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Physicochemical and Microbiological Evaluation of *Agbo* (herbal decoction) Sold in Some Parts of Warri Metropolis, Delta-State, Nigeria

Ejukonemu E. Francis, Isiosio O. Isaac

***Abstract*— Agbo which is the Yoruba name for herbal medicine, is an aqueous decoction produced from mixtures of several plant parts such as leaves, stems, roots and barks that is used in treating illnesses and diseases. The study was carried out to ascertain the microbial load, level of heavy metal contamination and antibiotics sensitivity of bacteria isolated from agbo sold in Warri, Delta State. A total of twenty –five (25) agbo samples collected from five (5) locations were analyzed. The pH values of the various agbo samples ranges from 3.38 ± 1.05 to 7.23 ± 1.02. Concentration of Iron ranges from 6.085 ± 1.00 - 21.904 ± 3.04 mg/l, Lead ranges from 0.006 ± 0.00 - 0.021 ± 0.08 mg/l, Cadmium ranges from 0.028 ± 0.10 - 0.102 ± 0.11 mg/l, Mercury ranges from 0.003 ± 0.00 - 0.010 ± 0.08 mg/l, Arsenic ranges from 0.002 ± 0.00 - 0.007 ± 0.05 mg/l and Chromium ranges from 0.012 ± 0.03 - 0.034 ± 0.01 mg/l respectively. The mean total viable count ranges between 25 ± 1.03 X 104 - 44 ± 0.06 X 104 cfu/ml, total coliforn count ranges between 0.15 ± 0.51 X 104 – 0.30 ± 0.11 X 104 cfu/ml and total fungal count ranges between 25 ± 1.06 - 49 ± 1.05 X 104 cfu/ml respectively. S.auerus (29.4%) was the predominant bacteria. This was followed by Klebsiella species (23.5%), E.coli (17.6%), Salmonella species (11.8%), Proteus mirabilis (11.8%) and Enterobacter species (5.9%). Aspergillus species (23.8) was the predominant fungi. This was followed by Candida species (19.0%), Penicillium spp (14.3%), Mucor (14.3%), Botrytis (9.5%), Fusarium spp (9.5%), Geotrichum spp (4.8%) and Phoma spp (4.8%). All the isolates were resistant to ampicillin. S.areus was susceptible to augmentin, gentamycin and ceporex, E. coli was susceptible to ciprofloxacin, gentamycin and nalidixic acid. Klebsiella spp was susceptible to ciprofloxacin and getamycin, Salmonella spp was susceptible to ciprofloxacin, P.mirabilis was susceptible to ciprofloxacin and gentamycin, and Enterobacter spp was susceptible to pefloxacine, augmentin and gentamycin.**

***Index Terms*— Agbo, Heavy metals, Herbal medicine, Microbial load.**

I. INTRODUCTION

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 contains parts of plant and other plant materials as its active ingredients that is used in treating illness and diseases (Oluyemi *et al*., 2016). Herbal medicines have been used to treat many conditions such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue and irritable bowel syndrome (Falodun *et al*., 2013)

In Nigeria, the increasing cost of orthodox medication coupled with poverty, has forced a large population to resort to the use of herbal medicines to relief themselves of ailments (Esinome *et al*., 2001). 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).

Agbo which is the Yoruba name for herbal medicine. It is an aqueous decoction produced from mixture of several plant parts such as leaves, stems, roots and barks. It is prepared by soaking the plants parts in either water or alcohol in bottles and allowed to stand for days or boiling the plant parts after which it is poured into bottles for drinking purpose. The producers and sellers are mostly Yoruba women who hawk the product in Warri and its environs. The product is dispensed in disposable plastic cups to users (Stafford *et al*., 2008).

Herbal medicines are perceived as being natural and therefore safe; however, they are not free from adverse effects, which may be due to factors such as adulteration, contamination, misidentification, lack of standardization, incorrect preparation and dosage (Oluyege and Oluyege, 2010). By their origin, herbs are subjected to contamination by pathogenic microorganisms from the soil, air and water. In addition, microbial contaminants may also be introduced during harvesting, handling, preparation and storage of herbal medicine. (Ola *et al*., 2013). Heavy metals such as lead, cadmium, mercury, arsenic, chromium and iron are constituent of the environment like air, water and soil. Furthermore, they are produced by technical and industrial processes, thus medicinal plant growing in nature may bioaccumulate toxic heavy metals to a certain extent depending on their individual properties and concentration of the heavy metals in soil, air and water (Osadolor *et al*., 2015). In addition, high levels of heavy metals can be found in plants when agricultural expedients such as cadmium fertilizers, organic mercury or lead pesticides are used (Caldas *et al*., 2004).

Pathogenic microorganisms and heavy metals can occur in herbal medicines when prepared with contaminated herbs. The contamination of these herbal medicines reduces the effectiveness and also poses serious health hazard to the consumers. Heavy metals if consumed can accumulate in different organs of the body leading to unwanted side effects while pathogenic microorganisms cause various diseases.

Ejukonemu E. Francis, Department of Science Laboratory Technology, Delta State Polytechnic, Otefe- Oghara
Isiosio O. Isaac, Department of Science Laboratory Technology, Delta State Polytechnic, Otefe- Oghara

(MacDonald, 2015)

The aim of this study was to evaluate the level of heavy metals and microbial contamination of agbo sold in Warri – Delta state as well as to test the antibiotics sensitivity of these isolates to commercial antibiotics.

II. MATERIALS AND METHODS

A. Collection of samples

A set of agbo for the treatment of five (5) disease conditions: Typhoid fever, Malaria fever, Erectile dysfunction, general Infections and Rheumatism were randomly purchased at point of sales from sellers at five (5) locations in Warri – Delta State: Okere market, Pessu market, Igbudu market, Effurun market and Main market. 10ml of each sample was dispensed into sterile containers and taken immediately to the laboratory for analysis.

B. Determination of pH

The pH value of each sample was obtained using a pH meter (search tech PHS – 3C model) at a temperature of 28.5^0C.

C. Heavy metals analysis

The amount of heavy metals in each sample was analysed with Electrothermal Atomic Absorption Spectrometer (Perkin Elmer analyst 800, Norwalk, U.S.A) by adopting the methods of Olmedo *et al.*, (2010)

D. Microbial analysis

In the laboratory, samples were shaken vigorously to ensure uniform distribution of microorganisms. 5ml of each sample was pipetted into 95ml of sterile distilled water for stock sample preparation and was subjected to tenfold serial dilution in sterile test tubes. 1ml of each stock sample was aseptically transferred and mixed in 9ml of sterile distilled water (10^{-1}) up to 10^{-10}. All media used were prepared according to manufacturer's instructions. For total viable bacterial count and coliform count, 1ml of dilutions 10^{-2} and 10^{-4} were transferred into sterile duplicate plates and 15 – 20ml of nutrient and MacConkey agar were added and mixed immediately. For fungal count, 1ml of dilutions 10^{-2} and 10^{-4} were spread over duplicate plates of pre – prepared dried potato dextrose agar (PDA). Plates were incubated at 32^0C for 48hours for total bacterial count, at 37^0C for 24 hours for coliform count and at 25^0C for 5 days for fungi count. Plates containing 25 – 250 CFU /ml were enumerated for total bacterial count whereas plates containing 15 – 150 CFU /ml were enumerated for coliform and fungal count (Christen *et al*., 1992). Other media with selective and differential characteristics used were Eosine Methylene Blue (EMB) and Kligler Iron Agar (KIA). Pure cultures bacterial isolates were identified using cultural, morphological and biochemical characterization. Fungal isolates were identified on the basis of macroscopic and microscopic characteristics by slide culture techniques and lactophenol staining. The scheme of Barnet and Hunter (1998) was used for the fungal identification. Bacterial identification to general level was based on Bergey's manual of determinative bacteriology. (Buchanna, and Gibbon, 1974).

E. Antibiotics sensitivity

Antibiotics sensitivity test was determine by the disc diffusion method as described by Bauer et al. (1966) in accordance with Mcfarland standard. Ten different commercially prepared antibiotics discs containing Ofloxacin(10µg), Pefloxacine (10µg), Ciprofloxacine (10µg), Augmentin (30µg), gentamcin (10µg), Streptomycin (30µg), Ceporex (10µg), Nalidixic Acid (30µg), Septrin (30µg), and Ampicillin (30µg) were used and after 18 hrs incubation at 37^0c, the size of the zone of inhibition was measured and interpreted by comparing with the standard antibiotic sensitivity chart to determine their resistance patterns.

F. Statistical analysis

The mean and standard deviation of the results were done using SPSS. The results were expressed as mean ± S.D. The results were also represented using descriptive statistics such as pie charts.

III. RESULTS AND DISCUSSIONS

Table 1: pH values of each samples

Sample source	Sample type	pH (mean ± S.D)
Main market	Erectile dysfunction	7.23 ± 1.02
	Rheumatism	3.79 ± 0.03
	Malaria	4.77 ± 0.07
	Typhoid	4.18 ± 0.04
	Infection	5.83 ± 1.00
Pessu market	Erectile dysfunction	4.42 ± 0.07
	Rheumatism	4.58 ± 0.03
	Malaria	5.48 ± 0.02
	Typhoid	4.60 ± 0.05
	Infection	5.53 ± 1.03
Effurun market	Erectile dysfunction	5.41 ± 0.08
	Rheumatism	4.68 ± 0.05
	Malaria	4.78 ± 0.01
	Typhoid	4.02 ± 0.05
	Infection	5.88 ± 1.00

	Erectile dysfunction	3.38 ± 1.05
Okere market	Rheumatism	3.66 ± 1.03
	Malaria	4.87 ± 0.45
	Typhoid	4.26 ± 0.01
	Infection	6.55 ± 0.08
Igbudu market	Erectile dysfunction	3.88 ± 0.01
	Rheumatism	4.65 ± 0.08
	Malaria	5.54 ± 0.05
	Typhoid	4.93 ± 0.45
	General Infection	5.75 ± 0.07

Table 2: The Values of heavy metals present in herbal medicine

Sample source	Sample type	Heavy metals (mean ± S.D Mg/l) Fe	Pb	Cd	Hg	As	Cr
Main Market	Erectile-dysfunction	8.452 ± 1.01	0.008 ± 0.04	0.039 ± 0.05	0.004 ± 0.02	0.003 ± 0.04	0.013 ± 0.02
	Rheumatism	10.333 ± 0.09	0.010 ± 0.05	0.048 ± 0.03	0.005 ± 0.09	0.004 ± 0.00	0.016 ± 0.05
	Malaria	21.671 ± 2.03	0.021 ± 0.08	0.101 ± 0.06	0.010 ± 0.01	0.007 ± 0.03	0.034 ± 0.01
	Typhoid	8.397 ± 1.00	0.008 ± 0.03	0.039 ± 0.08	0.004 ± 0.00	0.003 ± 0.05	0.013 ± 0.09
	Infection	16.655 ± 3.00	0.016 ± 0.05	0.078 ± 0.05	0.008 ± 0.04	0.006 ± 0.00	0.026 ± 0.04
		10.951 ±	0.011 ±	0.051 ±	0.005 ±	0.004 ±	0.017 ±
Pessu Market	Erectile dysfunction	1.06	0.08	0.06	0.01	0.01	0.02
	Rheumatism	19.365 ± 1.07	0.019 ± 0.07	0.090 ± 0.01	0.009 ± 0.03	0.007 ± 0.05	0.030 ± 0.01
	Malaria	16.296 ± 1.01	0.016 ± 0.09	0.076 ± 0.08	0.007 ± 0.01	0.006 ± 0.07	0.026 ± 0.03
	Typhoid	21.057 ± 3.02	0.021 ± 0.00	0.098 ± 0.05	0.010 ± 0.08	0.007 ± 0.09	0.033 ± 0.10
	Infection	9.005 ± 1.04	0.009 ± 0.00	0.042 ± 0.03	0.004 ± 0.00	0.003 ± 0.00	0.014 ± 0.09
	Erectile dysfunction	9.419 ±	0.009 ±	0.044 ±	0.004 ±	0.003 ±	0.015 ±
Effurun Market		1.08	0.10	0.10	0.03	0.05	0.10
	Rheumatism	11.745 ± 1.10	0.011 ± 0.11	0.055 ± 0.07	0.005 ± 0.05	0.004 ± 0.05	0.018 ± 0.09
	Malaria	21.904 ± 3.04	0.021 ± 0.02	0.102 ± 0.11	0.010 ± 0.01	0.007 ± 0.03	0.034 ± 0.00
	Typhoid	7.407 ± 2.06	0.007 ± 0.08	0.035 ± 0.11	0.003 ± 0.02	0.003 ± 0.08	0.012 ± 0.03
	Infection	8.507 ± 1.09	0.008 ± 0.04	0.040 ± 0.04	0.004 ± 0.03	0.003 ± 0.01	0.013 ± 0.04
	Erectile dysfunction	6.085 ±	0.006 ±	0.028 ±	0.003 ±	0.002 ±	0.010 ±
Okere Market		1.00	0.00	0.10	0.00	0.00	0.03
	Rheumatism	7.725 ± 1.07	0.008 ± 0.01	0.036 ± 0.01	0.003 ± 0.00	0.003± 0.00	0.012 ± 0.05
	Malaria	9.906 ± 1.07	0.010 ± 0.07	0.046 ± 0.02	0.004 ± 0.01	0.003 ± 0.01	0.016 ± 0.02
	Typhoid	19.576 ± 2.05	0.019 ± 0.09	0.091 ± 0.04	0.009 ± 0.03	0.007 ± 0.06	0.031 ± 0.03
	Infection	11.419 ± 2.10	0.011 ± 0.06	0.053 ± 0.04	0.005 ± 0.06	0.004± 0.03	0.018 ± 0.01
	Erectile dysfunction	9.062 ±	0.009 ±	0.042 ±	0.004 ±	0.003 ±	0.014 ±
Igbudu		1.05	0.05	0.03	0.00	0.08	0.04

Market	Rheumatism	8.519 ± 1.02	0.008 ± 0.00	0.040 ± 0.01	0.004 ± 0.07	0.003 ± 0.07	0.013 ± 0.09
	Malaria	15.926 ± 2.03	0.016 ± 0.02	0.074 ± 0.06	0.007 ± 0.01	0.005 ± 0.05	0.025 ± 0.03
	Typhoid	14.603 ± 3.10	0.014 ± 0.01	0.068 ± 0.07	0.007 ± 0.02	0.005 ± 0.06	0.023 ± 0.10
	Infection	10.370 ± 1.10	0.010 ± 0.03	0.048 ± 0.02	0.005 ± 0.05	0.004 ± 0.02	0.016 ± 0.01

Key: Fe – Iron, Pb – Lead, Cd – Cadmium, Hg – Mercury, As – Arsenic
Cr - Chromium

Table 3: Total viable bacterial count, coliform count and fungi count (mean ± S.D × 10^4 cfu/ml)

Sample source	Sample type	Total bacterial count	Coliform count	Fungi count
Main Market	Erectile dysfunction	25 ± 2.05^{b}	0.17 ± 0.09^{a}	35 ± 0.15^{c}
	Rheumatism	32 ± 1.08^{c}	0.19 ± 2.03^{a}	20 ± 1.00^{b}
	Malaria fever	34 ± 0.06^{c}	0.18 ± 1.03^{a}	37 ± 1.50^{c}
	Typhoid fever	38 ± 3.03^{c}	0.23 ± 1.08^{a}	40 ± 1.00^{c}
	General Infection	28 ± 2.02^{b}	0.20 ± 1.00^{a}	31 ± 2.01^{c}
Pessu market	Erectile dysfunction	35 ± 1.05^{c}	0.26 ± 1.04^{a}	29 ± 1.09^{b}
	Rheumatism	29 ± 2.03^{b}	0.15 ± 0.05^{a}	32 ± 0.51^{c}
	Malaria fever	30 ± 1.01^{c}	0.16 ± 2.04^{a}	38 ± 1.07^{c}
	Typhoid fever	28 ± 1.03^{b}	0.29 ± 1.01^{a}	31 ± 1.00^{c}
	General Infection	31 ± 0.09^{c}	0.30 ± 0.11^{a}	35 ± 0.51^{c}
Effurun market	Erectile dysfunction	32 ± 1.03^{c}	0.18 ± 0.07^{a}	29 ± 2.06^{b}
	Rheumatism	25 ± 2.05^{b}	0.19 ± 2.09^{a}	30 ± 1.03^{b}
	Malaria fever	44 ± 0.06^{c}	0.17 ± 2.00^{a}	38 ± 1.51^{c}
	Typhoid fever	40 ± 1.00^{c}	0.21 ± 1.03^{a}	49 ± 1.05^{c}
	General Infection	42 ± 2.01^{c}	0.22 ± 1.00^{a}	35 ± 0.09^{c}
Okere Market	Erectile dysfunction	31 ± 2.03^{c}	0.18 ± 2.08^{a}	28 ± 0.50^{b}
	Rheumatism	38 ± 1.02^{c}	0.27 ± 1.06^{a}	37 ± 1.04^{c}
	Malaria fever	35 ± 1.06^{c}	0.15 ± 3.05^{a}	31 ± 0.51^{c}
	Typhoid fever	30 ± 3.00^{c}	0.16 ± 2.09^{a}	31 ± 0.50^{c}
	General Infection	33 ± 0.09^{c}	0.21 ± 1.10^{a}	40 ± 2.00^{c}
Igbudu market	Erectile dysfunction	36 ± 1.00^{c}	0.16 ± 1.08^{a}	25 ± 1.06^{b}
	Rheumatism	33 ± 2.07^{c}	0.19 ± 0.10^{a}	35 ± 0.11^{c}
	Malaria fever	38 ± 1.03^{c}	0.15 ± 0.51^{a}	48 ± 3.01^{c}
	Typhoid fever	37 ± 0.08^{c}	0.18 ± 0.00^{a}	46 ± 2.50^{c}
	General Infection	34 ± 1.03^{c}	0.17 ± 2.01^{a}	36 ± 1.04^{c}

The superscripts b = c (the difference between b and c is not statistically significant)
But b ≠ a and c ≠ a (the difference between b&a and also c&a are statistically significant $P< 0.05$)
b = c ≠ a* (a is significantly different from b and c at $P< 0.05$)

Table 4: bacterial and fungal isolates

Sample source	Identified bacterial isolates	Identified fungal isolates
Main Market	*Salmonella species*	*Aspergillus species*
	Klebsiella species	*yeast species*
	Eschericha coli	*Penicillium*
	Staphylococcus aureus	*Botrytis species*
Pessu market	*Klebsiella species*	*Aspergillus species*
	Staphylococcus aureus	*yeast species*
		Mucor

Effurun market	*Klebsiella species* *Eschericha coli* *Salmonella species* *Staphylococcus aureus*	*Aspergillus species* *Penicillium* *Fusarium spp.*
Okere Market	*Klebsiella species* *Proteus mirabilis* *Eschericha coli* *Staphylococcus aureus*	*Aspergillus species* *yeast species* *Mucor* *Fusarium spp.*
Igbudu market	*Enterobacter species* *Staphylococcus aureus* *Proteus mirabilis*	*Aspergillus species* *yeast species* *Penicillium* *Mucor* *Botrytis species* *Geotrichum spp* *Phoma spp.*

Table 5: Antibiotics susceptibility of bacterial isolates

	Inhibition zone diameter (mean ± S.D mm)									
Bacterial Isolates	**OFX (10µg)**	**PEF (10µg)**	**CPF (10µg)**	**AU (30µg)**	**CN (10µg)**	**S (30µg)**	**CEP (10µg)**	**NA (30µg)**	**SXT (30µg)**	**PN (30µg)**
S. aureus	18±1.0 (R)	20±3.1 (I)	18±3.0 (I)	32±3.1 (S)	20±1.1 (S)	14±0.0 (R)	23±3.1 (S)	-	18±1.1 (R)	28±1.3 (R)
E. coli	20±3.3 (I)	16±1.0 (R)	24±0.0 (S)	21±1.4 (I)	21±2.3 (S)	12±1.1 (R)	14±0.0 (R)	24±3.0 (S)	-	18±0.0 (R)
Klebsiella spp.	19±1.1 (I)	18±3.4 (I)	25±3.1 (S)	16±2.3 (R)	28±1.6 (S)	16±3.1 (I)	14±1.0 (R)	16±2.4 (R)	18±1.3 (R)	12±2.6 (R)
Salmonella spp.	18±3.0 (I)	18±2.2 (I)	27±0.8 (S)	16±0.1 (R)	18±1.1 (I)	14±2.0 (R)	16±3.4 (R)	-	-	14±0.5 (R)
P. mirabilis	20±1.5 (I)	20±0.8 (I)	26±1.8 (S)	-	25±3.3 (S)	16±2.4 (R)	18±3.0 (R)	16±0.0 (R)	-	16±3.3 (R)
Enteobacter spp.	16±0.0 (R)	23±0.8 (S)	19±3.3 (I)	24±0.0 (S)	22±1.3 (S)	-	18±1.5 (I)	20±3.5 (R)	-	14±1.4 (R)

Key:
OFX=Ofloxacin, PEF=Pefloxacine, CPX=Ciprofloxacin, AU=Augmentin, CN=Gentamycin, S=Streptomycin, CEP=Ceporex, NA=Nalidixic Acid, SXT=Septrin, PN=Ampicillin,

No inhibition, S = Susceptible, I = Intermediate, R = Resistant
Note that IZD's were interpreted using Kirby-Bauer antibiogram table.

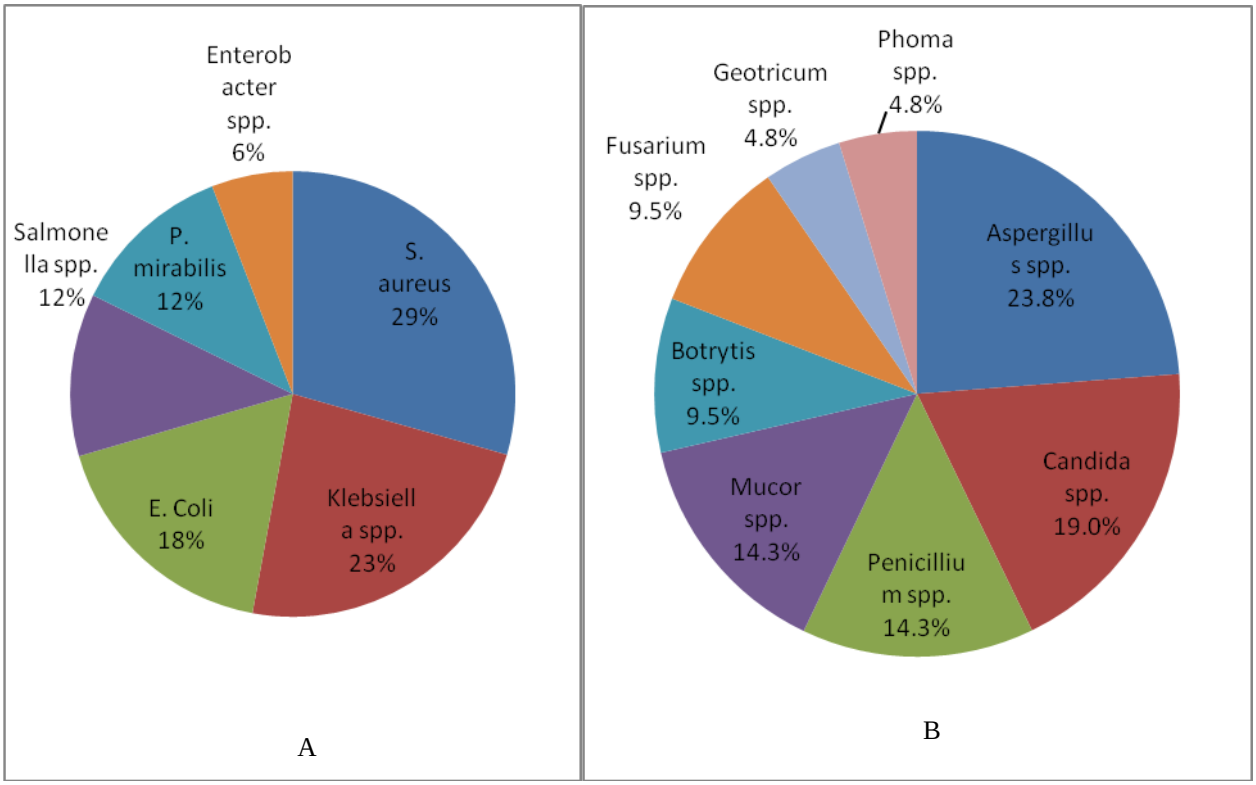

Figure 1: Percentage occurrence of bacterial isolates (A) Fungal isolates (B)

The pH values of the various agbo samples ranges from 3.38 ± 1.05 to 7.23 ± 1.02 as shown in table 1. These pH values are in the acidic region except for one having pH of 7.23 ± 1.02 which is in basic region of the pH scale. Richard (2017) reported that the human body needs to take in the right amount of acidic and alkalizing nutrients to maintain a healthy pH balance. The recommended daily intake should be 20% of acidic and 80% alkalizing. He stated further that if the body acid/alkaline balance is not maintained, the body has to compensate by robbing minerals from other parts of the body such as the bones, joints, muscles, gall bladder and mucosal lining of the digestive tract. Thus, when the body robs calcium from the bones, one can develop a weak back and if left untreated, this can develop into osteoporosis (low bone density). Therefore, people who regularly consume those agbo samples are at risk of developing acidic/alkalinic imbalance which can result to various diseases

Table 2. Shows concentration of heavy metals in the various agbo samples sold in Warri. Concentration of Iron ranges from 6.085 ± 1.00 - 21.904 ± 3.04 mg/l, Lead ranges from 0.006 ± 0.00 - 0.021 ± 0.08 mg/l, Cadmium ranges from 0.028 ± 0.10 - 0.102 ± 0.11 mg/l, Mercury ranges from 0.003 ± 0.00 - 0.010 ± 0.08 mg/l, Arsenic ranges from 0.002 ± 0.00 - 0.007 ± 0.05 mg/l and Chromium ranges from 0.012 ± 0.03 - 0.034 ± 0.01 mg/l respectively. The World Health Organization (WHO) limits for Arsenic, Cadmium, Lead and Chromium in herbal preparation are 0.01mg/l, 0.003mg/l, 0.001mg/l and 0.02mg/l respectively, while 0.002mg/l is the limit for Mercury set by the Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) (Osadolor *et al*, 2015). The concentration of heavy metals in all the agbo samples are above the limit. Persistent users may accumulate these heavy metals to levels that are toxic to organs within the body with time. According to Osadolor et al., (2015), adult with high blood lead levels (0.04mg/l) may have impaired haemsynthesis, chronic kidney disease and impairment of cognitive function. Increased exposure to mercury can alter brain functions and lead to shyness, tremors, memory problems, irritability, and changes in vision or hearing (Monisha *et al*., 2014). Ingestion of large amounts of arsenic can lead to gastrointestinal symptoms such as severe vomiting, disturbances of the blood and circulation, damage to the nervous system and eventually death (Manju, 2015). Iron produces free radicals that results in cellular damage, mutation and malignant transformations which in turn can cause an array of diseases (Monisha *et al*, 2014). Cadmium damages a specific structure of the functional unit of the kidney.

Table 3 shows the estimation of the total viable bacterial count, total coliform counts and total fungi count of the various agbo samples on nutrient agar, MacConkey agar and potato Dextrose agar. The mean total viable count ranges between 25 ± 1.03 X 10^4 - 44 ± 0.06 X 10^4 cfu/ml, total coliforn count ranges between 0.15 ± 0.51 X 10^4 – 0.30 ± 0.11 X 10^4 cfu/ml and total fungal count ranges between 25 ± 1.06 - 49 ± 1.05 X 10^4 cfu/ml respectively. The microbial counts detected were in the order of10^4 cfu/ml. The World Health Organization (WHO), British Pharmacopoeia and the United State Pharmacopoeia have recommended tolerable microbial limits in non – sterile pharmaceutical products which include 10^7 cfu/ml bacteria and 10^5 cfu/ml fungi. All the agbo samples were able to meet this limit. However, the detection of bacteria such as *S. aureus* andcoliforms which are food borne pathogens in the samples shows high level of contamination from different sources which makes the quality of the samples unacceptable. The occurrence of *S. aureus* in majority of the samples could be from the nose where it is commonly found, hands, skin and clothing of

handlers. Coliform contamination could result from the water used in preparing or diluting the agbo and poor personal hygiene during preparation. Some of the fungi species of Aspergillus are known to produce mycotoxins that are harmful to man and as such, their incidence in agbo is undesirable. The presence of these fungi isolated may be due to poor harvesting conditions and post – harvestingpreservation techniques used for the herbs. These observations are in agreement with the work of Odedara and Memuletiwon, (2014) who recorded total bacterial count of 8.5 X 10^5 cfu/ml

Table 4 shows the distribution of bacterial and fungal pathogens isolated from agbo samples sold in Warri metropolis. Six bacteria and eight fungi were isolated.

Figure 1 showsthepercentage of occurrence of the various bacteria and fungi isolates. *S.auerus* (29.4%) was the predominant bacteria. This was followed by *Klebsiella species* (23.5%), *E.coli* (17.6%), *Salmonella species* (11.8%), *Proteus mirabilis* (11.8%) and *Enterobacter species* (5.9%). *Aspergillus species* (23.8) was the predominant fungi. This was followed by *Candida species* (19.0%), *penicillium spp* (14.3%), *Mucor* (14.3%), Botrytis (9.5%), *Fusarium spp* (9.5%), *geotrichum spp* (4.8%) and *Phoma spp* (4.8%). This observation is in agreement with the work of MacDonald et al, (2015) who reported isolating fungal species such as *Fusarium spp, Aspergillus spp, Penicillium spp, Geotrichum spp, a*nd *Mucor f*rom poly – herbal products in Lagos. Esimone et al (2001) also reported the presence of bacteria such as *E. coli, Klebsiella spp, Proteus spp, Streptococus spp* and *Staphylococcus spp* from herbal preparations marketed in South East Nigeria

Table 5 shows the antibiotics sensitivity of the various bacteria isolated from the agbo samples. All the isolates were resistant to ampicillin. *S.areus* was susceptible to augmentin, gentamycin and ceporex, *E. coli* was susceptible to ciprofloxacin, gentamycin and nalidixic acid. *Klebsiella spp* was susceptible to ciprofloxacin and getamycin, *Salmonella spp* was susceptible to ciprofloxacin, *P.mirabilis* was susceptible to ciprofloxacin and gentamycin, and *Enterobacter spp* was susceptible to pefloxacine, augmentin and gentamycin. These antibiotics have been in the Nigerian market for a long time and might have been exposed to use and abuse which might account for the levels of resistance observed in the study. Therefore, gentamycin and ciprofloxacin are antibiotics options that can be used to treat bacterial infections acquired from the consumption of agbo sold in Warri, Delta state.

IV. CONCLUSION

This study has revealed that agbo sold in Warri metropolis contain relatively high amounts of heavy metals above the limit and by implication, frequent consumption may increase the body burden of these metals, which have been reported to be toxic to the body. The study also reveal the presence of pathogenic bacteria and fungi in the agbo samples which could have resulted from contaminated soils, plants and its products, preparation processes, quality of water, storage containers and processing equipment. This stands as a great threat to human health as these organisms can cause diseases. The study also revealed the antibiotic sensitivity of the bacterial isolates. The presence of antibiotic resistant organisms raises concerns to health as it may implicate the clinical management of diseases caused by the resistant organism in human. The use of agbo as an alternative drug could be a source of multiple antibiotics resistance microorganisms in consumers. Efforts on the part of producers should be made to enhance proper hygienic conditions in all the preparation processes starting from plant collection, processing, packaging and storage in order to reduce incidence of pathogenic microorganisms and spread of resistance strains

REFERENCES

[1] Barnett H.L. and Hunter, B.B. (1998). Illustrated Genera of Imperfect Fungi (4th Edition). Published by Amer Phytopathological Society. Pp 106, 130

[2] Bauer, A.M., Kirby, W.M.M., Shermis, T.C. and Truck, M. (1966). Antibiotics Susceptibility Testing by A Standard Single Disk Method. *Am. J. Clin. Pathol*. 45:493 – 496

[3] Buchanna, R.E. and Gibbon, N.E.(1974). Bergy's Manual of Determination Bacteriology, Williams and Wilkin Baltimore, USA.

[4] Caldas L.L. Machado (2004). Cadmium, Mercury and Lead in medicinal herbs in Brazil. *Food and Chemical Toxicology*. Vol 42, 599 – 603pp

[5] Christen, G.L., Davidson, P.M., McAllister, J.S. and Roth, L.A. (1992). Coliform and other indicator bacteria in: Standard Method for the Examination of Dairy product, Marshall, T.R. (Ed.) American Public Health Association, Washington, DC. Pp: 247 – 267

[6] Esimone C.O; Chach K.F, and Ikejide S.C. (2001). Microbiological quality of herbal preparations marked in South East Nigeria. *Journal of natural remedies*. 2: 42-48.

[7] Falodun A. and Imieje V. (2013). Herbal Medicine in Nigeria; holistic overview. *Nigeria journal of science and environment.* 12(1)

[8] Macdonald Idu, Ouvakporie Uvo Oghale, Adeola Jimoh (2015). Heavy metals contamination of some poly herbal products from Lagos State, Nigeria. *Journal of Ayurvedic and Herbal medicine.* 1(2): 45 – 50pp

[9] Macdonald I., Ouvakporie U. O., Adeola J. (2015). Microbial load of some poly- herbal products from Lagos State, Nigeria. *International Journal of Ethnobiology and Ethnomedicine.* 1: issue 1

[10] Manju M. (2015). Effects of heavy metals on human health. International Journal of research Granthalayah. 1 - 7pp

[11] Monisha J. T.T., Naresh A. Blessing B., Mathew K. and Beeregowda N. (2014). Toxicity, mechanism and health effects of some heavy metals. Journal of interdisciplinary toxicology. Vol 7(2): 60 – 72pp

[12] Odedera, O.O. and Memuletiwon, E.J. (2014). Microbial quality of some locally consumed herbal concoctions in Abeokuta metropolis, Nigeria. Journal of National Science, Engineering and Technology. 13:58 – 66

[13] Ola I.O.. Omomowo I.O., Anina D.A., Majolagbe N.O. and Oladipo E.K. (2013). Bacteriological evaluation and antimicrobial sensitivity test of common herbal concoction in ogbomoso Metropolis. *African journal of microbiology research* 7(48):5479-5483.

[14] Olmedo, P., Pedro F., Fernado, G., Antonio, H. and Antonio, P. (2010). Biomonitorization of cadmium, chromium, manganese, nickel and lead in whole blood, urine, axillary hair and saliva in an occupational exposed population.

[15] Oluyege, J.O. and Oluyege, J.O. (2010). Microbial contamination of some hawked herbal products in Ado – Ekiti, Nigeria. *Continental Journal Microbiology* 4:8-14

[16] Oluyemi J., Yinusa M.,, Abdullateef R. and Adejoke J. (2016). Utilization of herbal medicine among inhanitants of an urban centre in North – central Nieria. *Algerian journal of natural products*, 4(3): 367 – 378

[17] Osadolor, H.B., Igharo, O.G., Onuyon-Adatire, A.E., David, O.A. and Akpomiemie, V.O. (2015). Evaluation of Levels of Toxic Metals in commonly used Herbal Medicines in Bennin City, South- South, Nigeria. Annala of Biomedical Sciences 14:82-89

[18] Richard K.C. (2017). Microbial and Heavy Contaminations in selected Herbal Medicinal Products sold in Nairobi, Kenya. International Journal of Pharmaceutical Research. 8(1): 135- 138

[19] Stafford G.I, Redersen M.E., Van Stadew and Jager K. (2008). Review on plants with CNS – effects used in traditional South African medicine against mental diseases. *Journal of Ethropharmacology*. 119(3). 513 – 57pp