JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH

RESEARCH ARTICLE

Quality and Toxicity Assessment of Some Oral Liquid Herbal Formulations (*Agbo*) Consumed in Abuja, Nigeria

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ABSTRACT

Aim: To evaluate the microbiological quality and toxicity potential of commonly consumed oral herbal liquid preparations (*Agbo*) in some areas of Abuja, Nigeria.

Methods: Herbal liquid preparations (*Agbo*) were collected from different locations of the Municipal Area of Abuja, Nigeria. Samples were analyzed for elemental content using a flame atomic absorption spectrophotometer. Microbiological quality assessment was determined by suitability and sterility testing, and the contaminants were characterized to genera level. A portion of the samples (20 %) were subjected to *in vitro* hemolysis testing using mammalian (goat) blood.

Results: Minerals like chromium and manganese were found to be absent, while calcium, copper, iron, potassium, sodium, magnesium, lead (Pb), and zinc were detected at variable concentrations. Eleven percent of the samples contained Pb above WHO permissible limit. Sixty percent of the samples were found to be contaminated with microbes like *Bacillus*, *Staphylococci*, *Escherichia coli*, *Pseudomonas*, *Salmonella*, and *Proteus*, besides, fungi like *Microsporum*, *Rhizopus*, *Aspergillus*, and yeast were also found. *In vitro* hemolysis showed that half of the samples had hemolysis values greater than 50%. One of the samples had a considerably high value (>100 %) revealing the substantial extent of red blood cell lysis due to these *Agbo* preparations.

Conclusion: This study is a pointer to the potential harm that the consumption of *Agbo* can cause as it is purported to be safe and regularly consumed in liberal amounts.

KEYWORDS:
Agbo; hemolysis; herbal formulations; microbial quality; mineral content; toxicity

ARTICLE HISTORY:
Received August 04, 2020
Accepted January 03, 2021
Published April 14, 2021

DOI:
10.5455/jcmr.2021.12.01.04

VOLUME: 12
ISSUE: 1
ISSN: 2146-8397

INTRODUCTION

The use of herbal medicines has a long history. Presently, there has been increased interest in herbal medicines because of their fewer adverse effects compared to synthetic medicines. According to the World Health Organization (WHO), about 65–80% of the people living in developing countries rely majorly on plants for their primary health care. In developing countries like Ghana, Mali, Nigeria, and Zambia, the first-line treatment for about 60% of high-fever pediatric cases because of malaria is the use of herbal remedies administered at home.[1] This significant dependence on herbal medicine can be attributed to the ease of acceptability of natural and indigenous products, relative cost-effectiveness, and inaccessibility or unavailability of modern health facilities. Some developed countries like China also use herbal medicines as part of their first-line treatment for various diseases.

Despite the international diversity and adoption of herbal medicines in many countries, no international standards have been developed. .[2] This has been attributed to the inappropriateness of policies that govern the regulation of the practices, practitioners, and the products because of categorization variations of these herbal remedies.[3] The indiscriminate and unregulated use of herbal medicines could pose a health risk to the users.[4] While there is significant research data on individual medicinal plants, however, only limited data demonstrate the safety of many polyherbal formulations. Besides, practices used in harvesting, handling, storage, production, and distribution could cause microbial contamination of herbal products.[5] This has generated interest in the standardization of these phytotherapeutic agents with a specific focus on efficacy, safety, and quality.[6]

Oral liquid herbal remedies are the predominant available forms of herbal medications because of the ease with which they can be produced. In Nigeria, these oral liquid herbal formulations are popularly called *Agbo*, prepared by maceration of the plant materials in suitable solvents like water or alcohol, hand-filled into bottles, and hawked. The vendors would usually dispense portions into a cup as the "dose" required for the various common ailments treated like malaria, typhoid fever, sexually transmitted diseases, aphrodisiacs, diabetes, hypertension, weight loss remedies, etc.

Agbo is affordable, available, and superstitiously believed to be more efficacious than conventional medications.[7] 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.[8] Besides, pathogenic microorganisms or natural toxins could also contaminate these products resulting in damage to vital excretory organs like the liver and the kidney.[9,10]

Studies have shown that some commonly consumed herbal remedies are contaminated with various microorganisms and residues that are outside the official permissible limits. [11-13] The cell lysis capability of plants and their extracts has been shown in studies on the hemolytic and toxic effects of herbal preparations.[14-17] These preparations are also frequently and regularly (daily in most cases) consumed by a significant proportion of the population in many African countries. It is therefore imperative to evaluate their quality and toxicity potential. The *in vitro* method of assessing hemolytic activity is a quick and effective initial determiner of any substance's toxicity potential. The red pigmentation resulting from the lysis of red blood cells (RBCs) with consequent release of cellular contents into the surrounding fluids is an indication that the tested substance can cause extrinsic cell toxicity and cell death.[18] This involves the incorporation of the investigating sample into the suspension of erythrocytes and the determination of its ability to lyse the RBCs. The destruction of the RBC because of rupture of the membrane lipid bilayer and consequent release of the cellular components into the surrounding environment is the indicator of hemolysis and is evaluated as a marker for the safe use of such investigated materials. This selective index is a rapid tool for the toxicological assessment of such substances.[19]

This study aimed to assess the microbiological quality determine the mineral content, and hemolytic effect of commonly consumed *Agbo* circulating in the Municipal Area of Abuja, Nigeria.

MATERIALS AND METHODS

MATERIALS

Nutrient agar and nutrient broth, Sigma-Aldrich, (St. Louis, MO) tryptic soy agar, tryptic soy broth, potato dextrose agar, potato dextrose broth were procured from Sigma-Aldrich (St. Louis, MO). Standard solutions of calcium (Ca), copper (Cu), chromium (Cr), iron (Fe), potassium (K), manganese (Mn), lead (Pb), and zinc (Zn), nitric acid (HNO_3), mammalian (goat) blood, sodium dihydrogen orthophosphate was provided by BDH, chemicals Ltd. (Poole, England). Sodium citrate, sodium hydroxide pellets and, tween 80 used in this study were of analytical grades.

Hundred *Agbo* samples were purchased from hawkers around the Municipal Area of Abuja, Nigeria and were coded as A1 to A100. These samples were stored in sterile bottles and were transported to the National Institute for Pharmaceutical Research and Development (NIPRD) laboratory for further analysis.

METHOD

Determination of mineral content

Calibration standards were prepared from standard solutions of Ca, Cu, Cr, Fe, K, Mn, Pb, and Zn at 1000 µg/mL in 5% HNO_3 for the sample digestion. After which they were analyzed using the flame atomic absorption spectrophotometer (Model: GBC Avanta, version 2.0, GBC Scientific equipment, Hampshire, IL)

Table 1 Instrument operating condition for the analysis.

Element	Wavelength (nm)	Slit width	Lamp current
Ca	422.70	0.50	5.00
Cu	324.70	0.50	3.00
Cr	428.90	0.20	6.00
K	404.40	0.20	6.00
Fe	248.30	0.20	7.00
Mg	202.60	1.00	3.00
Mn	403.10	0.20	5.00
Na	589.60	0.50	5.00
Pb	217.00	1.00	5.00
Zn	213.90	0.50	5.00

based on the operating conditions mentioned in Table 1. The data obtained was processed using the Equation (1).

$$\text{Metal}(\mu g/mL) = C \times V \quad (1)$$

where C is the concentration of the sample analyte in μg/mL and V is the volume of the sample solutions in cm^3.

Suitability testing

The herbal preparations were analyzed for growth promotion by bacteriostasis and fungistasis.[20] Aliquots of the samples (1 mL) were added to 19 mL Tryptic and Saboraud agar for bacteria and fungi, respectively, and were inoculated with 100 µL of *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, Aspergillus niger*, and *Candida albicans* of 10^2 cfu/mL. The plates were incubated for three and five days for bacteria and fungi, respectively and later observed for the growth of these test organisms.

Sterility testing

The sterility testing of the samples was carried out by direct inoculation. [20] One milliliter of each sample was diluted in sterile normal saline up to 10^{-6}. About 0.5 mL each of 10^{-2}, 10^{-4}, and 10^{-6} dilutions were suspended into a sterile molten tryptic soy agar and potato dextrose agar for bacteria and fungi isolations, respectively. These plates were incubated at 37oC for 14 days. Colonies that appeared post incubation were counted on the colony counter and recorded as total colony-forming bacteria. A presumptive bacteria colony was used for bacteria identification by Gram staining and biochemical analysis (catalase, indole, coagulase, oxidase, urease, citrase, triple sugar iron, and methyl red Voges-Proskauer tests). The isolated organisms were then inoculated on differential media: mannitol salt agar, MacConkey agar, eosin methylene blue, cetrimide, and salmonella shigella agar (Sigma-Aldrich).

Hemolysis testing

A sample of the preparation (*Agbo*) was used for the hemolysis testing, as previously mentioned with some modification.[21] Goat blood (50 mL) collected from a slaughterhouse was poured into an appropriate container containing trisodium citrate (2 %) and was immediately transported to the laboratory. The blood sample was centrifuged at 3500 rpm for 10 min, the supernatant was discarded, and the sediments (erythrocytes) were rinsed with phosphate buffer solution (PBS; pH 7.4) twice by centrifuging for another 10 min. The resulting sediments (1 mL) were diluted to 50 mL PBS, and then 2 mL of the dispersion was mixed with the *Agbo* samples (1:1). These were incubated in the isotherm incubator (Thermo Fisher Scientific Co., Waltham, MA) at 37°C for 2 h. Later the incubated samples were centrifuged at 3500 rpm for 10 min and 0.2 mL of the supernatant was diluted to 3 mL with PBS and its absorbance was determined at 415 nm using an ultraviolet-visible spectrophotometer (Agilent). Preparations containing Tween 80 and PBS were used as positive and negative controls, respectively. The absorbance was used to calculate percentage hemolysis by using Equation (2):

$$H\,\% = As\text{-}An / Ap\text{-}An \quad (2)$$

As = absorbance of test sample, An = absorbance of negative control, and Ap = absorbance of positive control

RESULTS

DETERMINATION OF MINERAL CONTENT

The analysis showed that Ca, Cu, K, Fe, Mg, Na, Zn, and Pb were detected in 75, 89, 100, 96, 93, 100, and 83% of the samples, respectively (Figure 1). Cr and Mn were not detected in any of the samples (Table 2).

MICROBIOLOGICAL QUALITY ASSESSMENT

Four of the herbal preparations contained inhibitory substances as they did not support the growth and reproduction of test organisms. The substances were neutralized/inactivated by the serial dilution technique.

The sterility testing of different herbal products analyzed by microbial load assay showed that 42 (61 %) of the products were contaminated with different bacteria species (Table 3). Total bacterial count in contaminated products ranged from 1.20×10^2 to 2.26×10^4. Bacterial colonies isolated and identified by colony morphology, growth description on differential media, and biochemical characteristics are presented in Table 3. Organisms isolated in different products were identified as *Bacillus spp, Staphylococcus spp, Klebsiella spp, Salmonella, E. coli*, and *Proteus*. Seventeen samples (25 %) were contaminated with different fungal and yeast species. The distribution and percentage of occurrence of the isolated organisms are shown in Figure 2.

IN VITRO *HEMOLYSIS*

Hemolysis (%) calculated from of the tested *Agbo* samples are shown in Table 4 and Figure 3. Blood cell lysis was found to be between 33 and 130% across the tested samples.

Table 2 Elemental composition of the liquid herbal products (*Agbo*).

Sample Codes	Mean concentration of mineral elements (µg/mL)									
	Ca	Cu	Cr	K	Fe	Mg	Mn	Na	Pb	Zn
A1	ND	0.58	ND	13.64	4.95	42.03	ND	14.43	3.55	1.80
A2	40.53	0.23	ND	23.92	3.04	88.06	ND	8.76	ND	0.45
A3	36.11	0.61	ND	20.08	1.81	5.72	ND	15.06	7.13	0.23
A4	24.86	0.55	ND	18.11	1.92	58.59	ND	14.70	7.13	ND
A5	17.11	0.64	ND	29.82	3.43	22.20	ND	7.23	ND	ND
A6	57.28	0.63	ND	15.30	ND	33.27	ND	12.35	ND	0.03
A7	4.36	0.58	ND	17.11	ND	ND	ND	14.39	ND	0.60
A8	60.18	ND	ND	34.77	1.71	40.26	ND	7.81	ND	0.12
A9	15.95	0.62	ND	18.34	2.40	26.76	ND	12.21	ND	0.27
A10	ND	0.79	ND	25.96	2.83	49.23	ND	7.34	ND	0.73
A11	ND	0.31	ND	3.79	1.17	21.83	ND	5.65	ND	ND
A12	ND	0.75	ND	18.95	2.75	ND	ND	14.41	7.13	0.86
A13	6.23	2.48	ND	18.66	12.14	23.96	ND	11.74	ND	0.99
A14	8.00	0.19	ND	16.17	1.74	52.97	ND	10.19	ND	0.57
A15	ND	1.32	ND	17.41	5.32	7.05	ND	9.80	ND	1.21
A16	36.80	ND	ND	12.86	3.82	2.97	ND	27.86	ND	0.25
A17	13.93	0.78	ND	2.22	6.98	47.31	ND	16.87	ND	0.72
A18	0.55	0.22	ND	19.51	5.00	120.43	ND	13.72	3.55	1.90
A19	ND	ND	ND	31.95	2.97	23.23	ND	11.70	ND	ND
A20	15.80	0.51	ND	20.41	9.16	41.34	ND	9.93	ND	1.90
A21	ND	0.44	ND	19.16	3.94	45.21	ND	2.99	ND	0.29
A22	47.16	0.76	ND	15.49	2.01	3.88	ND	31.21	ND	ND
A23	10.73	0.11	ND	15.99	2.51	65.21	ND	7.43	ND	ND
A24	21.62	0.73	ND	20.08	20.93	121.90	ND	8.33	ND	1.00
A25	35.21	0.59	ND	32.95	1.70	27.22	ND	28.42	ND	0.43
A26	17.13	0.55	ND	12.34	15.36	50.44	ND	17.88	ND	0.91
A27	19.86	0.82	ND	9.76	1.05	32.41	ND	13.90	7.13	ND
A28	18.50	0.63	ND	20.84	13.42	102.35	ND	13.36	ND	5.00
A29	24.63	0.60	ND	17.21	2.25	62.81	ND	12.26	ND	0.02
A30	8.52	0.74	ND	19.03	4.24	44.53	ND	13.81	7.13	0.25
A31	36.36	0.62	ND	19.44	3.69	77.38	ND	14.41	ND	0.65
A32	35.26	0.64	ND	41.59	5.02	80.21	ND	21.50	3.55	0.97
A33	ND	0.53	ND	23.58	4.93	50.44	ND	14.71	ND	2.65
A34	8.28	0.96	ND	19.90	2.04	46.07	ND	8.48	ND	2.19
A35	33.95	0.67	ND	47.26	0.25	30.08	ND	8.12	3.55	0.30
A36	ND	0.80	ND	39.39	2.31	2.34	ND	8.38	ND	ND
A37	ND	2.23	ND	14.39	5.44	55.18	ND	9.37	ND	4.67
A38	9.35	1.32	ND	22.12	20.27	67.68	ND	30.55	7.13	2.07
A39	62.66	0.58	ND	9.13	2.49	69.55	ND	4.57	ND	0.07
A40	4.05	0.48	ND	15.51	2.90	80.87	ND	12.49	ND	1.29

Sample Codes	Mean concentration of mineral elements (µg/mL)									
	Ca	Cu	Cr	K	Fe	Mg	Mn	Na	Pb	Zn
A41	47.53	ND	ND	17.50	5.18	40.51	ND	7.34	ND	0.66
A42	ND	0.39	ND	7.03	0.05	49.93	ND	12.69	ND	ND
A43	28.98	0.59	ND	16.99	4.97	22.91	ND	1.43	ND	0.50
A44	8.94	1.08	ND	21.20	7.75	59.86	ND	28.87	ND	1.23
A45	12.26	0.92	ND	19.52	8.73	25.22	ND	14.98	3.55	0.80
A46	4.78	0.67	ND	13.85	32.17	9.12	ND	13.09	3.55	8.29
A47	6.65	ND	ND	18.82	5.31	59.27	ND	19.14	ND	0.44
A48	2.51	0.73	ND	17.00	6.24	9.60	ND	11.68	ND	0.45
A49	21.50	ND	ND	43.75	1.90	ND	ND	7.38	7.13	0.46
A50	23.70	0.73	ND	11.92	1.17	28.60	ND	6.09	ND	0.15
A51	6.86	0.48	ND	20.21	5.20	24.13	ND	12.52	ND	0.44
A52	1.13	0.50	ND	19.93	2.37	105.94	ND	14.06	10.69	0.18
A53	46.98	0.52	ND	15.95	2.55	14.66	ND	15.91	ND	0.31
A54	36.26	0.27	ND	20.38	5.89	77.07	ND	30.28	ND	1.47
A55	ND	0.57	ND	32.07	ND	35.78	ND	0.06	ND	ND
A56	36.38	0.70	ND	15.57	3.92	62.98	ND	2.63	ND	ND
A57	6.65	0.59	ND	7.16	ND	48.49	ND	11.92	ND	0.52
A58	ND	0.76	ND	35.18	3.90	43.09	ND	5.64	ND	0.30
A59	15.38	1.06	ND	20.39	15.93	119.67	ND	10.00	ND	1.29
A60	32.73	0.49	ND	10.73	2.47	77.33	ND	8.49	3.55	0.16
A61	18.63	ND	ND	6.59	4.72	87.34	ND	8.10	ND	ND
A62	24.15	0.40	ND	13.06	2.98	ND	ND	9.71	ND	ND
A63	9.35	0.70	ND	19.09	7.14	74.63	ND	13.74	ND	0.42
A64	47.88	0.61	ND	10.31	9.61	0.05	ND	29.54	ND	0.59
A65	18.00	0.64	ND	13.80	5.84	16.15	ND	5.35	ND	1.24
A66	25.61	0.71	ND	11.79	1.40	49.32	ND	10.13	ND	0.11
A67	ND	0.75	ND	5.392	4.78	75.96	ND	8.90	ND	0.52
A68	4.98	0.91	ND	20.31	8.16	29.08	ND	12.98	ND	0.81
A69	13.72	0.79	ND	20.31	19.08	ND	ND	11.21	ND	0.66
A70	19.31	0.15	ND	16.10	2.41	61.09	ND	29.93	ND	0.14
A71	ND	0.34	ND	20.08	2.99	98.69	ND	14.69	7.13	0.84
A72	33.83	0.75	ND	14.11	6.02	54.54	ND	9.18	ND	8.02
A73	45.41	0.55	ND	6.56	8.78	12.39	ND	13.86	3.55	ND
A74	7.63	0.45	ND	5.48	8.62	55.25	ND	15.02	3.55	0.45
A75	ND	ND	ND	42.42	2.90	54.05	ND	14.13	ND	ND
A76	ND	0.65	ND	24.09	2.96	3.64	ND	0.33	ND	0.29
A77	ND	0.34	ND	20.50	4.38	111.60	ND	11.05	ND	5.65
A78	10.81	0.86	ND	19.83	4.69	26.65	ND	25.48	7.13	0.47
A79	0.73	0.60	ND	14.56	2.62	22.81	ND	31.59	ND	ND
A80	4.36	0.82	ND	17.81	5.65	1.65	ND	30.41	3.55	1.42
A81	20.99	0.67	ND	20.19	8.83	67.07	ND	8.87	ND	0.86

(*Continued*)

Table 2 Continued

Sample Codes	Mean concentration of mineral elements (µg/mL)									
	Ca	Cu	Cr	K	Fe	Mg	Mn	Na	Pb	Zn
A82	ND	ND	ND	8.51	3.83	27.03	ND	5.38	10.69	0.32
A83	54.10	0.63	ND	0.54	4.92	45.57	ND	13.15	ND	1.57
A84	32.66	0.51	ND	11.87	0.14	58.73	ND	26.59	ND	0.20
A85	10.35	1.09	ND	18.98	3.73	62.79	ND	11.02	ND	1.12
A86	ND	0.66	ND	7.46	5.15	1.88	ND	7.47	ND	0.82
A87	ND	0.75	ND	4.24	0.73	ND	ND	21.61	ND	0.14
A88	ND	ND	ND	3.94	0.55	111.60	ND	8.24	ND	2.38
A89	ND	0.32	ND	17.72	1.87	76.43	ND	15.29	ND	1.08
A90	4.98	0.61	ND	15.48	3.08	45.56	ND	11.53	ND	0.11
A91	ND	0.46	ND	18.49	2.83	22.57	ND	7.44	ND	0.43
A92	ND	1.02	ND	8.53	4.02	1.915	ND	15.84	ND	1.06
A93	8.86	0.64	ND	20.16	0.05	6.44	ND	9.99	ND	ND
A94	7.98	0.88	ND	18.37	3.66	32.88	ND	8.91	ND	1.29
A95	43.03	0.55	ND	13.50	3.28	28.73	ND	1378.14	ND	0.82
A96	29.53	1.00	ND	19.07	17.73	12.29	ND	15.79	ND	1.69
A97	30.47	0.94	ND	31.64	0.46	67.54	ND	7.70	7.13	0.14
A98	50.91	1.11	ND	20.05	4.80	108.22	ND	6.25	ND	0.08
A99	31.90	0.56	ND	8.54	5.07	24.50	ND	11.06	ND	0.95
A100	ND	0.51	ND	17.34	1.93	32.30	ND	14.67	ND	0.03

ND, not detected.

DISCUSSION

DETERMINATION OF MINERAL CONTENT

Cr and Mn were absent in all the samples, while Ca, Cu, K, Fe, Mg, Pb, Na, and Zn were detected in various concentrations. Eleven percent of the sample contained Pb above WHO permissible limit (10µg/ml) which is lower than what was reported in an earlier solid herbal preparation (33%).[22] Contamination with Pb was linked to the plant raw materials or water used in processing. Pb toxicity results in several health challenges ranging from colic, anemia, headache, convulsions, chronic nephritis of the kidneys, brain damage and central nervous system disorders. Other elements (Cu, Zn, Ca, K, Na, Fe, Mg) were found to be below WHO permissible limits.[23,24]

MICROBIOLOGICAL QUALITY ASSESSMENT

The herbal medicinal products were found to be grossly contaminated by aerobic spore-forming bacteria, fungi, and pathogenic-and indicator bacteria such as *E. coli*, *Proteus*, and *Salmonella* species (Table 3). A total of 42/69(61 %) herbal products were contaminated with bacteria of eight genera, namely *Bacillus*, *Staphylococci*, *Klebsiella*, *E. coli*, *Salmonella*, *Pseudomonas*, and *Proteus* species. *Bacillus spp*. was found in 69 % of the samples (Figure 2) and was the most common bacteria contaminant with others at varying levels of occurrence. The mean bacteria level of the herbal products was found to be above the WHO recommended limit for aerobic spore-forming bacteria like *Bacillus*.[25]

Seventeen of the 69 (20 %) herbal products were contaminated by different fungi. The colonial and microscopic characteristics of fungi isolated from the herbal remedies are as presented in Table 3 and identified on Sabouraud dextrose agar as *Aspergillus*, *Rhizopus*, *Microsporum*, *Candida*, and *Saccharomyces* species. A previous study showed that some of the fungi isolated from these herbal products could be from the soil, raw materials, water, equipment, and the environment.[26] Indicator organisms like *E. coli*, *Salmonella*, *Pseudomonas*, and *Proteus* are generally known to multiply in potable, distilled, and deionized water and serve as an index of possible human pathogen contamination.[11,27] The Fungi species produce potent mycotoxins that have been implicated in carcinogenicity, dermatitis, hepatotoxicity, and nephrotoxicity.[28, 29]

Fourteen of the tested samples were not contaminated by bacteria or fungi. Antibacterial susceptibility screening showed that 12 of these samples had antibacterial effect against *S. aureus, E. coli, P. aeruginosa, S. typhi*, and *Streptococcus pyrogenes* at 50% aqueous concentration. Majority of the herbal products analyzed were contaminated with bacteria and fungi species, and samples with no contaminants were possibly extracted with alcohol which could be responsible for the inhibition of microbial growth elicited by the tested samples.

Table 3 Number and types of bacteria and fungi present in *Agbo* samples.

Sample code	Types of bacteria	Bacteria colony count	Types of fungi
A1	*Bacillus spp.*	2.20×10^{2}	–
A2	*Bacillus spp.*	5.20×10^{2}	–
A3	*Bacillus* and *Staphylococcus spp.*	6.00×10^{2}	–
A4	*Bacillus spp.*	4.80×10^{2}	–
A5	*Bacillus* and *Salmonella spp.*	2.30×10^{3}	–
A6	*Bacillus* and *Salmonella spp.*	1.40×10^{2}	*Aspergillus niger spp.*
A7	*Bacillus spp.*	9.00×10^{2}	–
A8	*Bacillus spp.*	2.60×10^{2}	–
A9	*Bacillus, Klebsiella,* and *other coliforms*	1.08×10^{4}	*Rhizopus oryzae*
A10	*Bacillus spp.*	2.60×10^{4}	*Aspergillus spp.*
A11	*Bacillus spp.*	1.20×10^{2}	–
A12	*Escherichia coli* and *Klebsiella spp.*	3.60×10^{3}	*Microsporum gypseum, R. oryzae*, and *A. fumigatus*
A13	*Bacillus spp.*	2.00×10^{2}	*Aspergillus spp.*
A14	*Bacillus, Klebsiella,* and *Salmonella spp*	3.82×10^{3}	*A. flavus*
A15	*Bacillus* and *Salmonella spp.*	NG	*R. Oryzae, Microsporum spp*, and *A. flavus*
A16	NG	2.20×10^{3}	–
A17	NG	2.26×10^{4}	*R. oryzae*
A18	NG	NG	–
A19	*Bacillus spp*	8.00×10^{2}	–
A20	NG	NG	–
A21	NG	NIL	–
A22	*Bacillus subtilis* and *Klebsiella* and *Staphylococcus spp.*	NG	–
A23	NG	7.00×10^{2}	–
A24	*Bacillus spp*	NG	–
A25	*Bacillus spp*	NG	–
A26	NG	NG	–
A27	*Bacillus spp*	3.00×10^{4}	–
A28	*Bacillus spp*	Uncountable	–
A29	*Bacillus spp*	8.00×10^{2}	–
A30	*Bacillus spp*	$2.90\times1\ 0^{3}$	–
A31	*Bacillus spp*	2.00×10^{2}	–
A32	NG	NG	–
A33	*B. subtilis* and *Staphylococcus spp Pseudomonas*	4.00×10^{3}	*Candida albicans*
A34	NG	NG	–
A35	*Bacillus, Klebsiella, Salmonella, Proteus, Staphylococcus*, and *Pseudomonas spp*	Uncountable	*Microsporum spp and A. niger*
A36	*Bacillus, Salmonella,* and *Proteus spp.*	1.10×10^{3}	–
A37	*B. subtilis* and others *Bacillus spp.*	Uncountable	–
A38	*B. subtilis*	6.00×10^{2}	–
A39	*B. subtilis* and other *bacillus spp.*	Uncountable	–

(*Continued*)

Table 2 Continued

Sample code	Types of bacteria	Bacteria colony count	Types of fungi
A40	*B. subtilis*	1.00×10^{3}	–
A41	NG	NG	–
A42	*B. subtilis*	NG	–
A43	*B. subtilis*	4.00×10^{2}	–
A44	*B. subtilis* and other *bacillus spp.*	2.305×10^{4}	–
A45	*B. subtilis, Salmonella, Klebsiella* and *E. coli*	Uncountable	–
A46	*Bacillus spp.* and *Klebsiella.*	Uncountable	–
A47	*Bacillus spp.*	4.00×10^{2}	–
A48	NG	NG	*Saccharomyces and C. albicans*
A49	*B. subtilis* and *Proteus.*	Uncountable	*R. oryzae*
A50	*B. subtilis*	2.60×10^{3}	*Saccharomyces*
A51	*B. subtilis*	2.46×10^{4}	–
A52	*B. subtilis*	4.00×10^{2}	–
A53	*B. subtilis*	2.60×10^{3}	–
A54	NG	NG	–
A55	*B. subtilis*	1.60×10^{3}	–
A56	NG	NG	–
A57	NG	NG	–
A58	*B. subtilis*	5.00×10^{2}	–
A59	NG	NG	–
A60	NG	NG	–
A61	NG	NG	*C. albicans*
A62	*B. subtilis*	2.00×10^{2}	–
A63	*B. subtilis*	9.00×10^{2}	–
A64	*B. subtilis and E. coli*	Uncountable	–
A65	*B. subtilis*	4.10×10^{3}	–
A66	*B. subtilis*	4.60×10^{3}	–
A67	*B. subtilis* and *E. coli*	1.90×10^{3}	*C. albicans*
A68	*B. subtilis* and other *bacillus spp.*	3.47×10^{4}	–
A69	*B. subtilis*	2.00×10^{3}	–

NG, no growth.

EVALUATION OF IN VITRO *HEMOLYSIS*

In this study, absorbance values were used to demonstrate the degree of cell rupture in the incubated suspension. Tween 80 and PBS were used as the positive and negative response indicators to show the extent of cell rupture in the absence of the *Agbo* samples.

The results showed varying absorbance values ranging from 0.076 to 2.479 for the tested samples (Table 4). All the herbal products caused cell lysis with absorbance values higher than that of the negative control (0.076). One of the samples had an absorbance value (2.479) higher than that of the positive control (1.881), indicating a substantial level of cell lysis, and four of the samples had values (1.094 to 1.234) close to that of the positive control. These outcomes shows that all the herbal formulations tested caused cell rupture but to varying degrees, which is a cause for concern, as there is a high possibility that these samples will cause toxicity, including hemolysis, when ingested.

Hemolysis of all the samples was between 33 and 131%, with about half of them causing more than 50 % hemolysis (Figure 3). Sample A3 was found to have considerably high potential for toxicity based on the degree of hemolysis (130.08 %).

Plants contain many constituents, and some of them possess properties that induce hemolysis of RBCs in humans. This effect is attributed to the disruption of the RBC membranes and idiosyncratic fragility of the cell membrane which could

Table 4 Absorbance and hemolysis of *Agbo* samples.

Sample	Absorbance	Hemolysis (%)
A1	0.312 ± 0.00	80.16
A2	0.233 ± 0.04	84.23
A3	2.479 ± 0.00	130.56
A4	0.230 ± 0.09	84.36
A5	0.168 ± 0.03	87.55
A6	0.529 ± 0.20	69.05
A7	0.769 ± 0.15	56.82
A8	1.234 ± 0.26	33.04
A9	0.952 ± 0.14	47.44
A10	1.094 ± 0.17	40.23
A11	1.170 ± 0.14	36.33
A12	1.217 ± 0.27	33.95
A13	0.965 ± 0.11	46.79
A14	0.912 ± 0.16	49.51
PBS	0.076 ± 0.00	–
Tween 80	1.881 ± 0.00	–

lead to hemolytic anemia.[30,31] Hemoglobin released during the process of hemolysis produces vasoactive and redox-active proteins that are toxic to the vascular, myocardial, central nervous tissues, and these eventually induce anemia with other diseases.[32]

A relationship has been reported between the chronic consumption of onion and hemolytic anemia observed in rats, and dogs and was attributed to the ability of some of their constituents to damage the cell membrane. [33-35] Benjamin et al.[36] showed that incubation of human RBCs in saline tea extracts caused alterations in the blood cell membrane. This was proved by the report of a young adult whose hemolytic profile decreased after chronic consumption of the herbal tea. Consumption of large doses of the leaf extract of *Gingko biloba* has been found to cause cell fragility and destroy RBCs. Another study showed that daily consumption of a weight-loss product containing green leaf extract caused a substantial decrease in platelet count.[37,38] The effect of sub-chronic consumption of a herbal formulation has been shown to cause anemia in rats, while copious administration of the aqueous extract of *Viola tricolor* resulted in glucose-6-phosphate-dehydrogenase deficiency.[39,40]

CONCLUSION

This study of oral liquid herbal formulations marketed in Abuja municipal areas showed the presence of a toxic element (Pb) in concentrations above the official permissible level. Most of the herbal products were contaminated with bacteria and fungi species. All the *Agbo* samples were toxic to mammalian (goat) blood cells, causing hemolysis. With one having remarkably high hemolysis (130.08 %), an indication of a high potential toxicity. This article underscores the need for caution in the consumption of *Agbo* and increased regulatory activity to safe guard the user's health.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Pharmaceutical Technology and Raw Materials Development, Department of Microbiology and Biotechnology, and Department of Medicinal Chemistry and Quality Control of the NIPRD for their technical support.

FUNDING

This was a self-funded study.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the author.

REFERENCES

1 World Health Organization (WHO). The Africa malaria report. WHO/CDS/MAL/2003 [Internet]. 2003. Available from: https://apps.who.int/iris/handle/10665/67869

2 Abt AB, Oh JY, Huntington RA, Burkhart KK. Chinese herbal medicine induced acute renal failure. Arch Intern Med. 1995 Jan 23;155(2):211-2.

3 Oshikoya KA, Njokanma OF, Chukwura HA, Ojo OI. Adverse drug reactions in Nigerian children. Paediatr Perinat Epidemiol. 2007;8:81-8.

4 Nnorom IC, Osibanjo O, Eleke C. Evaluation of human exposureto lead and cadmium from some local Nigerian medicinal preparations. J Appl. Sci. 2006;6:2907-11.

5 Roy AK, Kumari S. Complementary medicines in Europe. BMJ.1999; 309:107-11.

6 Karadeniz C, Pinarli FG, Oğuz A, Gürsel T, Canter B. Complementary alternative medicine use in a paediatric oncology unit in Turkey. Pediatr Blood Cancer.2007;48:540-3.

7 Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz J Med Biol Res.2000; 33:179-89.

8 Phillips S, Balge M. Heavy metal toxicity. Texas (SA): New Fields; 2007. 1-30 pp.

9 Abou-Arab AAK, Kawther MS, El Tantawy EM, Badeaa RI, Khayria N. Quantity estimation of some contamination in commonly used medicinal plants in the Egyptian market. Food Chem. 1999; 67:357-63. http://doi.org/10.1016/S0308-8146(99)00082-5

10 Kaplowitz N. Hepatotoxicity of herbal remedies: Insight into the intricacies of plant-animal warfare and cell death. Gastroenterology.1997;113;1408-12. https://doi.org/10.1053/gast.1997.v113.agast971131408.

11 Esimone CO, Chah KF, Ikejide SC. Microbiological quality of herbal preparations marketed in South East Nigeria. J Nat Remedies. 2002; 2: 42-48.

12 Agbulu CO, Ameh EA, Ocharifu SA. Microbiological quality of cough syrups and herbal solutions, 'Agbo' sold in Makurdi metropolis of Benue State, Nigeria. J Microbiol Microb Technol. 2016;1:6. http://doi.org/10.13188/2474-4530.1000001

13 Nwankwo CC, Olima T. Microbial quality of herbal preparationssold in some parts of Nigeria. GSCBPS. 2019; 6(3):76-84.

14 Uwaifo AO. The mutagenicity of seven coumarin derivatives and a furan derivative isolated from three medicinal plants. J Toxicol Environ Health. 1983;13: 521–30. http://doi.org/10.1080/15287398409530517.

15 Sharma P, Sharma JD. Evaluation of *in vitro* schizontocidal activity of plant parts of *Calotropis procera*–an ethnobotanical approach. J Ethnopharmacol. 1999;23:42. https://doi.org/10.1016/S0378-8741(99)00052-5.

16 Omonhinmin AC, Dike IP, Rotimi SO. Phytochemical, cytotoxicity and antioxidant activities of five anti-malaria plants. Res J Med Plant. 2015;9:81–89. http://doiorg/10.3923/rjmp.2015.81.89.

17 Joujeh D, Lahdo R, Gherwaty A. Evaluation of hemolytic and antihemolytic activity of the aerial parts of *Sonchous oleraceus* extracts. Int J Pharm Sci Nanotechnol. 2017;10: 3745–51.

18 Lemery L. Oh, no! It's hemolyzed! What, why, who, how. Advance for Medical Laboratory Professionals, Feb 15. 1998; 24–25.

19 Gandhi VM, Cherian KM. Red cell haemolysis test as an in vitro approach for the assessment of toxicity of Karanja oil. *Toxicology In Vitro*. 2000;14(6): 513–6.

20 United States Pharmacopoeia (USP). 1990, 22 ed. 1684-1685.

21 Nair S, Xu C, Shen G, Hebbar V, Gopalakrishnan A, Hu R, et al. Toxicogenomics of endoplasmic reticulum stress inducer tunicamycin in the small intestine and liver of Nrf2 knockout and C57BL/6 J mice. Toxicol Lett. 2007; 168: 21–39. http://doi.org/10.1016/j.toxlet.2006.10.012.

22 Samali A, Mohammed M, Ibrahim MB, Gamaniel KS. Metal content determination of some sexual dysfunction medicine in Northern Nigeria. BAJOPAS. 2017;10(1): 234–8.

23 Alemika TE, Ojerinde OS, Samali A, Mustapha KB, Gamaniel KS. Nutriceutical potential of Nigerian grown *Citrullus lanatus* (watermelon) seed. J Pharm. 2017; 14(2): 253–9. http://doi.org/10.4314/jpb.v14i2.20

24 World Health Organization/Food Agricultural Organization (WHO/FAO, 2001). WHO/FAO food addictive data system. Evaluation by the Joint WHO/FAO Expert Committee on Food Additives 1956–1984. FAO Food and Nutrition Paper 30/Review, Rome. pp. 2–51.

25 World Health Organization (WHO). Regulatory situation of herbal medicine: A worldwide review. Geneva:WHO; 1998.

26 Prescott LM, Harley JP, Klein DA. Isolation of pure bacterialculture from specimen: Microbiology international. 4th ed. Boston: WCB McGraw's Hill Companies; 1997.714–96 pp.

27 Frazier WF, Westhoff PC. Food microbiology. 3rd ed. New Delhi, India: Tata McGraw Hill Publishing Co-Limited; 1978.17–64, 456 pp.

28 Pelczar MJ, Chan ECS. Laboratory Exercise in Microbiology. New York, USA: Bank Pot. Inc; 1977.

29 De Freitas MV, Netto RC, Da Costa Huss JC, De Souza TM, Costa JO, Firmino CB, et al. Influence of aqueous crude extracts of medicinal plants on the osmotic stability of human erythrocytes. Toxicol In Vitro. 2008; 22:219–24. http://doi.org/10.1016/j. tiv.2007.07.010.

30 Manthey JA, Grohmann K, Guthrie N. Biological properties of citrus flavonoids pertaining to cancer and inflammation. Curr Med Chem. 2001; 8 (2): 135–53. http://doi.org/10.2174/0929867013373723.

31 Buehler PW, D'Agnillo F. Toxicological consequences of extracellular hemoglobin: Biochemical and physiological perspectives. Antioxid Redox Signal. 2010; 12: 275–91.

32 Salami HA, John AI, Ekanem AU. The effect of aqueous preparation of *Allium Cepa* (onion) and *Allium Sativa* (garlic) on erythrocyte osmotic fragility in wistar rats: *in vivo* and *in vitro* studies. Niger J Physiol Sci. 2012;27: 29–34. http://doi.org/10.11648/j.ajls.20140205.15.

33 Umar IA, Omage JJ, Lawal JL, Igbokwe IO, Mode S. Prevention of beer-induced hyperlipaemia by the essential oil of garlic (*Allium Sativum* Linn). Biosci Biotechnol Res Commun. 1996; 8: 273–7.

34 Yamamoto D, Maede Y. Susceptibility of onion-induced haemolysis in dogs with hereditary high erythrocyte reduced glutathione and potassium concentration. Am J Vet Res. 1992; 53:134–7.

35 Benjamin LJ, Goldstein BD, Distenfeld A, Troll W. Production paroxysmal nocturnal hemoglobinuria-like red blood cells by tea. Am J Hematol. 1977; 2: 245–9.

36 He J, Lin J, Li J, Zhang J, Sun X, Zeng C. Dual effects of *Ginkgo biloba* leaf extract on human red blood cells. Basic Clin Pharmacol Toxicol. 2009; 104(2):138–44.

37 Liatsos GD, Moulakakis A, Ketikoglou I, Klonari S. Possible green tea-induced thrombotic thrombocytopenic purpura. Am J Health-Syst Pharm. 2010;67:531–4. http://doi.org/10.2146/ ajhp080673.

38 Kingsley CPI, Kpobari WN. Toxicity effect of sub-chronic oral administration of class bitters® - a polyherbal formula on serum electrolytes and hematological indices in male Wistar albino rats. J Xenobiot. 2015; 5: 20–3. https://doi.org/10.4081/xeno.2015.5369

39 Kheir A, Gaber I, Gafer S, Ahmed W. Life-threatening haemolysis induced by henna in a Sudanese child with glucose-6-phosphate dehydrogenase deficiency. East Mediterr Health J. 2017;23(1): 28–30.

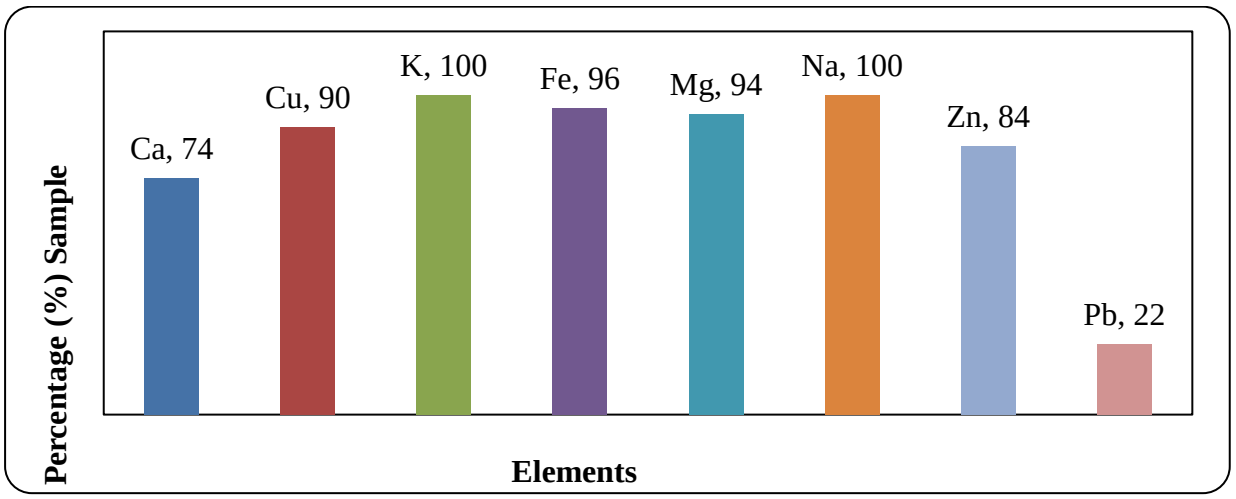

Fig.1: Distribution of mineral elements in Agbo samples

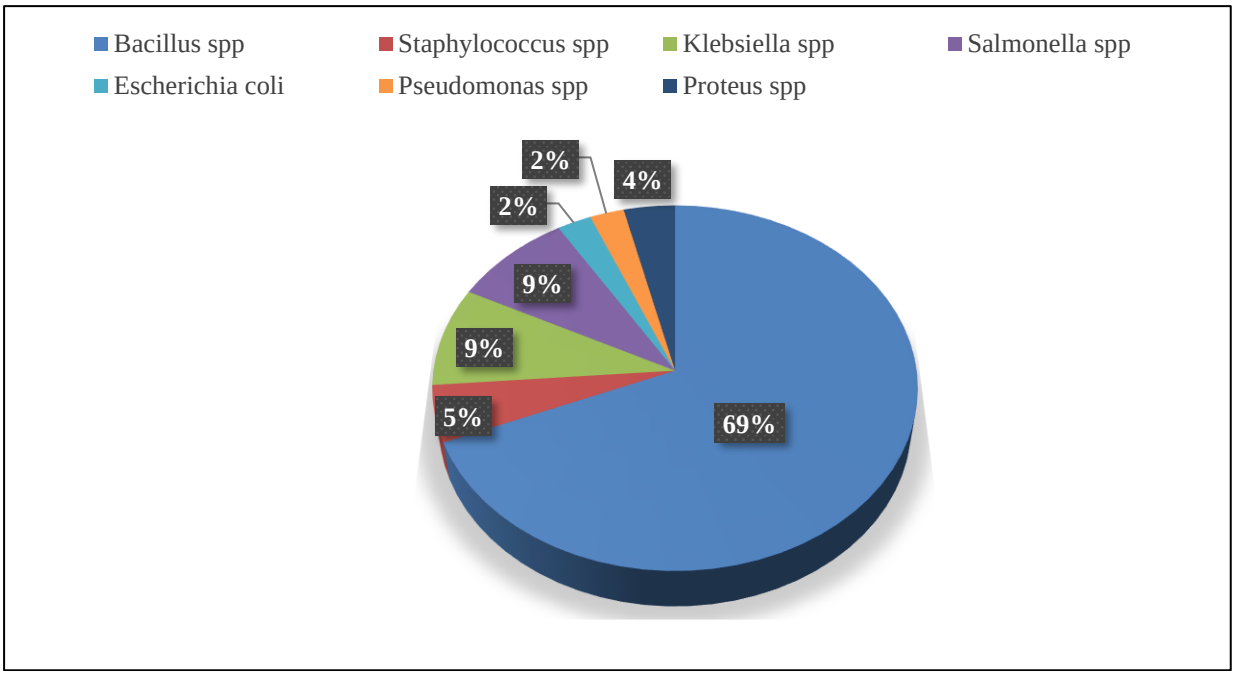

Fig.2: Distribution of bacteria contaminants in tested samples

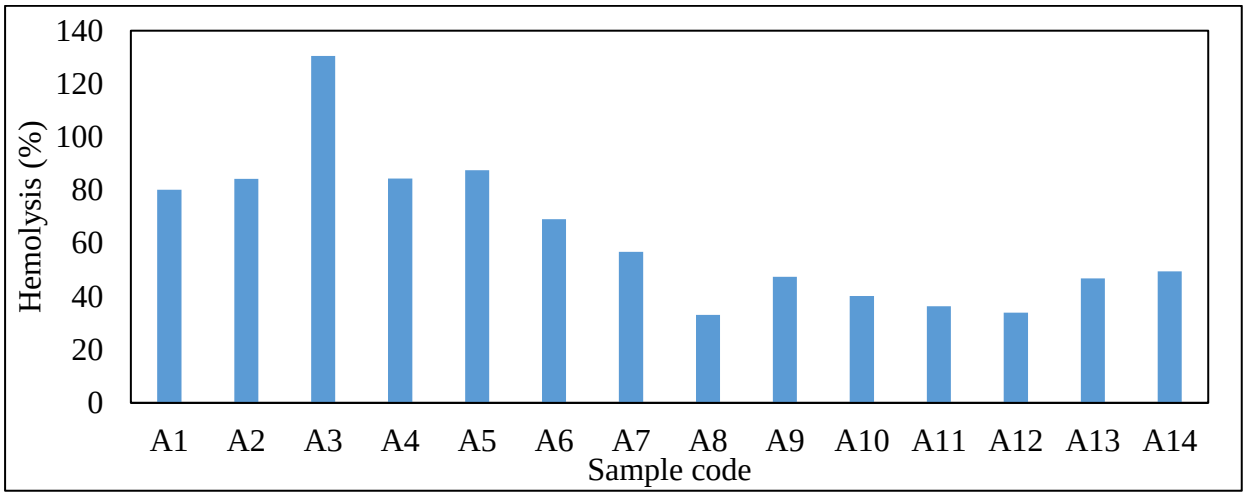

Fig.3: Hemolysis (%) of Agbo samples