obtain the Total Heterotrophic Counts (THC), Total Coliform Counts (TCC) and Total Feacal Counts (TFC) respectively on the plates. Thereafter, the colonies were subcultured onto plates of nutrient agar and incubated at 37℃ for 24 hr. Pure cultures of isolates were streaked onto nutrient agar slants, incubated at 37℃ for 24 hr and stored in the refrigerator at 4℃ for characterization and identification. All isolates were Gram stained and subjected to various biochemical tests using standard methods [16-18].

2.3 Antibiotic Susceptibility Testing and Multidrug Resistant (MDR) Bacteria

Sixty-six (66) bacterial isolates obtained from the food samples were kept on nutrient agar slants at room temperature and their susceptibility to antibiotics were determined using disc diffusion method according to [19]. Zero point one (0.1) ml of each bacterial isolates prepared directly from an overnight agar plate adjusted to 0.5 McFarland Turbidity Standard was inoculated using sterile pipette onto each of the plates containing Mueller-Hinton Agar (MHA). The inoculated Mueller-Hinton Agar plates were then allowed to stay for about 3-5 min for the surface of the agar to air dry. The discs containing ten antibiotics: Penicillin (PN, 10 µg), Ceftazidime, (CEP, 30 µg), Streptomycin (S, 30 µg), Pefloxacin (PEF, 5 µg), Gentamycin (CN, 10 µg), Ofloxacin (OFL, 5 µg), Nalidixic acid (NA, 5 µg), Augmentin (AU, 10 µg), Ciprofloxacin (CPX, 5 µg) and Cotrimoxazole (SXT, 30 µg) (Oxoid, UK) were aseptically placed onto the surfaces of the Mueller-Hinton agar plates using a sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37℃ for 18 hr and the zones of inhibition after incubation were observed and the diameters of inhibitory zones were measured in millimeters (mm) using a ruler. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer's standard zone size interpretative manual. The intermediate readings were considered as sensitive for the assessment of the data. Bacterial isolates that were resistant to three or more antibiotics were taken as multiple antibiotic resistant bacteria [20].

2.4 Sources of *Acalypha hispida*

Acalypha hispida was obtained in Uyo, Akwa Ibom State. This plant was identified at Department of Botany and Ecological Studies, University of Uyo and later transferred to Pharmacognosy and Natural Medicine Laboratory, Faculty of Pharmacy, University of Uyo for processing. The plant was washed under running tap water and with distilled water in order to remove extraneous matters (sand e.t.c), and air-dried at room temperature for one month. The dried plant part was pulverized and stored in polythene bag until required.

2.5 Preparation and Concentration of Plant Extracts

The powdered *A. hispida* (about 3 kg) was exhaustively extracted by Soxhlet Apparatus using aqueous. The filtrate was evaporated using a rotary evaporator attached to a vacuum pump (Model type 3492, Corning Ltd). After complete evaporation, the extract was weighed and preserved at 4℃. The graded concentrations (20, 40 and 80) $mgml^{-1}$ of the extracts were prepared using 100 ml of dimethyl sulphoxide.

2.6 Phytochemical Screening of Aqueous Leaf Extract of *Acalypha hispida* (ALEAH)

The phytochemical components of ALEAH were determined using the methods of [21-23].

Fig. 1. *Acalypha hispida*

2.6.1 Test for saponins

Half a gram 0.5 g of the filtered ALEAH was put in a test tube and 2 ml of distilled water was added and shaken vigorously. Formation of frothing which persisted on warming was taken as preliminary evidence for the presence of saponins.

2.6.2 Test for tannin

Half a gram (0.5 g) of the filtered ALEAH was stirred with 5 ml of distilled water and 5% Ferric Chloride reagent added. A blue-black colouration indicated a positive test.

2.6.3 Test for phlobatanins

Half a gram (0.5 g) of ALEAH was added to 3 drops of 40% formaldehyde, 6 drops of dilute HCl was also added to boiling and cool. A precipitate was formed, if positive and washed with hot water; this left a colourless residue after washing, indicating the presence of phlobatanins.

2.6.4 Test for cardiac glycoside

Half a gram (0.5 g) of ALEAH was dissolved in 2 ml of chloroform concentrated sulphuric acid (H_2SO_4) was carefully added to form a lower layer. A reddish-brown colour at the interface indicated a positive test.

2.6.5 Test for anthraquinones

Half a gram (0.5 g) of ALEAH was boiled with 5 ml of 10% sulphuric acid (H_2SO_4) and filtered. The filtrate was cooled in ice and shaken with 2.5 ml benzene, the benzene layer separated and half its own volume of 10% ammonium hydroxide (NH_4OH) was added. A pink, red or violet coloration in ammonia (lower) phase indicated a positive test.

2.6.6 Test for flavonoids

Few pieces of magnesium metal strip were added to 5 ml of the filtrate plant extract with concentrated hydrochloric acid (5 ml). The formation of orange, red, crimson or magenta was taken as a positive test.

2.6.7 Test for terpenes

Half a gram (0.5 g) of ALEAH was dissolved in 3 ml of chloroform and filtered. 10 drops of acetic anhydride were added to the filtrate with 2 drops of concentrated sulphuric acid (H_2SO_4), pink colour at the interphase was taken as the positive test.

2.6.8 Test for deoxy-sugar

Half a gram (0.5 g) of the filtered ALEAH was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride. It was then underplayed with 1 ml of concentrated sulphuric acid (H_2SO_4). Violet ring observed which settled after few minutes was an indication of a positive test.

2.6.9 Test for alkaloids

Half a gram (0.5 g) of ALEAH was added with a few drops of picric acid reagent. A white or yellow precipitate indicated a positive test.

2.6.10 Test for phenolics

To 2 ml of ALEAH, 1 ml of ferric chloride solution was added. Blue or green colour indicated a positive test.

2.7 Preparation and Sterilization of Sensitivity Discs

Discs of 6 mm diameter were punched out using Whatman No. 1 filter paper with the aid of a paper punch and placed in Petri dishes. The Petri dishes containing the discs were then sterilized in an oven at 180℃ for 1 hr, after which they were allowed to cool before used.

2.8 Susceptibility Testing of MDR Bacteria to Aqueous Leaf Extracts *A. hispida* (ALEAH)

The antibacterial potency of ALEAH on MDR bacteria obtained from the food samples was evaluated using disc diffusion method [24,25]. Sterile filter paper discs of 6 mm diameter were

impregnated with each ALEAH solution of graded concentrations (20, 40 and 80) $mgml^{-1}$ and carefully placed using sterilized forceps onto plates of Mueller – Hinton agar which had previously been inoculated with 0.1 ml of MDR bacterial isolates prepared directly from an overnight agar plate and adjusted to 0.5 McFarland Turbidity Standard. The plates were then incubated at 37℃ for 18 hr. Each ALEAH concentration was replicated thrice and the mean zone of inhibition diameter (in millimeters) was determined in each case. The ALEAH was tested *in vitro* for purity by plating out on Petri dishes containing nutrient agar and incubated at 37℃ for 24 hr.

3. RESULTS

The Total Heterotrophic Counts (THC) obtained in boiled rice samples RI-01, RI-02 and RI-03 were $6.2x10^3$, $1.0x10^4$ and $1.9x10^4$ (CFU/g), respectively, while their Total Coliform Counts (TCC) ranged from $2.3x10^3$ to $3.6x10^3$ (CFU/g). Samples RI-04, RI-05 and RI-06 had THC of $4.5x10^3$, $4.8x10^3$ and $1.0x10^4$ (CFU/g), respectively. Samples RI-04, RI-05 and RI-06 had THC of $4.5x10^3$, $4.8x10^3$ and $1.0x10^4$ (CFU/g), respectively. Samples RI-01, RI-02, RI-04, RI-05 and RI-06 had no Total Feacal Counts (TFC). Highest TFC ($1.6x\ 10^2$) CFU/g was obtained in sample RI-12, while lowest TFC ($1.0x\ 10^2$) CFU/g was obtained in sample RI-09 (Table 1). The bacterial counts of 'garri' and 'fufu' are shown in Tables 2 and 3. In 'garri', the highest THC of $2.0x\ 10^4$ (CFU/g) was obtained in sample GA-03, followed by GA-12 with THC of $1.5x\ 10^4$ (CFU/g), while the highest TCC of $1.2x\ 10^4$ (CFU/g) was obtained in sample GA-03 and while lowest TCC of $2.0x\ 10^3$ (CFU/g) was obtained in samples GA-04 and GA-11. Total faecal counts ranged from $1.3x\ 10^2$ to $2.2x\ 10^3$ (CFU/g). In 'fufu', 75% of the samples had no TFC, while THC and TCC ranged from $7.3x\ 10^3$ to $9.5x\ 10^3$ (CFU/g) and $2.6x\ 10^3$ to $4.6x\ 10^3$ (CFU/g), respectively. "In fufu' highest THC and TCC were obtained in samples FU-11 (Table 3).

Table 1. Bacterial counts of ready to eat food (Boiled Rice)

Sample code	Total heterotrophic count (THC) (CFU/g)	Total coliform count (TCC) (CFU/g)	Total faecal count (TFC) (CFU/g)
RI-01	6.2×10^3	2.3×10^3	-
RI-02	1.0×10^4	2.9×10^3	-
RI-03	1.9×10^4	3.6×10^3	1.2×10^2
RI-04	4.5×10^3	-	-
RI-05	4.8×10^3	2.1×10^3	-
RI-06	1.0×10^4	2.6×10^3	-
RI-07	1.2×10^4	3.0×10^3	1.1×10^2
RI-08	3.9×10^3	1.9×10^3	-
RI-09	1.6×10^4	3.3×10^3	1.0×10^2
RI-10	5.0×10^3	2.0×10^3	-
RI-11	3.3×10^3	-	-
RI-12	1.4×10^4	2.9×10^3	1.6×10^2

Table 2. Bacterial counts of ready to eat food (Garri)

Samples code	Total heterotrophic counts (THC) (CFU/g)	Total coliform counts (TCC) (CFU/g)	Total faecal counts (TFC) (CFU/g)
GA-01	5.7×10^3	3.1×10^3	-
GA-02	1.3×10^4	3.4×10^3	-
GA-03	2.0×10^4	1.2×10^3	1.7×10^2
GA-04	6.6×10^3	2.0×10^3	-
GA-05	5.2×10^3	2.9×10^3	-
GA-06	1.3×10^4	3.3×10^3	-
GA-07	1.3×10^4	3.8×10^3	2.2×10^2
GA-08	7.4×10^3	3.0×10^3	-
GA-09	1.2×10^4	4.5×10^3	1.3×10^2
GA-10	7.0×10^3	2.9×10^3	-
GA-11	6.5×10^3	2.0×10^3	-
GA-12	1.5×10^4	3.8×10^3	2.6×10^2

Table 3. Bacterial counts of ready to eat food (Fufu)

Samples code	Total heterotrophic counts (THC) (CFU/g)	Total coliform counts (TCC) (CFU/g)	Total faecal counts (TFC) (CFU/g)
FU-01	8.5×10^3	2.7×10^3	-
FU-02	1.4×10^4	4.1×10^3	-
FU-03	2.1×10^4	4.0×10^3	1.9×10^2
FU-04	7.9×10^3	3.5×10^3	-
FU-05	1.0×10^4	3.5×10^3	-
FU-06	1.2×10^4	4.2×10^3	-
FU-07	1.8×10^4	2.6×10^3	2.2×10^2
FU-08	7.3×10^3	2.7×10^3	-
FU-09	1.6×10^4	3.9×10^3	-
FU-10	8.2×10^3	3.0×10^3	-
FU-11	9.5×10^3	4.6×10^3	-
FU-12	1.0×10^4	3.4×10^3	2.5×10^2

A total of thirty- six (36) food samples comprising boiled rice (N=12), 'fufu' (N=12) and 'garri' (N=12) were analyzed for the presence of bacterial contaminants. The bacteriological analysis of the 'fufu' revealed the percentage occurrences of the isolates as follows: *E. coli* 3 (25.0%), *Staphylococcus aureus* 5 (41.7%), *Enterobacter* spp. 2 (16.7%), *Streptococcus pyogenes* 4 (33.3%), *Klebsiella* spp. 4 (33.3%), *Proteus* spp 2 (16.7%), *Pseudomonas aeruginosa* 3 (25.0%) and *Salmonella* spp 2 (16.7%) (Table 4). In 'garri', bacterial isolate with the highest percentage of occurrence was *S. aureus* 5 (41.7%), followed by *E. coli* 4 (33.3%) and the lowest percentage of occurrences were obtained from *Proteus* spp 1 (8.3%) and *Salmonella* spp 1 (8.3%). A total of nineteen bacterial isolates belonging to six genera (*Escherichia*, *Staphylococcus, Streptococcus, Klebsiella, Proteus* and *Pseudomonas*) were obtained from the boiled rice, while both *Salmonella* spp and *Enterobacter* spp were not isolated (Table 4).

Of the 36 food samples collected, only 15 (41.7%) of the samples showed growth of single bacterial isolate, co-contamination with two and three bacterial isolates were observed in 12 (33.3%) and 9 (25.0%) samples, respectively (Table 5). The results also showed that out of the 12 'garri' samples collected 6 (50.0%) had growth of single bacterial isolate, 2 (16.7%) had growth of two bacterial isolates and 4 (33.3%) had growth of three bacterial isolates. Only 5 (41.7%) of 'fufu' and rice samples had growth of two bacterial isolates, while poly bacterial growth were present in 1 (8.3%) of rice and 4 (33.3%) of 'fufu' samples (Table 6). The phytochemical screening results showed that ALEAH contained very high concentrations of alkaloids and cardiac glycoside, high concentrations of saponins and tannin. Small amounts of flavonoids, phenolics, deoxy- sugar, anthraquinones and phlobatanins were detected, while terpenes was not detected (Table 6). Of 9/15 (56.3%) MDR *S. aureus* obtained from the boiled rice, fufu' and 'garri' only SAF4 and SAR3 had the same antibiotic resistant patterns (PEF-AU-SXT-S), while 6 /10 (60.0%) of *S. pyogenes* were resistant to 3 to 7 antibiotics (Table 7). Multidrug resistant patterns of the Gram negative bacteria from the food samples are shown in Table 8. Only 5/11 (45.5%) *E. coli,* 2/4 (50.0%) *Enterobacter* spp, 4/8 (50.0%) *Klebsiella* spp, *Proteus* spp 2/4 (50.0%), *P. aeruginosa* 5/10 (50.0%) and *Salmonella* spp 2/3 (66.7%) were multidrug resistant. Of these Gram negative bacteria, *E.coli* with codes ECG3 and ECR8 had the same resistant patterns, while both *Klebsiella* spp KSF6 and KSG7 were resistant to NA-AU-SXT (Table 8).

All the MDR *S. aureus* and *S. pyogenes* were sensitive to 80 $mgml^{-1}$ of ALEAH. The lowest and highest inhibitory zones of 6.7±1.8 mm and 12.2±1.0 mm were obtained, respectively. *S. pyogenes* SPF1 and SPF3 had the same antibiotic resistant patterns (NA-CN-AU-SXT-S-PN) and were resistant to both 20 $mgml^{-1}$ and 40 $mgml^{-1}$ of ALEAH (Table 7). Of the entire MDR Gram negative bacteria obtained, only *E coli* ECG3 and *P. aeruginosa* PAF2 were resistant to the three different concentrations of ALEAH. The used disc diffusion methods showed that the zones of inhibition increased on increasing the concentrations of the extracts, thus, exhibiting concentration dependent activity. The discs containing 80 $mgml^{-1}$ ALEAH showed the highest activity against MDR *Enterobacter* spp ETF3 and *E. coli* ECR1 with zones of 13.6±1.0 mm and

13.3±2.0 mm, respectively, while disc containing 20 $mgml^{-1}$ showed the lowest activity against MDR *Salmonella* spp SSG4 with zone of 6.9±2.5 mm (Table 8).

4. DISCUSSION

Foods meant for human consumption also serve as rich media for growth of microorganisms, which can result in deterioration, spoilage and food borne illnesses [26,27]. The occurrence of pathogenic micro-organisms in human foods could be a public health concern [28]. The properties of food such as moisture content, hydrogen-ion concentration, temperature, and nutritive values determine the types of microorganisms and the extent to which they are present in a food material [29,30].

Table 4. Distribution and proportion of bacterial contaminants of ready to eat food

Bacterial isolates	Boiled rice (N=12) No (%) of occurrences	Fufu' (N=12) No (%) of occurrences	Garri' (N=12) No (%) of occurrences	Total (N = 36) No (%)
Escherichia coli	4 (33.3)	3 (25.0)	4 (33.3)	11 (30.6)
S. aureus	6 (50.0)	5 (41.7)	5 (41.7)	16 (44.4)
Enterobacter spp	0 (0.0)	2 (16.7)	2 (16.7)	4 (11.1)
S. pyogenes	3 (25.0)	4 (33.3)	3 (25.0)	10 (27.8)
Klebsiella spp	2 (16.7)	4 (33.3)	2 (16.7)	8 (22.2)
Proteus spp	1 (8.3)	2 (16.7)	1 (8.3)	4 (11.1)
P. aeruginosa	3 (25.0)	3 (25.0)	4 (25.0)	10 (27.8)
Salmonella spp	0 (0.0)	2 (16.7)	1 (8.3)	3 (8.3)
Total	19	25	22	66

Values in parenthesis are percentages

Table 5. Prevalence of single and mixed bacterial contamination of ready-to-eat food samples

Source	No of samples collected	No (%) of samples with one isolate	No (%) of samples with two isolate	No (%) of samples with three isolate	Total No (%) of isolates
Garri	12	6 (50.0)	2 (16.7)	4 (33.3)	22 (33.3)
Fufu	12	3 (25.0)	5 (41.7)	4 (33.3)	25 (37.9)
Rice	12	6 (50.0)	5 (41.7)	1 (8.3)	19 (28.8)
Total	36	15 (41.7)	12 (33.3)	9 (25.0)	66 (100)

Values in parenthesis are percentages

Table 6. Phytochemical Constituents of Aqueous Leaf Extract of *A. hispida*

Test	Constituents	Results
Dragendorff's Test	Alkaloids	+++
General Test	Saponins	++
Lieberman's Test	Cardiac Glycoside	+++
General Test	Flavonoids	+
General Test	Tannins	++
General Test	Deoxy- sugar	+
General Test	Phlobatanins	+
General Test	Terpenes	ND
General Test	Anthraquinones	+
Frothing Test	Phenolics	+

Keys: +++: Present in very high concentration
++: Present in moderately high concentration
+: Present in low concentration
- : Not detected

Table 7. Susceptibility of multidrug resistant gram positive bacteria to aqueous leaf extracts of *A. hispida*

Bacterial isolates	Isolates code	Zones of inhibition			Antibiotic resistant pattern
		20 mg/ml	40 mg/ml	80 mg/ml	
S. aureus	SAG1	NZ	7.8 ± 1.2^a	9.5 ± 2.0^b	CN-AU-CPX-SXT-S-PN-CEP
	SAG2	9.2 ± 0.7^b	10.3 ± 2.5^c	12.0 ± 0.5^c	CN-CPX-S
	SAF4	9.0 ± 1.5^b	9.8 ± 1.5^b	11.1 ± 2.0^c	PEF-AU-SXT-S
	SAR3	8.4 ± 1.0^a	10.1 ± 0.5^c	12.2 ± 1.0^c	PEF-AU-SXT-S
	SAG9	NZ	7.5 ± 2.5^a	9.1 ± 2.5^b	NA-CN-AU-CPX-S-PN-CEP
	SAR6	6.7 ± 1.8^a	8.7 ± 0.6^b	10.5 ± 1.0^c	OFX-NA-CN-CEP
	SAF5	8.1 ± 1.5^a	10.4 ± 0.5^c	12.0 ± 2.0^c	SXT-S-PN-CEP
	SAR12	NZ	8.1 ± 0.7^a	11.3 ± 1.0^c	AU-CPX-SXT-S-PN
	SAF10	NZ	8.5 ± 1.2^a	10.7 ± 1.0^c	OFX-PEF-CN-CPX-SXT-PN-CEP
S. pyogenes	SPF1	NZ	NZ	7.4 ± 2.2^a	NA-CN-AU-SXT-S-PN
	SPF3	NZ	NZ	7.6 ± 1.5^a	NA-CN-AU-SXT-S-PN
	SPR5	7.3 ± 2.7^a	8.2 ± 1.0^a	9.9 ± 1.0^b	PEF-AU-CPX-PN-CEP
	SPF8	8.6 ± 0.5^b	9.6 ± 1.5^b	11.6 ± 1.0^c	OFX-CN-CPX-S
	SPF10	NZ	7.0 ± 2.4^a	9.3 ± 1.5^b	OFX-NA-AU-SXT-S-PN-CEP
	SPR9	8.0 ± 1.3^a	8.9 ± 1.0^b	10.6 ± 2.0^c	CPX-S-CEP

Key: NZ: No zone of inhibition; values in parenthesis are percentages; each inhibitory zone included 6 mm diameter of the disc., SD: Standard Deviation. Each value represents the mean of three replicates and standard deviation. Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test (P <0.05)

Table 8. Susceptibility of multidrug resistant gram negative bacteria to aqueous leaf extracts of *A. hispida*

Bacterial isolates	Isolates code	Zones of inhibition			Antibiotic resistant pattern
		20 mg/ml	40 mg/ml	80 mg/ml	
E. coli	ECR1	9.2 ± 1.6^b	11.7 ± 1.0^c	13.3 ± 2.0^c	NA-CN-AU-S
	ECG3	NZ	NZ	NZ	PEF-AU-CPX-SXT-S-PN-CEP
	ECR4	7.9 ± 1.0^a	9.0 ± 0.8^b	10.4 ± 2.5^c	PEF-CPX-PN
	ECF6	8.2 ± 2.3^a	8.8 ± 2.0^b	10.8 ± 1.0^c	NA-PEF-CPX-SXT
	ECR8	NZ	NZ	7.5 ± 1.5^a	PEF-AU- CPX-SXT-S-PN-CEP
Enterobacter spp	ETF1	7.7 ± 1.0^a	9.0 ± 1.0^b	9.7 ± 2.5^b	NA-PEF-CPX-PN-CEP
	ETF3	9.4 ± 1.5^b	11.2 ± 1.0^c	13.6 ± 1.0^c	OFX-AU-CEP
Klebsiella spp	KSR1	NZ	8.0 ± 0.5^a	9.1 ± 1.3^b	NA-CN-AU-SXT-S-PN
	KSR3	NZ	6.9 ± 2.0^a	8.4 ± 1.0^a	OFX-PEF-AU-SXT-CEP
	KSF6	9.4 ± 2.2^b	10.7 ± 1.0^c	12.5 ± 1.6^c	NA-AU-SXT
	KSG7	8.9 ± 1.7^a	10.1 ± 1.0^c	12.9 ± 0.5^c	NA-AU-SXT
Proteus spp	PSF4	NZ	7.0 ± 1.4^a	7.3 ± 2.5^a	NA-AU-CPX-SXT-S-PN-CEP
	PSR2	NZ	7.0 ± 2.1^a	7.6 ± 1.0^a	NA-PEF-AU-CPX-PN
P. aeruginosa	PAG1	8.1 ± 1.0^a	9.9 ± 1.0^b	10.2 ± 2.0^c	CN-AU-CPX-SXT-S-PN
	PAF2	NZ	NZ	NZ	OFX-NA-PEF-AU-S-CPX-CEP
	PAG3	8.5 ± 0.5^a	11.5 ± 1.0^c	11.9 ± 2.5^c	CN-CPX-SXT-S
	PAR5	7.2 ± 0.5^a	8.1 ± 1.0^a	9.7 ± 2.0^b	OFX-CN-SXT-S-PN
	PAR10	8.5 ± 1.0^a	10.3 ± 1.0^c	11.0 ± 1.5^c	NA-PEF-AU-SXT
Salmonella spp	SSG4	6.9 ± 2.5^a	8.0 ± 2.0^a	9.5 ± 1.0^b	CN-AU-S-PN-SXT
	SSF11	NZ	7.4 ± 1.5^a	8.7 ± 2.2^b	CN-AU-CPX-SXT-S-PN-CEP

Key: NZ: No zone of inhibition; values in parenthesis are percentages; each inhibitory zone included 6 mm diameter of the disc., SD: Standard Deviation. Each value represents the mean of three replicates and standard deviation. Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test (P <0.05)

In the present study, the Total Heterotrophic Counts (THC) obtained in boiled rice and 'garri' samples ranged from $3.3x10^3$ to $1.9x10^4$ (CFU/g) and $5.2x10^3$ to $1.5x10^4$ (CFU/g), respectively; and these values were observably still below recommended limit of bacterial counts (≤ 10^5

CFU/g) of the International Standards for Microorganisms in Foods [31,32]. Thus, the results of these plate counts indicated that the foods had low microbial counts. Our findings showed that different 'fufu' samples from the different restaurants differ in microbial load as FU-03 has the highest load of 2.1x 10^4 (CFU/g), while FU-03 has the lowest load of 7.9x10^3 (CFU/g). These variations in microbial loads may be attributed to the hygienic conditions of the personnel in contact with the food, the environment and the raw material from which the 'fufu' are produced.

In this study, *E. coli, S. aureus, Enterobacter* spp, *S. pyogenes, Klebsiella* spp, *Proteus* spp., *P. aeruginosa* and *Salmonella* spp were isolated from the cooked rice, 'garri' and 'fufu' collected. This finding showed *S. aureus* as the most prevalent bacteria in the food samples and this differs from the previous reports by [33] in which *K. pneumoniae* were predominant in the food samples from restaurants / fast food centres. The occurrence of *E. coli* in the food samples are indication of faecal contamination. Although cooking or heating is expected to reduce the microbial load of cooked foods, but such foods can also become contaminated due to cross contamination and environmental sources such as air and dust, food utensils and food handlers [34]. Isolation of the genera *Staphylococcus, Streptococcus, Pseudomonas* and *Proteus* from the cooked rice is in conformity with the findings of [27] who isolated *S. aureus, Pseudomonas* spp and *Proteus* spp from cooked rice. Findings by [35] showed the occurrence of *E. coli* in cooked rice sold in restaurants and our findings confirmed this.

The antibiotic susceptibility results also indicated multiple resistance patterns among both Gram positive and Gram negative bacteria isolated from the cooked rice, garri and 'fufu' samples. Previous studies by [36] have also shown the occurrence of MDR-bacteria in food samples as 50% of their isolates from food samples were resistant to six out of the eight antibiotics tested.

Phytochemical screening of ALEAH revealed the presence of flavonoids, deoxy-sugar, phenols, anthraquinones, cardiac glycosides, alkaloids, tannins, phlobatanins and saponins. This result agrees favorably with the result previously obtained by [37]. Antibacterial activities exhibited by the ALEAH in this study also confirm the preliminary investigations of [38,39]. The results obtained showed that ALEAH exhibited inhibitory activities against the MDR bacteria with different degrees as demonstrated by measuring the diameters of inhibitory zones and these results are in conformity with the results obtained by [12,40]. Plant extracts are rich in many phyto compounds which are the cause of their bioactivities. The mechanisms of actions of many antimicrobials are complex and may not be the consequence of their action on a single target. The mechanisms of antimicrobial action of phenolic compound include membrane disruption, proteins binding, inhibition of proteins synthesis, enzymes inhibition, production of cell wall complexes, formation of disulfide bridges and intercalation with cell wall [12,41], while the action of alkaloids could be intercalation with cell wall and / or DNA constituents [42]. Hence, the presence of these secondary metabolites such as anthraquinones, cardiac glycosides, saponins, tannins, alkaloids, flavonoids and phenolics in ALEAH may be responsible for its potential use as drug against MDR food pathogens.

5. CONCLUSSION

Conclusively, owing to non-adherence to food safety practices and the indiscriminate establishment of fast food centres / restaurants in most developing and underdeveloped countries, there is need to evaluate the bacteriological quality of the food sold in the fast food centres / restaurants from time to time, train the food handlers on food safety practices, enact and enforce the laws on the establishment of fast food centres / restaurants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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