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Microbial content and elemental analysis of some locally prepared herbal remedies (*Agbo*) in Ibadan metropolis

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Abstract

This study assessed the safety of two herbal remedies, *Agbo Iba* and *Agbo Jedi* commonly sold in Ibadan metropolis. The samples were analyzed for phytochemical composition, microbial contamination, pH level, and elemental content. Phytochemical screening identified alkaloids, cardiac glycosides, saponins, steroids, tannins and glycosides. Elemental analysis revealed sodium concentration ranging from 186.67 ± 1.7 to 283.33 ± 4.41 mg/100 g in *Agbo Iba* and *Agbo Jedi*, respectively. Lead content ranged from 0.04 ± 0.01 to 0.10 ± 0.00 mg/100 g in *Agbo Iba* and 0.03 ± 0.00 to 0.05 ± 0.00 mg/100 g in *Agbo Jedi*, while cadmium content ranged from 0.01 ± 0.00 to 0.02 ± 0.00 mg/100 g in *Agbo Iba*, it was absent in *Agbo Jedi*. The pH of all samples was acidic ranging from 2.79 to 4.41. Bacterial counts in *Agbo Iba* and *Agbo Jedi* exceeded European pharmacopoeia contamination limits ranging from 2×10^8 to 88×10^8 cfu/mL and 2×10^8 to 126×10^8 cfu/mL respectively. These findings highlight potential health hazards associated with these herbal remedies despite their rich phytochemical content. Stringent quality assurance and standardization by government agencies are essential to ensure safety and efficacy of herbal remedies for public use.

Keywords: Phytochemical analysis; Elemental analysis; Microbial contamination; pH level; Herbal remedies

INTRODUCTION

Humans have used herbal medicine as a source of health care needs for thousands of years. An 80% estimation of the people living in developing countries rely on herbal medicinal products (HMPs) for their primary health care requirements (WHO, 2002) implying a huge reliance on herbal medicine in these populations. In sub-Saharan Africa, it has been reported that there is one traditional healer for every 500 individuals, but only one medical doctor is available for every 40,000 people [1]. The continuous usage of herbal medicines persists for several compelling reasons. These include easy accessibility, cultural approval and affordability, and in some cases the exorbitant costs of conventional drugs [2]. Also, orthodox medicines often fall short in providing cure for some diseases and infections (e.g. HIV/AIDS) prompting individuals to seek alternative therapies [3]. Additionally, the alarming rise of antibiotic resistance [4] has spurred interest in herbal remedies as they are perceived as

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having a lower propensity to induce resistance. These factors highlight the continued interest in the use of herbal medicines as a viable alternative or complementary approach in healthcare. Medicinal plants are known to contain substances that aid primarily in the reproduction, growth, disease prevention, health promotion and efficient functioning of living organisms [5, 6]. Elemental analysis has revealed that many plant families contain substantial amounts of macro- and microelements as well as phytochemicals. These naturally occurring biologically active constituents confer health benefits to human [7].

Herbal remedies are generally considered safe unless a significant risk in humans has been established [8, 9]. However, there are a number of reported cases of adverse reactions in the usage of herbal remedies [10]. The increase in worldwide use has also raised worries and fears among various national and international health agencies and the general public regarding the quality, efficacy, and safety of herbal remedies available in the market [11-13]. These herbal remedies could be contaminated with heavy metals, chemical toxins, adulterated with undeclared allopathic contents [14] as well as other particulate contaminants [15, 16].

The toxic effects resulting from the use of low-quality herbal remedies vary from nausea to diverse complications affecting the gastrointestinal, hematological, cardiovascular and neurological systems which can ultimately result to death. The severity of these effects is contingent on the quantity and duration of use of the herbal remedies [17, 18]. The presence of toxic metals as impurities is determined by the nature of the sample as well as the contaminants or residues. The permissible limits for toxic metals and microbial contamination in several types of herbal products differ across countries and depends on factors such as the specific type of herb whether it's in its raw form or a finished product [19, 20]. Therefore, to protect public health, screening, confirmation and regulation of traditional remedies for efficacy and safety has been encouraged [10, 19].

Agbo Iba and *Agbo Jedi* are Yoruba terms that refers to a blend of various medicinal herbs commonly prepared by local herb sellers in South-Western part of Nigeria. These two herbal remedies are usually sold in liquid form, packaged in bottles or dispensed in small polythene bags. The preparation involves careful selection of various medicinal herbs and plant materials which are then subjected to a process of extraction typically by boiling or steeping in water resulting in concentrated herbal decoction or infusion. They are used orally, either in measured doses or as directed by the vendor or herbal practitioner. Depending on the specific concoction and intended use, they may be sold in ready-to-drink forms or as concentrated extracts that needs to be diluted with water before use. *Agbo Iba* is usually prescribed by vendors for the treatment of malaria, while *Agbo Jedi* is prescribed for treating hemorrhoid. These two remedies are mostly sold in motor parks, open markets and artisanal workshops and are used indiscriminately without any medical test to diagnose either of the two ailments. Although, some herbal remedies have promising potential, information on the comparative toxicological implications of their use are not available to the general public [10]. In this light, the evaluation of herbal remedies in a study such as this, offers information for patient counselling and public health interventions.

This study was carried out to determine the phytochemicals present in these herbal concoctions and to evaluate their microbial and elemental content. The results of the evaluation will serve as a useful document for intervention among the herb sellers and users. In addition, malaria and haemorrhoid are significant public health problems which requires proper treatment. Any herbal

remedies offered to the public for treating such ailments requires monitoring and evaluation to be sure that complications do not arise and fatalities are prevented.

EXPERIMENTAL METHODS

Study Area, sample collection. This study was conducted in Ibadan Metropolis, Oyo state, Nigeria. A total of eight (8) local preparations were purchased at random from four markets. The markets and the samples collected from them were as follows: Egbeda Market (Agbo Iba 1 and Agbo Jedi 1), Bodija Market (Agbo Iba 2 and Agbo Jedi 2), Bode Market (Agbo Iba 3 and Agbo Jedi 3), Oje Market (Agbo Iba 4 and Agbo Jedi 4). All eight samples were in liquid form.

Qualitative and Quantitative Phytochemical Analysis. The presence of secondary metabolites in all the samples of *Agbo Iba* and *Agbo Jedi* were evaluated using standard method described by Evans and Trease [21]. The method of Harborne and Williams [22] was used for the quantitative phytochemical analysis.

Determination of pH. The pH of the different herbal samples was analysed using a pre-calibrated Bench-top pH meter (pH-016A model). About 50 mL of each herbal product was measured into a beaker and the electrode was dipped inside and gently stirred. The pH was read off on the digital display screen of the pH meter.

Preparation of media. All media for microbiological analysis were prepared according to standard procedures [23], then mixed with 200 mL of distilled water and dissolved. The media was sterilized for 15 minutes at 121°C in an autoclave. The sterile media was poured into sterilized Petri dishes and cooled.

Total aerobic bacteria plate count. A volume of 1 mL of each herbal sample was mixed with 9 mL of freshly prepared sterile peptone water and shaken thoroughly to make a ten-fold serial dilution. Further dilution was repeated until a dilution of 10^{-7} was attained. A volume of 0.1 mL was pipetted onto the surface of each of the two petri dishes holding 20 mL solidified sterile media, ensuring even distribution. A duplicate was made for each media. At 37°C, the plates were incubated for up to 72 hours. The Stuart Digital colony counter (Cole-Parmer™, United Kingdom) was used to count bacterial colonies.

Elemental analysis of herbal remedies

The presence of elements like calcium, magnesium, iron, copper, zinc, cadmium and lead in the herbal samples was determined using Atomic Absorption Spectrometer (BUCK 200 AAS (AOAC, 975.23).

RESULTS

Quantitative Phytochemical analysis. The qualitative phytochemical screening of four samples each of Agbo Iba and Agbo Jedi revealed the presence of alkaloids, saponins, cardiac glycosides, glycosides, tannins, flavonoids, and steroids (Table 1).

Table 1: Phytochemicals detected in locally purchased herbal samples

Herbal Samples	Phytochemical							
	Alkaloids	Saponins	Anthraquinone	Cardiac Glycosides	Glycosides	Tannins	Flavonoids	Steroids
Agbo Iba 1	+	+	-	+	+	+	+	+
Agbo Iba 2	+	+	-	+	+	+	+	+
Agbo Iba 3	+	+	-	+	+	+	+	+
Agbo Iba 4	+	+	-	+	+	+	+	+
Agbo Jedi 1	+	+	-	+	+	+	-	+
Agbo Jedi 2	+	+	-	+	+	+	-	+
Agbo Jedi 3	+	+	-	+	+	+	-	+
Agbo Jedi 4	+	+	-	+	+	+	-	+

+: Present. **-:** Absent

Table 2: Quantitative estimation of phytochemicals detected in the locally purchased herbal samples

Herbal samples	Phytochemicals (mg/100 mL)				
	Alkaloids	Flavonoids	Steroids	Tannins	Cardiac Glycosides
Agbo Iba 1	453.3 ± 3.30	353.3 ± 6.00	71.7 ± 6.00	368.3 ± 4.40	1.7 ± 0.20
Agbo Iba 2	412.4 ± 0.30	340.3 ± 4.10	52.6 ± 3.30	353.4 ± 1.20	1.6 ± 0.30
Agbo Iba 3	385.5 ±1.60	383.6 ± 1.60	82.2 ± 1.60	406.2 ± 2.70	1.8 ± 0.00
Agbo Iba 4	472.3 ± 1.20	365.8 ±3.40	64.5 ± 5.60	391.1 ± 0.60	1.4 ± 0.20
Agbo Jedi 1	266.7 ± 4.40	458.3 ± 6.00	170.0 ± 2.90	708.3 ± 4.40	0.0 ± 0.00
Agbo Jedi 2	281.3 ± 0.10	358.9 ± 0.10	150.5 ± 3.30	656.1 ± 0.20	0.0 ± 0.00
Agbo Jedi 3	303.5 ± 1.10	397.9 ± 1.30	145.4 ± 4.40	596.5 ± 3.30	0.0 ± 0.00
Agbo Jedi 4	259.3 ± 0.10	365.9 ± 3.40	186.4 ± 6.10	621.4 ± 0.30	0.0 ± 0.00

Table 3: Elements detected in the locally purchased herbal remedies

Herbal samples	Elements (mg/100 g)									
	Calcium	Magnesium	Manganese	Potassium	Sodium	Iron	Copper	Cadmium	Zinc	Lead
Agbo Iba 1	139.7±2.60	63.3±1.70	0.02±0.00	26.7±1.70	258.3±4.40	7.4±0.20	0.40±0.10	0.01±0.00	0.77±0.03	0.10±0.00
Agbo Iba 2	75.0±2.90	36.7±1.70	0.05±0.00	15.0±2.90	186.7±1.70	9.1±0.10	0.3±0.00	0.00±0.00	0.37±0.03	0.04±0.01
Agbo Iba 3	115.0±2.90	61.7±1.70	0.03±0.00	30.0±2.90	283.3±4.40	12.2±0.10	0.5±0.03	0.02±0.00	0.83±0.03	0.83±0.03
Agbo Iba 4	161.7±4.40	75.0±2.90	0.01±0.00	11.7±1.70	278.3±1.70	12.7±0.10	0.3±0.00	0.01±0.00	0.67±0.03	0.09±0.00
Agbo Jedi 1	133.3±4.40	66.7±4.40	0.03±0.00	35.0±2.90	220.0±2.90	11.5±0.20	0.30±0.03	0.00±0.00	0.80±0.06	0.05±0.00
Agbo Jedi 2	76.7±1.70	33.3±1.70	0.02±0.00	25.0±2.90	173.3±1.70	8.7±0.20	0.23±0.03	0.00±0.00	0.47±0.03	0.04±0.00
Agbo Jedi 3	155.0±2.90	91.7±1.70	0.04±0.00	41.7±1.70	300.0±2.90	7.7±0.10	0.30±0.00	0.00±0.00	0.67±0.03	0.05±0.00
Agbo Jedi 4	85.0±2.90	41.7±1.70	0.05±0.00	26.7±1.70	256.7±4.40	8.6±0.10	0.20±0.00	0.00±0.00	0.43±0.03	0.03±0.00

Table 4: pH of the locally purchased herbal samples

Herbal samples	pH
Agbo Iba 1	4.52
Agbo Iba 2	4.05
Agbo Iba 3	4.41
Agbo Jedi 1	3.04
Agbo Jedi 2	2.79
Agbo Jedi 3	3.42
Agbo Jedi 4	3.00

Table 5: Total Viable Counts obtained for aerobic bacteria from *Agbo Iba* using different culture media

Herbal Remedies	Media	Average colony units × 10^8 cfu/mL		
		24 hours	48 hours	72 hours
Agbo Iba 1	MSA	0	0	65
	SSA	0	0	0
	EMB	0	0	10
	NA	14	14	73
Agbo Iba 2	MSA	0	13	17
	SSA	0	4	5
	EMB	11	14	16
	NA	13	14	24
Agbo Iba 3	MSA	0	2	64
	SSA	0	8	18
	EMB	0	0	15
	NA	13	15	88
Agbo Iba 4	MSA	0	0	2
	SSA	0	0	50
	EMB	0	9	34
	NA	3	14	88

Table 6: Total Viable Counts obtained for aerobic bacteria from *Agbo Jedi* using different culture media

Herbal Remedies	Media	Average colony units × 10^8 cfu/mL		
		24 hours	48 hours	72 hours
Agbo Jedi 1	MSA	0	0	3
	SSA	0	0	0
	EMB	0	0	0
	NA	3	8	11
Agbo Jedi 2	MSA	0	12	71
	SSA	0	0	0
	EMB	0	0	15
	NA	33	46	82
Agbo Jedi 3	MSA	2	16	38
	SSA	2	48	56
	EMB	48	56	58
	NA	48	104	126
Agbo Jedi 4	MSA	0	46	73
	SSA	0	0	0
	EMB	0	0	3
	NA	3	47	58

MSA=Mannitol Salt Agar, SSA= Salmonella Shigella Agar, EMB= Eosin Methylene Blue, NA= Nutrient Agar

The quantitative estimation of phytochemicals detected in the samples are shown in Table 2.

Determination of pH. Table 4 shows the pH values of the samples of *Agbo Iba* and *Agbo Jedi* ranging between 4.05-4.52 and 2.79-3.42 respectively.

Total aerobic bacteria plate count. The result of the microbial evaluation are shown in Tables 5 and 6. In the screened units of Agbo Iba and Agbo Jedi, the bacterial counts ranged from 2×10^8 to 88×10^8 cfu/mL and 2×10^8 to 126×10^8 cfu/mL respectively.

DISCUSSION

The qualitative phytochemical screening of four samples of *Agbo Iba* (Table 1) revealed the presence of alkaloids, saponins, cardiac glycosides, glycosides, tannins, flavonoids, and steroids but no anthraquinones, whereas the qualitative phytochemical screening of four samples of *Agbo Jedi* revealed the presence of alkaloids, saponins, glycosides, cardiac glycosides, tannins, and steroids but devoid of anthraquinones and flavonoids. The quantitative phytochemical screening of four samples of *Agbo Iba* (Table 2) revealed that alkaloids had the highest concentration, ranging from 385.50 ± 1.55 to 472.33 ± 1.24 mg/100 mL and cardiac glycosides had the lowest concentration, ranging from 1.360.02 to 1.820.04 mg/100 mL.

The quantitative phytochemical screening of four *Agbo Jedi* samples (Table 2) revealed that tannin had the highest concentration ranging from 596.46 ± 3.33 to 708.33 ± 4.41 mg/100 mL. Cardiac glycosides were not detected in the quantitative screening of the four *Agbo Jedi* samples perhaps they were present in trace amounts. Alkaloids have been linked to antibacterial activity in plants and have pharmaceutical potential as anaesthetics and central nervous system stimulants [24]. Flavonoids are thought to be antioxidants and free radical scavengers including coronary heart disease prevention, antiviral, hepatoprotective, anti-inflammatory, and anticancer activities [25, 26]. Saponins have been found to have anticancer, antioxidant, immunomodulatory, antimalarial, antibacterial, and analgesic properties [27]. The presence of these useful phytochemicals in the herbal remedies confirms the interest of the populace in purchasing them from the local vendors. It shows that these therapies may have the varying diseases ameliorative

potentials.

The result of elemental analysis of the samples of *Agbo Iba* and *Agbo Jedi* are shown in Table 3. Using atomic absorption spectrometry, it was possible to detect some micro minerals, trace minerals and potentially toxic heavy metals. The purpose of this analysis was firstly to confirm that the herbal remedies provide the necessary daily amounts of essential elements without surpassing them. Secondly the analysis sought to ensure that the samples do not contain constituents that could harm the body's organs. This is crucial in preventing potential toxicities (e.g., renal toxicity). As a result, there are specific allowable limits of these heavy metals in herbal medicines. For example, the level of lead should not exceed 2.0 ppm, mercury ≤ 0.5 ppm, cadmium ≤ 0.20 ppm and aluminum ≤ 0.20 ppm [28, 29]. There are also national limits specified by different countries. For example, Canada specified a maximum of 0.01 mg/day for arsenic, 0.02 mg/day for lead, chromium and mercury each and 0.06 mg/day for cadmium while Malaysia set a limit of 5 mg/kg for arsenic, 10 mg/kg for lead and 0.5 mg/kg for mercury. WHO however recommends 10 mg/kg for lead and 0.3 mg/kg for cadmium [30]. There are also maximum recommended doses allowed for essential elements. Zinc and iron should not exceed 5 ppm and 15 ppm respectively [28, 31]. Excess sodium and potassium should also be avoided in order to avoid hypertension, cardiac and metabolic issues [32, 33]. Of the ten (10) elements analyzed, eight essential elements with potential benefits were present. Sodium had the highest concentration range, ranging from 186.67 ± 1.67 to 283.33 ± 4.41 mg/100 g for *Agbo Iba* and 173.33 ± 1.67 to 300.00 ± 2.89 mg/100 g *Agbo Jedi.* Lead was detected in the samples with concentrations ranging from 0.04 ± 0.01 to 0.10±0.00 mg/100 g for *Agbo Iba* and 0.03 ± 0.00 to 0.05 ± 0.00 mg/100 g) for *Agbo Jedi*, respectively. Cadmium was not detected in *Agbo Jedi* but was present (0.01 ± 0.00 to 0.02 ± 0.00 mg/100g) in three samples of *Agbo Iba*. Other elements present include; calcium which is required in promoting strong bones and teeth and preventing osteoporosis, potassium for healthy nervous system, iron is essential for blood production, and zinc for immune boosting and performance of reproductive functions [28].

Table 4 shows the pH values of samples of *Agbo Iba* and *Agbo Jedi*. It was observed that all four (4) samples of *Agbo Iba* had variation in pH level as well as all four samples of *Agbo Jedi*. These slight variations in pH may occur as a result of multitude of factors including; difference in water quality, variation in ingredient proportion, method of preparation, storage conditions etc. The pH level of all eight (8) studied herbal samples was between 4.05-4.52 for *Agbo Iba* and 2.79-3.42 *Agbo Jedi* indicating acidity. If the patients are consistently exposed to this acidic pH without commensurate information of the need to use the medication during meals, then within a short time, they may likely develop ulcers of different categories.

The result of the microbial evaluation as shown in Tables 5 and 6 demonstrated that the samples exhibited varying degree of microbial contamination exceeding the European Pharmacopoeia stated limit. According to European pharmacopoeia, bacterial contamination limits are; total aerobic bacteria (10^5 cfu/g**)**, enterobacteria and other gram-negative organisms (10^3 cfu/g**)** [34]**.** In *Agbo Iba* and *Agbo Jedi*, the bacterial counts ranged from 2×10^8 to 88×10^8 cfu/mL and 2×10^8 to 126×10^8 cfu/mL respectively. Large numbers of pathogenic bacteria may be present in herbal remedies due to the unhygienic methods of preparation or the use of unsterile equipment and materials including personnel handling the raw materials during processing. As a result, the bacteriological quality of raw material is influenced by the harvesting, drying, storage, handling and soil type which in turn

determines the overall quality of the herbal remedies. Consequently, to preserve the quality, safety and efficacy of the final herbal remedies, manufacturers should ensure the greatest level of hygiene and the lowest level of contamination during manufacturing of the herbal remedies.

Conclusion. The herbal remedies evaluated in this study appear to contain phytochemicals which may be beneficial in antimicrobial therapy and antimalarial treatment even though they contain varying degree of contamination with pathogenic microorganisms. In addition, the pH of all the samples were in the acidic range and this could be detrimental to patient health. Furthermore, while the presence of micronutrients may be beneficial, lead and cadmium are not. Generally, herbal remedies require extreme hygiene during preparation to offer standard delivery system devoid of harm to the ultimate user. It is recommended that herbal remedies be prepared under the most hygenic conditions and imperative to establish standardised dosage regimens and conduct thorough vetting of vendors of these herbal remedies. This oversight should be conducted by relevant government agencies like NAFDAC to ensure consumer safety and quality product

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