Phytochemical properties and heavy metal contents of commonly consumed alcoholic beverages flavoured with herbal extract in Nigeria

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Abstract

There is proliferation of alcoholic beverages flavoured with herbal-extracts perceived to have medicinal values. Information on the phytochemical and heavy metal contents of these products is scarce. This study assessed the phytochemical properties and heavy metal contents of herbal-extract flavoured alcoholic beverages in major motor parks in Ibadan, Nigeria. The phytochemical properties of the beverages were determined in triplicate using standard methods while the heavy metal contents were assessed using atomic absorption spectrophotometry. Data were analyzed using descriptive statistics and means were compared using ANOVA at $p<0.05$. The pH range of the beverages was 3.28-6.57 and the alcohol content was 34.0-51.5%. Detected major phytochemicals and concentration ranges were phytic acid (0.72-2.37 mg/g), alkaloids (0.42-4.11 mg/g), flavonoids (0.22-3.64 mg rutin equivalents/g), total phenols (1.13-3.66 mg gallic acid equivalents/g), anthraquinones ((0.74-1.93 mg/g) and triterpenoids (0.74-1.93 mg/g). The phytochemical contents were within the acceptable limits while the heavy metals were: Pb (2.13-4.70 mg/L), Cd (0.06-0.07 mg/L), Co (0.12-0.23 mg/L), Zn (0.14-0.40 mg/L) and Fe (0.72-4.22

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mg/L); all except Pb and Cd were within permissible limits. The herbal-extract flavoured alcoholic beverages contain beneficial phytochemicals and traces of heavy metals. Safety awareness of these products for improved consumers' health would be of public health importance.

Key words: Phytochemical, heavy metals, flavoured alcoholic beverages, herbal extract.

Key Contribution: This article indicates the phytochemical properties and heavy metal contents of commonly consumed herbal-extract flavoured alcoholic beverages. There is increase in the consumption of these products in Ibadan, Nigeria and therefore, information on the contents will no doubt promote consumers' safety and health through necessary intervention and policy formulation. This article revealed the arrays of herbal-extract flavoured alcoholic beverages proliferating in Ibadan, Nigeria and the associated health risks.

1.0 Introduction

Beverages play important role in the diets of people and some beverages are flavoured with herbal products, based on the perceived health benefits of the herbs. Many consumers are increasingly engaged in this practice as initiative to obtain certain health benefits or preventing an illness rather than waiting to cure diseases [1]. Energy drinks refer to beverages that contain, besides calories, caffeine in combination with other presumed energy-enhancing ingredients such as taurine, herbal extracts, and B vitamins [2]. Though the product was introduced in Europe and Asia in 1960 primarily to satisfy consumers demand for a dietary supplement that would result in increased energy, it is now a regular consumption in many countries including Nigeria. Producers have initiated many innovative approaches to promote sales and increased consumption of energy drinks in Nigeria with shifting emphasis on increased energy to herbal extracts. This is particularly interesting following the resurgence in the use of herbal medicines in sub-Saharan Africa [3], perceived affordability, safety of herbal products compared to modern medicines [4] and its ready mix with the socio-cultural life of the people. Studies have shown that large number of the people in developing countries relies on herbal

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medicines for their primary health care [5,6]. As at 2008, 80% of the world's population was using herbal medicine for one form of primary health care or another and its health risk posed a major concern [7]. Many medicinal herbs are rich in a multitude of chemical compounds like alkaloids, taninns, saponins, flavonoids, resins and triterpenoids [8,9].

In addition to herbal extracts, reports have shown the presence of heavy metals in many beverages. Though some heavy metals could be beneficial, these metals possess deleterious effect when present or their levels in food and drinks exceed the tolerable limit [10]. Interestingly, both beverages and herbal extracts separately are noted as source of heavy metals and the additive effects of heavy metals from these sources could be particularly harmful to health. Studies have reported the presence of lead, cadmium, mercury, and arsenic in beverages which lead to progressing physical, muscular, and neurologically-degenerating disease conditions [11,12].

Phytochemical assessment is essential to evaluate the chemical components that may be responsible for the observed/perceived health benefits associated with the herbal beverages. Phytochemicals are naturally occurring compounds that contribute to the color, flavor and smell of plants and form part of a plant's natural defense mechanism against diseases. The therapeutic values of phytochemicals in human health and disease prevention have been reported [8,13]. Phytochemical screening is a tool by which the presence of these chemical compounds can be investigated in herbal products consumption of these beverages. Therefore, there is the need to identify the presence and the potentials of the phytochemical constituents of the beverages spiced with herbal medicines. This is particularly necessary to ensure safety following controversies with regulation and standardization in spite of the increasing use of herbal extracts in foods and drinks especially alcoholic beverages. Though, there are information on the chemical constituents of the beverages and the herbal mixtures separately, but information is scarce on the ready to consume form of the preparations. Therefore, the evaluation of heavy metals and phytochemical properties of commonly consumed beverages is essential to promote food safety and consumers' health. In addition, it will be useful in providing information that could lead to necessary intervention and policy formulation. This study is therefore, designed to assess the phytochemical properties and heavy metal contents of commonly consumed alcoholic beverages flavoured with herbal extract in Nigeria.

2.0 Materials and Methods

Collection of samples for laboratory analysis

A consumption survey of alcohol beverages flavoured with herbal extracts was conducted among young adults within selected motor parks in Ibadan, Nigeria to

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identify five most frequently consumed brands. The brand include: '*Baby oku dey man power'*; *Agbo jedi; Orijin;* Blackwood; and Alomo bitters. Samples of these five beverages were randomly purchased from the sale points in the major motor parks in Ibadan, Nigeria and maintained in the prescribed storage condition prior to analysis. The manufacturer, brand name, expiry date, regulatory agency registration number and percentage alcohol content were recorded.

Qualitative Analysis

The physical properties of the sampled beverages were determined using standard procedures. The specific gravity / alcoholic content (%) was determined using Food Safety Standards Authority of India method [14] and pH was determined using pH meter (Genwer 3500).

Chemical tests were conducted to identify the constituents in the sampled beverages using standard methods. Alkaloids presence was determined by the formation of precipitation using Harborne method [15]. Tannin and saponnins were determined using the procedure of Sofowora [16]. Flavonoids were confirmed using Cuillei method [17]. Terpenoids was determined using the Salkowski test [9]; anthraquinones using Trease and Evans method [18]; and total phenols using FolinCiocalteau assay [19].

Quantitative phytochemical analysis

Chemical tests were carried out on the samples to determine quantity of the previously identified constituents using standard procedures. Quantitative estimation of alkaloids content was conducted by alkaline precipitation gravimetric method [15] and expressed as mg alkaloid per ml of the sample. The aluminum chloride method was used for the determination of the total flavonoid content and absorbance at 415 nm after 30 minutes of incubation [9]. Total phenolic concentration was measured by FolinCiocalteau assay, absorbance was recorded at 750 nm and content was expressed as milligram per ml of gallic acid equivalents [19]. Saponin content was obtained using procedures of Edeoga [9]. Oxalate composition was determined at 590 nm absorbance using UV/VIS spectrophotometer [20] and expressed in milligrams per ml. Tannin content was determined using Butanol-HCl reagent and expressed in mg per ml [16]. Phytic acid concentration was determined using $FeCl_3$ as precipitant [21].

Determination of heavy metals

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A 50 ml volume of sample extract was concentrated to 25 ml volume using 2 ml concentrated HNO_3. Heavy metal contents of the drinks were determined spectrophotometrically by using Buck 200 atomic absorption spectrophotometer (Buck Scientific, Norwalk United Kingdom [22] and compared with absorption of standards of these minerals. All samples were determined in triplicate.

Statistical Analysis

Data were processed using the Statistical Package for Social Sciences (SPSS) for Windows 20.0 (SPSS, Chicago, Ill, USA) software. The results obtained were analysed using one way analysis of variance (ANOVA), and level of significance was set at $p < 0.05$.

3.0 Results

3.1. Physical properties of the alcoholic beverages

The physical properties of the samples are presented in Table 1. The pH range of the alcoholic beverages was 3.28 - 6.57 and the alcohol content (%) ranged from 34.0 to 51.5. For the samples, the alcoholic contents were higher than indicated on the beverages labels.

Table 1: pH and the alcohol contents of the alcoholic beverages

Samples	NAFDAC Reg. No.	pH	Alcohol Label claim (%)	Alcohol (%) determined in the laboratory
Sample 1	B1-4103L	3.28±0.04	42	41.1
Sample 2*	-	4.57±0.3	NA	34.0
Sample 3	B1-7529	6.57±0.07	40	42.0
Sample 4	08-0630	4.34±0.2	30	40.6
Sample 5	A1-8029	5.77±0.1	42	51.5

*An alcoholic beverage flavoured with herbal extract that is not packaged and branded

* NA-Not Available

3.2 Phytochemical qualitative and quantitative analysis of alcoholic beverages flavoured with herbal extracts

Phytochemical qualitative and the quantitative properties of the beverages in mg/ml are presented in Table 2 and 3 respectively. Phytic acid was present in samples: 1(*Baby oku dey man power*), 2 (*'Agbo jedi*) and 4 (*Orijin*) and concentrations in order of abundance was 2.43±0.1 mg/g, 2.37±0.30 mg/g and 0.72±0.1 mg/g for *Agbo jedi, 'Baby*

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oku dey man power' and *Orijin* respectively. Oxalates were not detected in all the samples. Alkaloids and flavonoids were present in all the samples. The concentration of alkaloids ranged from 0.42 to 4.11(mg/g). '*Baby oku dey man power*' had the highest concentration of alkaloids with mean concentration of 4.11±0.2 mg/g. Alkaloids concentration in '*Agbo jedi*', Alomo bitters, Orijin and Blackwood were 1.93±0.3 mg/g, 0.42±0.1 mg/g, 0.37±0.2 mg/g and 0.27±0.1 mg/g respectively in decreasing order of abundance.

Phenolic compounds were found in all the samples, except sample 4 (*Orijin*). Saponins were found in samples 1 (*Baby oku dey man power*) and 5 (*Alomo bitters*). Tanins were found in samples *Orijin* and *Alomo bitters*. Anthraquinones were found in 1 (*'Baby oku dey man power')* and sample 3 (Blackwood) while triterpenoids were found in samples 1 (*'Baby oku dey man power'),* 2 *('Agbo jedi)* and 3 (Blackwood). The mean concentration of flavonoids ranged from 0.22 to 3.64. '*Baby oku dey man power'* had the highest level of flavonoids with mean concentration of 3.64 ± 0.05 mg rutin quivalents/g and *'Orijin'* had the lowest level (0.22±0.01 mg rutin quivalents/g). Tannins content was 1.43±0.4 mg/g in *'Alomo bitters',* 0.12±0.4 mg/g in *'Orijin'* and was not detected in other samples.

The mean concentrations of saponins (mg diosgenin equivalents/g) were 0.22±0.01 and 0.17±0.02 for *'Alomo bitters' and 'Baby oku dey man power'* respectively. Total phenols was present in all samples except *'Orijin'* with a mean concentration of 3.66±0.05 mg gallic acid equivalents/g and 1.13±0.1 mg gallic acid equivalents/g for *'Baby oku dey man power'* and *'Agbo jedi'* respectively. Anthraquinones (mg/g) was found only in *'Baby oku dey man power'* (1.93±0.30) and Blackwood (0.74±0.01). Triterpenoids contents were 0.93±0.05, 0.24±0.1 and 0.11±0.01 for Blackwood, *'Agbo jedi'* and *'Baby oku dey' man power* respectively.

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Table 2: Phytochemical qualitative analysis of alcoholic beverages flavoured with herbal extracts

S/N	Parameters (mg/g)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	Phytic acid (mg/g)	++	++	-	+	-
2	Oxalate (mg/g)	-	-	-	-	-
3	Alkaloids (mg/g)	+++	++	+	+	+
4	Flavonoids (mg rutin quivalents/g)	+++	+	+	+	+
5	Tannins (mg/g)	-	-	-	+	++
6	Saponins (mg diosgenin equivalents/g)	+	-	-	-	+
7	Total Phenolic (mg gallic acid equivalents/g)	+++	++	++	-	++
8	Anthraquinones (mg/g)	++	-	+	-	-
9	Triterpenoids	+	+	+	-	-

- **Absent; + Present**

Sample 1: '*Baby oku dey man power*'; Sample 2: '*Agbo jedi*'; Sample 3: Blackwood; Sample 4: Orijin; Sample 5: Alomo bitters

Table 3: Phytochemical quantitative analysis of alcoholic beverages flavoured with herbal extracts

	Parameters	Sample 1	Sample 2*	Sample 3	Sample 4	Sample5
1	Phytic acid (mg/g)	2.37±0.30	2.43±0.1	ND	0.72±0.1	ND
2	Oxalate (mg/g)	ND	ND	ND	ND	ND
3	Alkaloids (mg/g)	4.11±0.2	1.93±0.3	0.27±0.1	0.37±0.2	0.42±0.1
4	Flavonoids (mg rutin equivalents/g)	3.64±0.05	0.77±0.1	0.68±0.1	0.22±1	0.52±0.2
5	Tannins (mg/g)	ND	ND	ND	0.12±0.4	1.43±0.4
6	Saponins (mg diosgenin equivalents/g)	0.17±0.02	ND	ND	ND	0.22±0.1
7	Total Phenolic (mg gallic acid equivalents/g)	3.66±0.05	1.13±0.1	1.58±0.2	ND	1.55±0.3
8	Anthraquinones (mg/g)	1.93±0.30	ND	0.74±0.1	ND	ND
9	Triterpenoids	0.11±0.01	0.24±0.1	0.93±0.5	ND	ND

*An alcoholic beverage flavoured with herbal extract that is not branded

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210 **3.3 Heavy metals content of the alcoholic beverages flavoured with herbal**
211 **extracts**

212 Heavy metals content of the alcoholic beverages flavoured with herbal extracts
213 samples is presented in Table 4. The Pb concentration ranged from 2.13 - 4.70 mg/L;
214 *Alomo bitters* had the highest concentration (4.70±0.5 mg/L) and '*Baby oku dey man*
215 *power'* had lowest (2.13±0.01 mg/L). The Cd content was similar in all samples and
216 ranged from 0.06 - 0.07 mg/L. The Cr concentration (mg/L) was highest in
217 Blackwood (0.35±0.02 mg/L) and undetected in *Baby oku dey man power.* The Co
218 content was highest in Blackwood (0.2±0.5 mg/L) and least in *Agbo jedi* (0.12±0.03
219 mg/L). Zinc and iron were present in all samples. *Baby oku dey man power* had the
220 highest Zn (0.40±0.02 mg/L) and Fe (4.22±.0.02 mg/L) contents.

221

222 **Table 4: Heavy metal content of the alcoholic beverages flavoured with herbal**
223 **extracts (mg/L)**

Samples	Pb	Cd	Cr	Co	Zn	Fe
1	2.13±0.01	0.07±0.02	ND	0.13±0.1	0.40±0.02	4.22±0.02
2*	2.39±0.01	0.06±0.03	0.15±0.02	0.12±0.03	0.19±0.04	0.83±0.01
3	3.91±0.01	0.06±0.03	0.35±0.02	0.23±0.01	0.14±0.03	1.01±0.01
4	2.51±0.04	0.06±0.01	0.22±0.04	0.13±0.02	0.17±0.01	0.72±0.05
5	4.70±0.50	0.07±0.01	0.31±0.01	0.20±0.50	0.22±0.01	1.10±0.10
WHO	10	0.3	-	-	60	100
RDA	0.3mg/week		10-60	-	100	10-60

224 Metals in mg/L, ND-Not detected
225 *An alcoholic beverage flavoured with herbal extract that is not branded
226

227 **3.0 Discussion**

228 This study presents the phytochemical properties and heavy metals content of five
229 commonly consumed herbal-flavoured beverages in Ibadan. The phytochemical
230 content of these beverages differ and alkaloids, flavonoid and total phenol
231 constituted the common and abundant phytochemicals in these products.
232 Phytochemicals are noted to be abundant in herbs, fruits and vegetables and their
233 health benefits include reduced risk of oxidative stress-related diseases and chronic
234 diseases, antioxidant properties, cell maintenance, DNA repair and promote
235 longevity [23-26]. The presence of these phytochemicals indicates that these
236 beverages could be potential sources of beneficial antioxidants and confer many
237 health benefits associated with these phytochemicals. The intake should however be
238 with caution as a study in Nigeria has linked intake to infertility [27]. Likewise,
239 phenolic compounds have high inhibitory effect on iron absorption and reduce

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protein and carbohydrate digestibility [28]. The beverages contain tannin in varying quantity, all within the permissible level. Tannins could reduce the bioavailability of protein by reducing its nutritional quality through hydrogen binding and hydrophobic interactions [29]. Saponins acts as chelators of transition metals (Cu^{2+} or Fe^{2+}) and results in diminished cellular sensitivity to oxidant damage [30,31]. Two of these beverages (*'Baby oku dey man power' and* Blackwood) contain Anthraquinones which possesses a variety of antimicrobial, antioxidant, anti-inflammatory, antiviral, or antitumor promoting biological activities [32,33]. The presence of triterpenoids in three beverages precisely *'Baby oku dey man power'*; *'Agbo jedi' and* Blackwood suggests the potential of the beverages exhibiting protective effects against cardiovascular disease and inflammation that are associated with this compound [34].

Phytic acid was found in three samples at levels below the antinutritional limit of 500 mg/100g. Phytic acid could play antinutritional role by forming a complex with calcium, iron, zinc and other minerals thereby reducing their bioavailability [35,36]. Hurrell [37] reported that a mole of phytic acid binds 6 moles ferric iron which is the major form of iron in plant foods. With phytic acid average content of about 2mg/g in some herbal-flavoured beverages, heavy consumers may be at risk of iron deficiency.

The alcoholic contents were higher than indicated on the beverages labels. This finding is of utmost concern as this could mislead the consumers. The high alcoholic contents of the products could cause intoxication and liver damage among consumers [1]. Heavy metals constitute health risks in human when intakes are higher than the permissible levels. In the present study, all the tested heavy metals were found in the herbal-flavoured beverages in varying proportions. The presence of these heavy metals conforms to earlier findings which reported the presence of impurities such as heavy metals including cadmium, copper, iron, nickel, selenium, zinc, lead and mercury in soft drinks, beverages and herbal products in Nigeria [38-41]. The lead concentration in the samples is above the permissible level (0.1 mg/L), which is of public health concern. The presence of lead in the entire samples suggests the need for stricter regulations on the production and marketing of herbal-flavoured drinks in Nigeria. Earlier studies have reported similar levels of lead in Nigerian drinks and herbal products [39, 41-43]. Lead toxicity can lead to kidney dysfunction, inhibition in haemoglobin synthesis and damage to cardiovascular and the central nervous system [12,41].

Cadmium level in this study is also above the WHO permissible limit and United States Environmental Protection agency recommended level of 0.005 mg/L. The level found in this study is higher than reported by Onianwa [38] and similar to the findings of some other authors that reported unsafe level of cadmium in Nigerian drinks [40,41,43]. Chromium contamination in this study agrees with earlier report showing the presence of chromium in Nigerian drinks [41,44]. This finding suggests

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a risk of lead, cadmium and chromium intoxication considering the frequency of consumption of these drinks.

Calcium and zinc constitute heavy metals with known biological importance in human nutrition and health, and with known recommended intake levels. Yet, intakes of these metals are expected to be within the recommended limits; otherwise, toxicity may occur with serious health implications. Zinc is a micronutrient of public health importance. Its presence in these beverages suggests the reduced likelihood of the consumers' susceptibility to zinc deficiency. Zinc deficiency is known to lead to anaemia and growth retardation and toxicity can result in vomiting, diarrhea, bloody urine, liver failure, kidney failure and anemia [39].

4.0 Conclusions

The major phytochemicals in the herbal-extract flavoured alcoholic beverages (in varying quantity) in Nigeria include phytic acid, alkaloids, flavonoids and tannins. The alcoholic contents of the products were quite high to cause intoxication and other health challenges. Heavy metals (Pb, Cd and Co) levels were above the permissible level and could pose health risks to the consumers. Public education to sensitize the consumers of herbal-extract flavoured alcoholic beverages on the health risk associated with these drinks is hereby suggested. Also, strict measures should be put in place to enhance quality of production of these products.

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