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Phenotypic characterization of pathogenic bacteria isolated from herbal mixtures (*agbo*) samples collected in Ibadan, Nigeria

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Abstract

Herbal mixtures called agbo are usually prepared by mixing different plant parts in water and/or alcohol. It is usually prepared by local women who have no education and knowledge of quality control measures. Therefore, the samples of agbo retailed by these women are commonly contaminated by spoilage and pathogenic organisms. The aim of the current study is to characterize microorganisms isolated from samples of agbo collected from different markets. The microbial load obtained for different samples of agbo ranged between 0 to 1.67x106 cfu/ml. The microorganisms isolated from the samples of agbo are E. coli, Salmonella typhi, Proteus vulgaris, Pseudomonads among others. Majority of the microbes isolated from agbo were resistant to antibiotic tested. Therefore, it could be concluded from this study that samples of agbo retailed in Ibadan are heavily contaminated by spoilage and pathogenic bacteria. Consumers are therefore advised to desist from taking agbo samples retailed in Ibadan.

Keywords: Herbal Mixtures; Agbo Samples; Pathogenic Bacteria; Spoilage Bacteria; Contamination; Ibadan

1. Introduction

An herb is a plant or plant part used for its scent, flavor, or therapeutic properties. Herb includes leaves, stems, flowers, fruits, seeds, roots, rhizomes and barks. The use of herbs for treating various diseases predates human history and forms the origin of most of the modern medicine. Long before the advent of modem medicine, herbs were the mainstream remedies for nearly all ailments (Barakat *et al*., 2013).

People commonly diagnosed their own illnesses, prepared and prescribed their own herbal medicines, or bought them from the local apothecaries. Herbal medicine is defined as a branch of science in which plant based formulations are used to alleviate diseases (Idu, 2011).

A herb is a plant or any part of a plant valued for its medicinal, aromatic and savory qualities. Herbal medicine refers to preparations and finished products that contain parts of plant and other plant materials as its active ingredients that is used in treating illness and diseases (Oluyemi *et al*., 2016).

It is estimated that approximately 80% of the population in developing countries uses traditional herbal medicinesas part of their primary health care (WHO, 2002 and Umair *et al*., 2017). This finding highlights the importance of research to support the development of traditional herbal medicine practices that provide appropriate, safe, and effective treatments (Umair *et al*., 2017; WHO, 2007 and Kosalec*et al*., 2009).

Among the main safety risks related to herbal medicines is contamination by microorganisms of various kindsthat may be adherent to leaves, stems, flowers, seeds, and roots from which herbal medicines are prepared. Alternatively,

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microorganisms can be introduced during harvesting, handling, open-air drying, preserving, and manufacturing (Kosalec*et al*., 2009; Danladi *et al.*, 2009 and Khattak, 2012).

The use of herbal medicine is prevalent among Nigerians because it is efficient, acts fast, resistant to pathogenic organisms, cheap and readily available (Oluyemi *et al*., 2016).

However, the possible adverse effects that could arise because of the mixture of various plant parts, the interaction between these plant parts and the solvents, or the contamination from handling are often overlooked. The raw materials used in producing *Agbo* could be contaminated or adulterated with toxic heavy metals or overloaded with essential mineral elements during growth, development, processing, at the sales point, or by other anthropogenic activities such as the addition of manures, sewage sludge, fertilizers, and pesticides (Phiips and Balge, 2007).

Besides, pathogenic microorganisms or natural toxins could also contaminate these products resulting in damage to vital excretory organs like the liver and the kidney (Olayemi *et al*., 2020).

Aim and objectives

The aim of the study is to determine the Phenotypic Characterization of Pathogenic Bacteria isolated from Agbo samples collected in Ibadan, Nigeria

While of the objectives of the study are:

- To isolate the pathogenic bacteria from Agbo samples
- To identify the pathogenic bacteria using biochemical methods

Material and methods

1.1. Collection of herbal samples

Ten (10) unregistered herbal oral liquid preparations produced and hawked by herb sellers were procured from major markets located in Ibadan. They were mostly aqueous decoctions produced from mixtures of several plant parts such as leaves, stems, roots and barks. The producers were found to be men and women, usually with no formal education. The markets included Bodija Market, Sango market and Ojo Market, The samples were purchased as packaged by the herb-sellers and transported to the laboratory.

1.2. Sample preparation and bacteriological analysis

1 ml/g each of the samples was dissolved in 9 ml of normal saline solution. Serial dilutions were made and viability assessed using spread plate method. The plates were incubated at 37^{o}C for 24 hrs for bacteriological analyses and 5 days for fungal analysis. After incubation visible colonies that developed were enumerated and recorded as colony forming units/ml (cfu/ml).

Total colony counts were recorded in cfu/ml using equation:

$$\text{Total colony counts (cfu/ml)} = \frac{\text{No. of colony formed}}{\text{Volume plated}} \text{x dilution factor}$$

1.3. Preparation of samples

The liquid sample was prepared by dispensing 1 ml of herbal sample in 9 ml of physiological saline under aseptic condition. The herbal suspension was steamed thoroughly while stirring using a sterile rod thereafter, a 10-fold serial dilution was performed following aseptic technique protocol.

1.4. Bacteria Count

At the end of the incubation period, the number of colony- forming units per gram (CFU/g) was calculated by multiplying the average number of colonies by the dilution factor. The obtained CFU/g of sample was compared with WHO standards. Samples that presented bacterial growth greater than 105 CFU in 1 g of herbal medicine were considered unsatisfactory or inadequate according to WHO guidelines for aerobic bacteria.

1.5. Biochemical Tests

Biochemical test description for the identification of microorganisms based on microscopic appearance and characterization was carried out using The Becton Dickinson Crystal Identification Systems Enteric/Non-Fermenter Machine.

1.6. Oxidase Test

A piece of clean filter paper was placed on a clean plate and 2-3 drops of oxidase reagent dropped on the filter paper and a loop full of organism of each isolates was streamed across the surface of the droplet. A positive reaction was indicated by the appearance of a purple color within 10 -15 second while a colorless appearance indicated a negative reaction. This test was carried out on 24 hour isolates.

1.7. Catalase Test

This test was done on a clean grease free slide whereby 2-3 drops of 3 % hydrogen peroxide were placed on the slide and a loop full of inoculums placed and mixed properly with hydrogen peroxide. A positive result is indicated by effervescence; a negative reaction showed absence of effervescence.

1.8. Indole Test

One ml (1ml) of Kovacs reagent was dispensed into a 48 hour broth culture of isolates and appearance of red ring on the surface of the broth indicated indole positive while yellow-green color indicated indole negative.

1.9. Motility Test

This was done by stabbing the test isolates into the motility broth and incubating at 37^0C for 48 hour. Motile organisms were shown by cloudy appearance of the organism around the broth (diffuse growth) while region stabbed that is clearly seen or limited to the line of inoculum was termed to be non- motile.

1.10. Methyl Red Test

The biological peptone water was prepared according to manufacturer's specification and was sterilizes in an autoclave for 15mins at 121°C after cooling, 2 ml was dropped into test tube and was inoculated with the bacteria organism and incubated at 37°C for 48hours. After which a drop of methyl red reagent was added to the tube (prepared by dissolving 0.04grams of methyl red in 100ml of ethyl alcohol). Development of red colour implies that the result is positive. Development of yellow color implies that the result is negative (Chibuzor *et al.,* 2019).

1.11. Sugar Fermentation Test

The principle of sugar fermentation states that the action of organism on a sugar substrate results in acidification of the medium, detected by a pH indicator dye. This was carried out to determine the ability of organism to ferment various sugars (sucrose, maltose, glucose etc). Peptone water was prepared according to manufacturer's specification and 3 drops of bromocresol blue was added. The broth was dispensed in 4.5 ml of a aliquot test tube containing an inverted Durham tube, the tubes were cotton plugged and autoclaved at 121°C for 15 mins, 25g of each sugar was dissolved in 200ml distilled water prepared separately, the sugars were automated at 121°C for 15mins and 0.5ml of broth was added to 4.5ml of peptone water aseptically. This was inoculated and incubated at 37°C for 24hr. Colour changes from red to yellow indicated a positive result (Nwachukwu and Osuacha, 2014).

1.12. Antimicrobial Susceptibility Testing

The antibiotic susceptibility test was performed by using disc diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines on Muller- Hinton agar plate. The antibiotic discs and their concentration were Septrin (SXT), Azithromycin (AZM), Oxacillin, Gentamycin, Vancomycin, Ciprofloxacin, Ceftriaxone, Ampicillin, Doxycycline, Cefuroxime, Erythromycin, and Augmentin. A sterile swab was placed into the broth culture of a specific organism and the excess water was removed gently by pressing or rotating the swab inside the tube. Before placing the antimicrobial disc, the swab with the bacterial suspension was distributed evenly over the entire surface of Mueller- Hinton plates. The plates were incubated at 37°C for 18 - 24 hour. The diameter of the zone of inhibition was measured and interpreted using standard chart as sensitive and resistant.

1.13. Statistical Analysis

Data was checked for completeness, cleaned manually, entered, and analyzed using SPSS version 20 statistical package. Analysis was made using frequency tables. Pearson's test and odds ratio with 95% CI were used for measures of association and *P* values less than 0.05 was considered as statistically significant.

2. Results and discussion

The microbial load detected in different samples is presented in table 1 andtheranges obtained for different samples are A ($2.18x10^3$ to $3.37x10^4$cfu/ml),B (0 to $3.45x10^4$cfu/ml), C (0 to $1.35x10^6$cfu/ml), D (0 to $1.67x10^6$cfu/ml) and E (0 to $3.82x10^5$). Nwankwo and Olime reported a total heterotrophic bacterial count of herbal mixture to range from 3.1x102 to 2.65x103 cfu/ml while the total fungal counts ranged from $1x10^1$ to $1.9x10^2$cfu/ml. In another study by Idu et al. (2015) total bacterial count ranged from $2.5x10^3$ to $6.4x10^9$ while fungal counts ranged from $9.5x10^3$ to $3.5x10^9$ cfu/ml. In table 2 the results of phenotypic identification is presented. The microorganisms identified include E.coli, Salmonella typhi, Proteus vulgaris among others. In a previous study different groups of microorganisms were reported which include E. coli, Salmonella spp., Pseudomonas aeriginosa and Staphylococcus aureus (Lima et al., 2017). In another study the organisms reported are S. aureus, E. coli, Salmonella and Pseudomonads (Okunlola et al., 2007). The fifty nine microbial isolates were subjected to antibiotic sensitivity tests. The results show that most of the organisms were resistant to the antibiotic tested. In a previous study the antibiotic resistant pattern of liquid herbal mixture was reported to range between 7 and 31mm.

Table 1 Microbial Loads

Code	NA count Cfu/ml	MSA count Cfu/ml	EMB countCfu/ml	MCA countCfu/ml	PDA count Cfu/ml
A	2.69x104	2.70x 104	3.37x104	1.15x104	2.18x103
B	2.09x103	0	0	0	3.45x104
C	6.27x103	8.36x103	4.54x102	0	1.35x106
D	7.18x103	4.73x103	0	0	1.67x106
E	3.82x105	1.64x104	0	0	1.82x103

Key: NA: Nutrient Agar; MSA: Mannitol Salt Agar; EMB: Eosin methyl blue; MCA: MacConkey agar; PDA: Potato Dextrose Agar; Sample A: Atosi; Sample B: Ale; Sample C: Opaeyin; Sample D: Kokoro inueje; Sample E: Agbo iba

Table 2 Morphological, Physiological and Biochemical Characterization of Agbo Samples

isolate code	Gram reaction	Gram reaction	Catalate test	Oxidase test	Sulphide test	Indole test	Motility test	Methyl red test	Voges proskaur	Urease	Glucose	Maltose	Lactose	Mannitol	Galactose	Sucrose	Probable organisms
NAI	-	rod	+	-	-	-	-	-	-	-	a	NC	NC	A	NC	A	*E. coli*
NAI	-	rod	-	-	-	-	-	-	-	+	NC	NC	NC	NC	NC	NC	*E.coli*
NAI	-	rod	+	-	-	-	-	-	+	-	A	NC	NC	NC	NC	A	*E.coli*
NAI	-	rod	+	-	-	-	-	-	+	-	NC	NC	NC	A	NC	NC	*Salmonelle typhi*
NAI	-	rod	-	+	-	-	-	-	-	-	AG	NC	A	A	NC	NC	*E.coli*
NAI	-	rod	+	-	-	-	-	-	+	-	AG	NC	A	A	NC	NC	*E.coli*
NAI	-	rod	+	-	-	-	-	-	+	-	A	NC	A	A	NC	NC	*E.coli*
NAI	-	rod	-	-	-	-	+	-	+	-	A	NC	A	A	A	A	*E.coli*
NAA	-	rod	+	-	-	-	-	-	-	-	NC	NC	NC	NC	NC	NC	*E.coli*
NAA	+	Cocci	+	-	-	-	+	-	-	-	A	A	NC	A	NC	NC	*Salmonella typhi*

NAA	-	rod	-	-	-	-	-	-	-	-	NC	NC	NC	A	NC	NC	*E.coli*
NAA	+	rod	+	-	-	-	+	-	-	-	NC	a	NC	A	NC	NC	*Proteus vulgaris*
NAT	+	rod	+	-	-	-	+	-	-	-	A	A	NC	AG	A	A	*E.coli*
NAT	-	rod	+	+	-	-	+	-	-	-	a	a	NC	A	A	A	*E.coli*
NAT	-	rod	+	-	-	-	+	-	-	-	A	A	NC	AG	A	A	*E.coli*
NAT	-	rod	+	-	-	-	+	-	-	-	AG	A	NC	AG	A	A	*E.coli*
NAT	-	rod	+	-	-	-	+	-	-	-	NC	a	NC	A	NC	A	*E.coli*
NAK	-	rod	+	+	-	-	-	-	-	-	NC	NC	NC	NC	NC	NC	*E.coli*
NAK	-	rod	+	-	-	-	+	-	-	-	NC	A	NC	NC	NC	NC	*E.coli*
NAK	-	rod	+	-	-	-	-	-	-	-	NC	a	NC	A	NC	NC	*E.coli*
NAK	-	rod	+	+	-	-	+	-	-	-	a	a	NC	A	NC	NC	*E.coli*
NAK	+	rod	+	-	-	-	-	-	-	-	NC	NC	NC	NC	NC	NC	*Salmonella typhi*
NAK	-	rod	-	-	-	-	-	-	-	-	a	NC	NC	A	NC	NC	*Proteus vulgaris*
NAO	-	rod	+	-	-	-	-	-	-	-	a	a	NC	NC	NC	NC	*E.coli*
NAO	-	rod	+	+	-	-	-	-	-	-	a	a	NC	A	NC	A	*E.coli*
NAO	-	rod	+	-	-	-	-	-	-	-	NC	a	NC	A	NC	A	*E.coli*
NAO	-	rod	+	-	-	-	-	-	-	-	NC	a	NC	A	NC	NC	*E.coli*
NAO	-	Rod	+	-	-	-	-	-	-	-	a	a	NC	A	NC	A	*E.coli*
NAO	+	rod	+	-	-	-	-	-	-	-	a	NC	NC	NC	NC	NC	*E.coli*
NAO	-	rod	+	+	-	-	-	-	-	-	A	NC	NC	NC	NC	-	*Proteus vulgaris*
NAO	-	rod	+	+	-	-	-	-	-	-	A	NC	NC	NC	NC	-	*E.coli*
MCT	-	rod	+	-	-	-	+	-	-	+	a	NC	NC	AG	A	-	*E.coli*
MCT	-	rod	+	-	-	-	+	-	+	+	A	A	A	AG	NC	-	Proteus vulgaris
MCT	-	rod	+	-	-	-	+	-	+	+	AG	A	AG	AG	NC	-	E. coli
MCT	+	rod	+	-	-	-	-	-	-	+	A	A	AG	AG	A	-	Salmonella typhi
MCT	-	rod	+	-	-	-	+	-	-	+	A	A	A	AG	A	-	E.coli
MCT	-	rod	+	-	-	-	+	-	+	+	AG	A	NC	NC	NC	-	E. coli
MSI	-	rod	-	-	-	-	+	-	+	+	a	NC	NC	a	NC	-	E. coli
MSI	+	rod	+	+	-	-	-	-	-	+	a	a	NC	NC	NC	-	E. coli
MSI	+	rod	+	-	-	-	-	-	-	+	NC	a	NC	AG	NC	-	Salmonella
MST	-	rod	+	-	-	-	-	-	+	+	AG	A	NC	AG	a	-	E. coli
MST	+	rod	+	-	-	-	+	-	-	+	AG	A	A	A	NC	-	Pseudomonas spp.
MST	+	rod	+	+	-	-	-	-	-	-	A	A	NC	A	NC	-	E coli
MSK	-	rod	+	-	-	-	-	-	-	+	AG	A	NC	A	NC	-	Salmonelle
MSK	+	rod	+	+	-	-	-	-	-	-	A	A	NC	A	NC	-	Pseudomoas
MSK	+	rod	+	-	-	-	-	-	-	+	a	A	a	A	NC	-	E coli
MSK	+	rod	+	+	-	-	-	-	-	+	A	A	a	A	A	-	Salmonelle
MSO	+	rod	+	-	-	-	+	-	-	+						-	Salmonelle

MSO	+	rod	+	-	-	+	+	-	-	+	AG	A	a	A	A	-	E coli
MSO	+	rod	+	-	-	-	-	-	-	+	A	A	a	A	NC	-	Pseudomoas
MSO	-	rod	+	+	-	-	+	-	-	-	AG	A	a	A	NC		Salmonelle
EMT	-	rod	+	-	-	-	+	-	-	+	A	A	NC	AG	A	-	E coli
EMA	-	rod	+	-	-	-	-	-	-	-	A	A	NC	A	NC	-	E coli
EMA	-	rod	+	-	-	-	+	-	-	-	A	A	NC	A	A	-	Salmonella
EMA	+	rod	+	-	-	-	+	-	-	-	AG	A	NC	A	NC	-	E. coli
EMO	+	rod	+	-	-	-	-	-	-	-	A	A	NC	A	A	-	E. coli
EMO	+	rod	+	-	-	-	+	-	-	-	AG	A	NC	NC	A	-	Salmonella
EMI	+	rod	+	-	-	-	+	-	-	+	-	-	-	-	-	-	E. c oli
EMI	-	rod	+	+	-	-	+	-	-	-	-	-	-	-	-	-	E. coli

Key: NAI: Nutrient agar agboibaNAA: Nutrient agar ale NAT: Nutrient agar atosiNAK:Nutrient agar kokoro Inu eje NAO: Nutrient agar opaeyin MCT:MacConkey atosiMST:Mannitolsalt atosiMSK: Mannitolsalt kokoroinuejeMSO Mannitolsalt opaeyinEMT: Eosin methyl blue atosiEMA:Eosin methyl blue ale EMO: Eosin methyl blue opaeyinEMI: Eosin methyl blue agboiba

Table 3 Antimicrobial susceptibility of bacterial Isolates

S/N	**SXT**	**SP**	**CH**	**CPX**	**AM**	**AU**	**CN**	**PEF**	**OFX**	**S**	**APX**	**Z**	**R**	**E**
1	9.00	9.00	4.50	0.50	1.00	1.00	0.50	3.00	4,50	9.00	ND	ND	ND	ND
2	4.00	2.50	8.00	3.00	2.50	0.50	6.00	8.00	8.00	3.50	ND	ND	ND	ND
3	0.00	0.00	7.50	0.00	0.00	0.00	4.50	7.00	7.00	3.00	ND	ND	ND	ND
4	6.00	5.00	6.00	6.00	8.00	8.00	9.00	9.00	9.00	7.00	ND	ND	ND	ND
5	5.00	9.00	9.00	5.00	6.50	7.00	4.00	4.00	5.00	6.00	ND	ND	ND	ND
6	5.00	5.00	8.00	8.00	10.00	6.00	8.00	8.00	6.00	4.00	ND	ND	ND	ND
7	9.00	9.00	6.00	0.00	4.00	7.00	6.00	6.00	6.00	6.00	ND	ND	ND	ND
8	5.00	7.00	7.00	5.00	5.00	5.00	6.00	9.00	11.00	8.00	ND	ND	ND	ND
9	3.50	3.50	8.00	2.50	2.00	1.50	5.00	6.00	5.00	4.00	ND	ND	ND	ND
10	7.00	ND	ND	ND	10.00	2.50	-	3.00	3.00	5.00	5.00	0.00	4.50	4.00
11	1.50	6.50	10.00	2.50	2.50	2.00	5.00	3.00	3.00	3.00	ND	ND	ND	ND
12	7.00	ND	ND	8.00	1.00	-	2.00	7.00	-	8.00	4.00	0.00	2.5-	6.00
13	4.00	ND	-	ss5.00	7.50	-	9.00	6.00	-	5.00	10.00	8.00	8.00	4.00
14	1.50	1.00	5.00	0.00	3.50	7.00	5.50	8.00	7.00	5.00	ND	ND	ND	ND
15	4.00	5.00	6.00	1.00	2.50	1.00	2.00	8.00	6.00	3.00	ND	ND	ND	ND
16	5.00	4.00	3.00	4.00	6.00	9.00	8.00	0.50	0.50	6.00	ND	ND	ND	ND
17	5.00	4.00	3.00	5.00	6.00	4.00	3.50	12.00	4.00	9.00	ND	ND	ND	ND
18	12.00	9.00	9.00	3.50	5.00	2.00	3.00	5.00	8.00	6.00	ND	ND	ND	ND
19	2.50	1.00	2.00	0.00	0.00	0.00	2.50	8.00	7.00	3.50	ND	ND	ND	ND
20	5.00	2.00	1.00	2.00	4.00.	6.00	8.00	15.00	12.50	6.50	ND	ND	ND	ND
21	3.00	7.00	3.50	0.00	0.00	6.00	2.50	7.00	4.50	2.50	ND	ND	ND	ND
22	4.00	-	-	9.00	4.50	-	4.50	5.00	-	7.00	0.00	3.00	1.00	5.00

23	4.00	5.00	7.00	7.00	8.00	11.00	6.50	7.00	6.00	3.00	ND	ND	ND	ND
24	5.00	3.00	7.00	6.00	7.00	9.00	5.00	6.00	4.00	1.00	ND	ND	ND	ND
25	7.00	9.50	14.00	5.00	3.00	6.00	3.00	1.00	2.00	4.00	ND	ND	ND	ND
26	4.00	10.00	7.00	5.00	3.00	3.00	4.00	7.00	10.00	10.00	ND	ND	ND	ND
27	4.00	1.00	1.00	4.00	9.00	9.00	9.00	10.00	13.00	8.50	ND	ND	ND	ND
28	4.50	9.50	12.00	6.50	3.50	4.00	2.00	1.00	2.00	4.00	ND	ND	ND	ND
29	8.00	-	-	9.00	2.00	-	8.00	8.00	-	10,00	1.50	4.50	5.00	8.00
30	5.00	10.00	9.00	5.00	4.00	5.00	2.00	1.00	1.00	1.00	ND	ND	ND	ND
31	6.00	8.00	9.00	9.00	9.00	8.00	6.00	6.00	6.00	5.00	ND	ND	ND	ND
32	6.00	8.00	9.00	7.00	2.00	6.00	5.00	6.00	5.00	4.00	ND	ND	ND	ND
33	6.00	8.00	8.00	8.00	7.50	9.00	5.00	8.00	7.00	5.50	ND	ND	ND	ND
34	6.00	-	-	8.00	5.50	-	7.00	8.00	-	7.00	4.50	5.00	6.00	7.00
35	4.00	7.00	5.00	7.00	4.00	5.00	1.00	2.00	1.00	1.00	ND	ND	ND	ND
36	6.00	8.00	9.00	4.50	7.00	6.50	6.00	6.00	5.00	2.00	ND	ND	ND	ND
37	3.00	4.00	7.00	5.00	3.00	1.00	3.00	4.00	2.00	4.00	ND	ND	ND	ND
38	7.00	-	-	5.00	7.00	-	6.00	5.00	-	9.00	1.00	5.00	3.00	7.00
39	5.00	-	-	6.00	7.00	-	6.00	7.00	-	5.00	2.00	2.50	4.00	5.00
40	9.00	6.00	4.00	3.50	5.00	6.00	8.00	10.00	12.00	5.50	ND	ND	ND	ND
41	8.00	-	-	7.00	5.00	-	8.00	9.00	-	8.00	4.00	2.00	2.00	7.00
42	9.00	-	-	8.00	4.00	-	6.00	5.00	-	7.00	5.00	4.00	4.00	3.00
43	7.00	5.00	7.00	4.00	5.00	4.00	5.00	4.00	3.00	1.00	ND	ND	ND	ND
44	9.00	-	-	8.00	5.00	-	6.00	4.00	-	7.00	4.00	1.00	1.00	2.00
45	4.00	-	-	6.00	3.00	-	4.00	3.00	-	5.00	2.00	2.00	1.00	2.00
46	5.00	-	-	7.00	4.00	-	3.00	4.00	-	6.00	2.00	1.00	2.00	1.00
47	9.00	-	-	5.00	7.00	-	4.00	6.00	-	7.00	5.00	3.00	2.00	2.00
48	8.00	-	-	5.00	7.00	-	5.00	4.00	-	6.00	4.00	5.00	3.00	4.00
49	7.00	-	-	5.00	6.00	-	6.00	5.00	-	7.00	5.00	4.00	2.00	5.00
50	9.00	-	-	5.00	7.00	-	8.00	6.00	-	9.00	3.00	7.00	4.00	7.00
51	5.00	7.00	8.00	6.00	4.00	6.00	5.00	6.00	5.00	7.00	3.00	7.00	4.00	7.00
52	8.00	5.00	5.00	7.00	5.00	4.00	6.00	5.00	3.00	5.00	4.00	5.00	2.00	2.00
53	5.00	7.00	4.00	6.00	7.00	5.00	3.00	6.00	5.00	7.00	4.00	1.00	2.00	7.00
54	9.00	6.00	5.00	4.00	8.00	6.00	5.00	4.00	6.00	5.00	3.00	2.00	4.00	7.00
55	9.00	9.00	500	600	5.00	9.00	7.00	8.00	6.00	4.00	5.00	2.00	2.00	7.00
56	7.00	-	-	8.00	1.00	-	2.00	7.00	-	8.00	3.00	0.00	3.00	4.00
57	4.00	-	-	5.00	6.00	-	8.00	3.00	-	6.00	7.00	4.00	3.00	5.00
58	4.00	-	-	8.00	5.00	-	6.00	3.00	-	6.00	2.00	4.00	2.00	1.00
59	5.00	6.00	4.00	6.00	4.00	6.00	5.00	4.00	6.00	4.00	2.00	4.00	2.00	1.00

Key: NA Agbo Iba 1-8; NA Ale 9-12; NA Atosi 13-17; NA Kokoro Inu eje 18-23; NA Opaeyin 24-31; MCA Atosi 32-37; MSA Agbo Iba 38-40; MSA Atosi 41-43; MSA Kokoro Inu eje 44-47; MSA Op eyin 48-51; mEMBAtosi 52; EMB Ale 53-55; EMB Opaeyin 56-58; EMB Agbo Iba 58-59

3. Conclusion

It could be concluded that all the samples of herbal mixtures (agbo) tested were contaminated with spoilage and pathogenic microorganisms. The organisms were also resistant to all the commonly use antibiotic that were tested. Therefore it is advised that people should stop the consumption of agbo samples.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to disclosed.

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