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# Phytochemical and Heavy Metal Screening of Fura da Nono, Ciklavit®, Katoka Mixtures®, and Yoyo Bitters®

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ABSTRACT

**Background**: Evaluation of the phytochemicals and heavy metals contained in four commonly consumed beverage and herbal products in Nigeria, was investigated. This was to ascertain the health benefits as well as potential for toxicity that may be associated with the consumption of these products.
**Methods**: The samples studied are *Fura da Nono*, Ciklavit®, Katoka Mixtures® and Yoyo Bitters®. The phytochemical screening of each product was done using standard procedures. The heavy metals screening was done using Agilent® 720 Inductively Coupled Plasma -Optical Emission Spectroscopy machine (ICP-OES). The Hazard quotient and Index were assessed using appropriate equations in line with United States Environmental Protection Agency (USEPA).
**Results:** All phytochemicals tested were found in *Fura da Nono* except Steroids and Terpenoids. Also, all the tested phytochemicals were detected in Ciklavit® except Alkaloids and Phlobatannins. Both Katoka Mixtures® and Yoyo Bitters® contained all other phytochemicals except Phlobatannins. Quantitatively, *Fura da Nono* was high in Cardiac glycosides, and Ciklavit® was found to be high in Flavonoid content. Both Katoka Mixtures® and Yoyo Bitters® are high in Phenols. The results of the heavy metal analysis revealed different concentrations (ppm) of arsenic, mercury, lead, cadmium, zinc, manganese, copper and nickel in the four products. Arsenic was not detected only in *Fura da Nono.* The heavy metal concentrations obtained were all within WHO maximum permissible limits. Further analysis for potential non-carcinogenic health risk using Hazard quotient on each of the four toxic heavy metals (arsenic, mercury, lead and cadmium) and Hazard Index (HI) on the same toxic heavy metals revealed that by the United States Environmental Protection Agency (USEPA) threshold value of HI $\leq 1$, Yoyo Bitters® (0.4684) and Ciklavit® (0.7870) had the lower and safer values for combined heavy metal risk while Katoka Mixtures® (0.9908) and *Fura da Nono* (1.4144) had the highest.
**Conclusion:** The phytochemical screening of the investigated products showed that the commonly consumed food drink and herbal products, are rich in phytochemicals. Even though the heavy metal screening indicates a fairly positive safety margin for three of the products. Caution should therefore be exercised in the use of these products.

## 1. Introduction

Historically, plants have been known to be crucial to the survival of man. They serve as sources of both food and medicine. According to the United Nations Educational, Scientific and Cultural Organization, UNESCO, up to 85 % of the world's food calories are still obtained from plant sources till date[1]. Also, herbal medicines are in high demand around the world. In 2016, the global herbal medicine market was valued at USD 71.19 billion, with the extract segment contributing approximately 38 % of the total value. The herbal extract segment accounted for USD 27.1 billion and has been projected to rise to USD 44.6 billion by 2024[2]. Furthermore, research has shown that herbal remedies are the most widely used consumer health products in Nigeria[3].

*Fura da Nono* is a locally produced beverage, popular in West Africa and widely consumed in northern Nigeria, serving as a mid-day meal [4,5]. It consists of a cereal, 'Fura', and a fermented milk product 'Nono.' *Fura da Nono* (Hausa for "millet ball and milk") is prepared by mashing balls of

*Fura in Nono*. Sugar may be added in the mixture, to taste[6]; and depending on its consistency, the beverage may be used as food, a refreshing drink or a weaning drink for infants [4,5].

Ciklavit®, a finished herbal product, is the most prominent and widely used of all drugs prepared from medicinal plants for the management of sickle cell anaemia[7]. It is a liquid herbal preparation made from the extracts of *Cajanus cajan* seeds and contains proteins (essential amino acids), vitamins such as vitamin C (ascorbic acid), and minerals such as zinc. Sickle-cell anaemia (also known as sickle-cell disorder or sickle-cell disease, SCD) is a common genetic condition. It is a haemoglobin disorder resulting from the inheritance of mutant haemoglobin genes from both parents. According to World Health Organization, about 150,000 children are born annually with sickle-cell anaemia in Nigeria alone, and the disease accounts for up to 16 % of under-five deaths in individual West African countries[8]. The public health implications of sickle-cell anaemia are thus significant.

Katoka Mixtures® is a finished herbal product made from *Olax subscorpioidea*, *Erthrophleum sauveolens*, *Abelmoschus esculentus*, *Canarium schweinfurthii*, *Xylopia aethiopica*, *Alstonia boonei*, *Morinda lucida*, *Nauclea latifolia*, M.S, *Parinari* spp., and water. According to the manufacturer, De-Katoka (W.A.) Ltd. (2019), the herbal mixture is effective in the treatment of fever, malaria, stomach ache and headache, as well as in the treatment of bacterial, microbial and fungal infection, oxidative stress, inflammation, anaphylaxis pain, bronchitis and asthma among other conditions.

Bitters are another important category in the Nigerian herbal medicine market. They are very common and are currently marketed as "cure-all" patent medicines[9]. Among the bitters available in the Nigerian market, Yoyo Bitters® is one of the most widely consumed[10,11]. Yoyo Bitters® is a galenical oral preparation made from a blend of various parts and fruits of plants such as *Aloe vera, Cinamum aromaticum, Citrus aurantifolia, Acinos arvensis* and *Chenopodium murale*. It also contains water soluble vitamins and minerals e.g., vitamins (B1, B2, B3, B6, and B12), mineral (copper, zinc, iron) added to fortify the herbal preparation[12]. Yoyo Bitters® was launched in 2003 by Abllat Company Nigeria, Limited. Yoyo Bitters® has been widely reported to have different effects and functions including the ability to help regulate blood pressure and dilate arteries, aid the process of digestion, prevent disorders like ulcers, gastritis, insomnia, stress and depression, and to also prevent kidney and bladder infections, overweight and excess body fats[13,14].

Heavy metal contamination is another phenomenon that has been linked to some of the health challenges associated with the consumption of foods and medicines. Luo et al.[15] found that 30.5 % of 1773 global herbal samples contained one over limit metal. Similarly, a local study conducted in southern Nigeria revealed that 17 out of 25 commercially available herbal formulations had metals above limits specified by the World Health Organization/Food and Agriculture Organization, WHO/FAO [16].

Methods of atomic spectrometry such as flame atomic absorption spectrometry (FAAS) and electrothermal atomic absorption spectrometry (ETAAS), as well as plasma-based techniques such as inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are typical instrumental techniques used for the determination of elemental composition[17]**.** But among these techniques, ICP-OES is an industry standard technique for trace metal analysis. It offers high sensitivity with relatively high freedom from non-spectral interference, extended linear working range and rapid multielement analysis with good detection capabilities[18]. An ICP-OES instrument was used in the metal analyses for this study.

The high level of dependence on plants and their derived products by people in developing countries, particularly in Nigeria, makes imperative the determination of the beneficial as well as toxic constituents of the plant-based beverages and herbal products consumed. This would provide essential information regarding the safety of these products, leading to the general improvement of the products and the protection of the health of the masses. This study was, thus, aimed at ascertaining both qualitatively and quantitatively, the phytochemical constituents and heavy metals present in the beverage *Fura da Nono*, and the herbal medicines, Ciklavit®, Katoka Mixtures® and Yoyo Bitters®.

## 2.0 Materials and Methods

### 2.1 Materials

The multi-element stock standard solutions containing the target analytes (arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), zinc (Zn), iron (Fe), manganese (Mn), chromium (Cr), copper (Cu), and nickel (Ni)) were provided by CTX-ION Analytical LTD, Oshodi, Lagos. The chemicals used are nitric acid (69%, BDH®, England) as well as sulfuric acid (BDH®, England), formaldehyde (BDH®, England), ammonia solution (BDH®, England), glacial acetic acid (BDH®, England), hydrochloric acid

(BDH®, England), acetic anhydride (BDH®, England), chloroform (BDH®, England), Fehling's solution A (BDH®, England), ethyl acetate (BDH®, England) and phosphomolybdic acid (BDH®, England), Mayer's reagent (Merck, Germany), ferric chloride (Merck, Germany), Dragendorff's reagent (Merck, Germany), Follins-Dennis reagent (Merck, Germany), Folin-Ciocalteu reagent (Merck, Germany) chromogen solution (Merck, Germany), alkaline pirate solution (Merck, Germany), tannic acid (Merck, Germany), gallic acid (Merck, Germany), sodium carbonate (Merck, Germany), sodium carbonate solution (Merck, Germany), isobutyl magnesium carbonate alcohol (Merck, Germany), alkaline copper reagent (Merck, Germany), Ethanol (Fisher, USA), deionized water (Fisher, USA) and olive oil (Fisher, USA)

### 2.1.1 Equipment and chemicals
Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) Equipment (Varian Agilent® ICP-OES 720, USA). analytical grade chemicals and reagents were used throughout the experiment.

### 2.1.2 Sample collection
*Fura da Nono* was randomly obtained from a local vendor. Katoka Mixtures®, Ciklavit® and Yoyo Bitters® were purchased directly from companies' sales representatives in Lagos, Nigeria (Table 1). This study was conducted in 2020.

**Table 1: Label details of the herbal medicines**

| Brand Name | Manufacturer | NAFDAC Reg. No. | Batch No. | Mfd Date | Exp. Date | Volume (mL) |
|---|---|---|---|---|---|---|
| **Katoka Mixtures®** | De-Katoka (W.A.) Ltd. | A7-1524L | Z070L | 24/11/2020 | 24/11/2022 | 250 |
| **Ciklavit®** | Neimeth International Pharmaceuticals Plc. | 04-2506L | 00836019A | 08/2020 | 02/2022 | 200 |
| **Yoyo Bitters®** | Abllat Company Nigeria Limited | A7-1055L | YYBL-2008 | 08/2020 | 08/2022 | 200 |

## 2.2 Methods

### 2.2.1 Phytochemical Screening

#### 2.2.1.1 Sample preparation
Exactly 100 mL of each sample were evaporated to dryness using the oven drying method, after which they were transferred into appropriately labelled sample bottles as stock. Tests were then carried out on the dried samples to determine the phytochemicals present using the standard procedures qualitatively and quantitatively as described by Harborne[19], Brunner[20], Trease and Evans[21] and Sofowara[22]. The phytochemicals examined were Tannins, Phlobatannins, Saponins, Flavonoids, Steroids, Terpenoids, Cardiac glycosides, Alkaloids, Reducing sugars and Phenols.

### 2.2.2 Heavy metal analysis

#### 2.2.2.1 Instrumentation
The Agilent 720 axial view ICP-OES instrument was used for the heavy metal analysis of the digestates. The ICP-OES Expert software was used to develop the method. Appropriate conditions and parameters were then set for the analytical process. A peristaltic pump was used for the introduction of digested samples into the ICP-OES system at a flow rate of 1.5 mL min$^{1}$. The output power and generator radiofrequency of the ICP-OES system were 1450 W and 40 MHz, respectively. The nebulizer argon gas flow rate was 0.70 L min$^{1}$, the auxiliary argon gas flow rate was 0.50 L min$^{1}$ and the plasma argon gas flow rate was 12 L min$^{1}$.

**2.2.2.2 Analysis of metals present in *Fura da Nono,* Ciklavit®, Katoka Mixtures® and Yoyo Bitters®**

The samples were compared to a multi-element standard curve to determine the ppm concentration of each analyte in the digested solution. Each sample was analysed three independent times on the spectrometer. The wavelengths used for the detection and measurement of As, Hg, Pb, Cd, Zn, Fe, Mn, Cr, Cu and Ni were 188.980, 184.887, 220.353, 214.439, 213.857, 238.204, 257.610, 267.716, 327.395, and 231.604 nm respectively.

**2.2.2.3 Sample preparation for heavy metal analysis of *Fura da Nono,* Ciklavit®, Katoka Mixtures® and Yoyo Bitters®**

Sample digestions were carried out by the wet digestion method using $HNO_3$, following a slightly modified method of Adepoju-Bello et al.[23]. Exactly 2.0 g of each sample was carefully weighed and transferred into a clean 250 mL beaker, and 10 mL of concentrated nitric acid ($HNO_3$) was added. The solution was then placed in a fume hood and heated over a hot plate at 60 °C. While each sample was still dark, another 10 mL of $HNO_3$ was added and the heating continued until the emission of red nitrous oxide fumes ceased. The colour change observed in the fumes were from brown to white until there were no fumes at all. At this point, the colourings cleared out.

Exactly 2 mL of $HNO_3$ was again added to each sample and they were further heated until almost dried. The residues were allowed to cool to room temperature, transferred into 50 mL volumetric flasks, made up to the mark with deionized water and then filtered. They were afterwards collected in appropriately labelled sample bottles for analyses by ICP-OES. The blank solutions were prepared following the same process.

**2.2.3 Risk assessment of toxic heavy metals in *Fura da Nono,* Ciklavit®, Katoka Mixtures® and Yoyo Bitters®**

The risk, to human health, of the intake of heavy metals in the screened herbal products was evaluated using the parameters: Estimated Daily Intake (EDI), Hazard quotient (HQ) and Hazard Index (HI). This assessment was carried out specifically for the toxic metals: arsenic, mercury, lead and cadmium. Using the adult population as an index of measurement, the estimated daily consumption of *Fura da Nono* and the recommended maximum daily dose for Ciklavit®, Katoka Mixtures® and Yoyo Bitters® were used to determine the projected daily loading of heavy metals into the body system. The estimated daily consumption of *Fura da Nono* is 750 mL. The recommended maximum daily doses (RMDD) of Ciklavit®, Katoka Mixtures® and Yoyo Bitters® are 40 mL, 50 mL and 200 mL respectively.

The value of EDI of heavy metal for each product was determined using Equation 1

$$EDI = \frac{Cmetal\ x\ IR}{BW} \ldots\ldots\ldots\ldots\ldots \quad \text{Equation 1}$$

Where Cmetal (mg/kg) is the average weighted heavy metal content in herbal product, IR (ingestion rate) is the average daily consumption of herbal preparation (g/day/person), and BW is the average body weight (kg). An average adult body weight of 70 Kg was employed.

**2.2.4 Hazard Quotient (HQ):** Hazard Quotient (HQ), which is used to assess the non-carcinogenic risk, to humans, of long-term exposure to heavy metals; was calculated as a fraction of the determined dose to the reference dose as shown in Equation 2 below[26]

$$HQ = \frac{EDI}{RfD} \ldots\ldots\ldots\ldots \quad \text{Equation 2}$$

Where, EDI is the average herbal product metal intake per day (mg/kg/day), and RfD is the oral reference dose of the metal (mg/kg/day).

The RfD approximates daily tolerable exposure to which a person is expected to have without any significant risk of harmful effects during a lifetime. RfD for As, Hg, Pb and Cd are 0.0003, 0.0036, 0.02, and 0.001 mg/Kg/day respectively[24,25].

An HQ of <1, signifies that no potential health effects are expected from the exposure, while

HQ > 1 signifies that there are potential health risks due to the exposure.

**2.2.5 Hazard Index (HI):** Hazard Index (HI) helps to evaluate the overall non-carcinogenic risk to human health of more than one heavy metal. Exposure to more than one pollutant results in additive effects. It is the sum of the hazardous quotient (HQ) of all heavy metals as described in Equation 3.

$$HI = \Sigma HQ(As) + HQ(Hg) + HQ(Pb) + HQ(Cd) \text{ ........ Equation 3}$$

**2.2.6 Data analysis**

Data generated were reported as means ± standard deviation.

## 3.0 Results

### 3.1 Qualitative and quantitative phytochemical screening of *Fura da Nono,* Ciklavit®, Katoka Mixtures® and Yoyo Bitters®

The phytochemical constituents of interest in this research work were Tannins, Phenols, Flavonoids, Reducing sugars, Cardiac glycosides, Phlobatannins, Steroids and Terpenoids. The results of the qualitative and quantitative phytochemical screening of the herbal products are contained in tables 2 and 3 respectively. In the study, Katoka Mixtures® and Yoyo Bitters® were found to contain the highest number of phytochemicals. Out of the ten phytochemical groups analysed, Katoka Mixtures® and Yoyo Bitters® contained nine phytochemicals each, while Ciklavit® and *Fura da Nono* contained eight phytochemicals each. The high number of phytochemicals identified in Katoka Mixtures® and Yoyo Bitters® may be responsible for their multiple indications for a wide array of pathophysiological conditions; for which, in local parlance, are referred to as 'gbogbonise' (Yoruba, Nigeria term for "cure-all").

**Table 2. Qualitative phytochemical screening of *Fura da Nono*, Katoka mixtures®, Ciklavit® and Yoyo Bitters®**

| Phytochemical | *Fura da Nono* | Ciklavit® | Katoka Mixtures® | Yoyo Bitters® |
|---|---|---|---|---|
| Tannin | + | + | + | + |
| Phenol | + | + | + | + |
| Flavonoid | + | + | + | + |
| Reducing Sugar | + | + | + | + |
| Cardiac Glycoside | + | + | + | + |
| Alkaloid | + | - | + | + |
| Phlobatannin | + | - | - | - |
| Steroid | - | + | + | + |
| Terpenoid | - | + | + | + |
| Saponin | + | + | + | + |

(+) Present (-) Absent

**Table 3. Quantitative phytochemical screening of *Fura da Nono*, Katoka mixtures®, Ciklavit® and Yoyo Bitters®**

| Phytochemical | Mean Concentration ± Standard Deviation in mg/100g | | | |
|---|---|---|---|---|
| | *Fura da Nono* | Ciklavit® | Katoka Mixtures® | Yoyo Bitters® |
| Tannin | 32.14 ± 0.08 | 40.92 ± 0.12 | 50.18 ± 0.12 | 46.62 ± 0.24 |
| Phenol | 44.19 ± 0.11 | 56.25 ± 0.16 | 68.97 ± 0.17 | 64.09 ± 0.33 |
| Flavonoid | 44.51 ± 0.26 | 57.45 ± 0.06 | 63.58 ± 0.38 | 53.95 ± 0.25 |
| Reducing Sugar | 23.58 ± 0.12 | 29.41 ± 0.20 | 25.49 ± 0.16 | 30.69 ± 0.12 |
| Cardiac Glycoside | 55.56 ± 0.23 | 39.79 ± 0.23 | 46.65 ± 0.23 | 57.19 ± 0.23 |
| Alkaloid | 34.16 ± 0.13 | Nil | 29.21 ± 0.25 | 36.55 ± 0.25 |
| Steroid | Nil | 27.64 ± 0.18 | 29.45 ± 0.29 | 23.81 ± 0.23 |
| Terpenoid | Nil | 25.88 ± 0.23 | 26.62 ± 0.11 | 20.07 ± 0.35 |
| Saponin | 40.15 ± 0.28 | 56.84 ± 0.28 | 42.91 ± 0.28 | 52.37 ± 0.46 |

Number of replicates = 3

### 3.2 Heavy metal analysis of *Fura da Nono,* Ciklavit®, Katoka Mixtures® and Yoyo Bitters®

Table 4 indicates different concentrations of heavy metals obtained. Arsenic was below detection limit in *Fura da Nono*. The estimated concentrations were found within the maximum tolerable limit in food and herbal medicine as recommended by WHO. The Estimated Daily Intake per body weight of an adult for arsenic, mercury, lead and cadmium in the four herbal products studied, are represented in Table 5. The results of the Hazard Quotients and the Hazard Index are all contained in Table 6. The Hazard Index (HI) for the four products based on Arsenic, Mercury, Lead and Cadmium showed that Ciklavit® (0.7870), Yoyo Bitters® (0.4684) and Katoka Mixtures® (0.9908) conformed to the limit while *Fura da Nono* (1.4144) had value above normal.

**Table 4. Concentration of heavy metals in ppm (mg/kg) in *Fura da Nono*, Katoka Mixtures®, Cikalvit and Yoyo Bitters®**

| **Element** | **Concentration in mg/kg** | | | | **Reference** |
|---|---|---|---|---|---|
| | ***Fura da Nono*** | **Ciklavit®** | **Katoka Mixtures®** | **Yoyo Bitters®** | |
| Arsenic | BDL | 0.26 | 0.41 | 0.08 | 5 |
| Mercury | 0.33 | 0.65 | 0.46 | 0.46 | 0.5 |
| Lead | 4.20 | 6.82 | 3.65 | 5.76 | 10 |
| Cadmium | 0.19 | 0.03 | 0.09 | 0.19 | 0.3 |
| Zinc | 12.31 | 4.25 | 18.18 | 18.02 | 50 |
| Manganese | 3.54 | 37.01 | 23.50 | 18.98 | 200 |
| Copper | 7.85 | 6.89 | 6.51 | 9.00 | 150 |
| Nickel | 1.33 | 1.35 | 8.84 | 7.28 | 10 |

BDL - Below Detection Limit.

**Table 5. Screening of the toxic heavy metals As, Hg, Pb and Cd Based on the Health Risk Assessment Parameters - Estimated Daily Intake (EDI) and Hazard Quotient (HQ).**

| Toxic Heavy Metals | *Fura da Nono* | | Ciklavit® | | Katoka Mixtures® | | Yoyo Bitters® | |
|---|---|---|---|---|---|---|---|---|
| | EDI (mg/kg/day) | HQ | EDI (mg/kg/day) | HQ | EDI (mg/kg/day) | HQ | EDI (mg/kg/day) | HQ |
| As | BDL | BDL | 0.0001 | 0.4856 | 0.0002 | 0.7559 | BDL | 0.1088 |
| Hg | 0.0009 | 0.2603 | 0.0003 | 0.0990 | 0.0002 | 0.0697 | 0.0001 | 0.0498 |
| Pb | 0.0400 | 0.6033 | 0.0037 | 0.1875 | 0.0073 | 0.1150 | 0.0047 | 0.2379 |
| Cd | 0.0005 | 0.5508 | BDL | 0.0149 | BDL | 0.0502 | BDL | 0.0719 |

*BDL – Below Detection Limit, EDI – Estimated Daily Intake HQ – Hazard Quotient

**Table 6: Hazard Quotient and Hazard Index (HI) for *Fura da Nono*, Ciklavit®, Katoka Mixtures® and Yoyo Bitters® based on Arsenic, Mercury, Lead and Cadmium**

| Herbal Products | HQ [As] | HQ [Hg] | HQ [Pb] | HQ [Cd] | HI | Comments based on HI Values |
|---|---|---|---|---|---|---|
| *Fura da Nono* | BDL | 0.2603 | 0.6033 | 0.5508 | 1.4144 | Overall non-carcinogenic health risk is high (>1) |
| Ciklavit® | 0.4856 | 0.0990 | 0.1875 | 0.0149 | 0.7870 | Overall non-carcinogenic health risk is low (<1). |
| Katoka Mixtures® | 0.7559 | 0.0697 | 0.1150 | 0.0502 | 0.9908 | Overall non-carcinogenic health risk is at borderline (~1). |
| Yoyo Bitters® | 0.1088 | 0.0498 | 0.2379 | 0.0719 | 0.4684 | Overall non-carcinogenic health risk is low (<1). |

## 4. Discussion

The similarity observed in the general phytochemical constituents of Katoka Mixtures® and Yoyo Bitters®, in this study, may suggest that they would produce similar biological effects. Expectedly, indications such as management of blood sugar and cholesterol, malaria, pile, arthritis, stomach discomfort and bacterial infections are common to both formulations[27,28]. In any case, their indications do not completely overlap since the formulations were constituted from different plant sources and may thus contain distinct chemical compounds within their shared phytochemical groups. Structural diversity within phytochemical groups has been found to have a restricted distribution in specific families, genera, or species[29].

The findings of this study are in line with Anionye and Onyeneke[9] in identifying Tannins, Flavonoids, Saponin, Alkaloids, Steroids and Terpenoids as phytochemical constituents of Yoyo Bitters®. However, they reported the presence of high concentrations of Phlobatannin, and the absence of Reducing sugars and cardiac glycosides, which directly contrast the results of this study. Imaga and Ogunnusi[30], nevertheless, reported finding cardiac glycosides and Phenols in Yoyo Bitters®.

The phytochemical components detected in *Fura da Nono* with the highest concentration is cardiac glycosides, having a concentration of 55.56 ± 0.23 mg/100 g. This suggests that the consumption of *Fura da Nono* may be good for preventing congestive heart failure and generally improving the health of the heart. Cardiac glycosides are known to exert specific powerful action on the cardiac muscles, increasing the force of heart contraction without a concomitant increase in oxygen consumption, making the myocardium more efficient at pumping blood [31]. The next substantial concentrations of phytochemicals found in *Fura da Nono* belong to the class of Flavonoids (44.51 ± 0.26 mg/100 g) and Phenols (44.19 ± 0.11 mg/100 g). Polyphenols show high antioxidant activity, play a vital role in boosting the body's immune system. They also act as anti-inflammatory and antiviral agents[32,33].

The WHO approved nutritional supplement, Ciklavit®, developed by Prof G. Ekeke in collaboration with Neimeth Pharmaceuticals, PLC., Lagos, Nigeria [34,35], which is widely used for the management of sickle cell disease was found to contain Flavonoids (57.45 ± 0.06 mg/100 g), Saponin (56.84 ± 0.28 mg/100 g) and Phenol (56.25 ± 0.16 mg/100 g) at high concentrations, with Tannin at a moderate concentration of 40.92 ± 0.12 mg/100 g. The highest occurring phytochemical groups in Ciklavit® thus belong to the larger family of Phenolics/Polyphenols. This is in line with studies on the methanol extract of *Cajanus cajan* seed, the primary plant material in Ciklavit®, which reveal that the plant has a high Total Phenol content[35].

The phenolic group, especially the flavonoids and tannins are known for their powerful antioxidant action[36]. Many chronic diseases such as atherosclerosis, cancer, Parkinson's disease, and Alzheimer's; including several hereditary disorders of erythrocytes such as, sickle cell disease, thalassemia and glucose-6- phosphate dehydrogenase (G6PD) deficiency have all been associated with oxidative stress[35].

The repeated polymerization and depolymerization of Sickle Haemoglobin (HbS) which is the hallmark of Sickle Cell Disease is an important trigger of oxidative stress. Also, oxidative-stress-affected erythrocytes (RBCs) are the cause of the joint inflammations experienced by patients during sickle cell crisis [35]. This is why antioxidant agents have been shown to be effective in reducing the pathological consequences of the disease[37].

The heavy metals that pose the most health concern when found in food, drugs (medicines), and dietary supplements are arsenic, cadmium, lead and mercury[38]. These toxic impurities produce harmful effects in the body even at significantly low concentrations. Authorities around the world thus attempt to protect public health by allowing them very low tolerable limits.

In this study, the concentration of toxic heavy metals (arsenic, mercury, lead and cadmium) evaluated in *Fura da Nono*, Ciklavit®, Katoka Mixtures® and Yoyo Bitters® were within WHO limits. Onwordi et al.[39] and Afieroho et al.[40] reported zero level of lead in Yoyo Bitters® in their work, which contrasts the result obtained from our study. These disparities may be due to inconsistent sources for obtaining raw materials used in the production of these herbal products. It is well established that plants cultivated in contaminated soils retain heavy metals due to bioaccumulation[41,42].

Other heavy metals evaluated are zinc, manganese, copper and nickel. These metals, although recognized as micronutrients with critical impacts on health, must be taken with limits because of the notable toxicity associated with their excessive intake. These micro-nutrients were all within stipulated limits in *Fura da Nono*, Ciklavit®, Katoka Mixtures® and Yoyo Bitters®.

However, a more practical approach to understanding the health implications of heavy metal concentration involves the evaluation for systemic toxicity (non-carcinogenic impact) and carcinogenic effect. This study there in, focused on the long-term risk of systemic or non-carcinogenic toxicity associated with the presence of these elements. The reference doses used were therefore, based on chronic exposure (365 days or more). Systemic toxicity is often studied using parameters such as Hazard Quotient (HQ) and Hazard Index (HI).

From the results obtained, HQ for each of the four toxic heavy metals (arsenic, mercury, lead, and cadmium) was below the acceptable level of 1 in *Fura da Nono,* Ciklavit®, Katoka Mixtures® and Yoyo Bitters®. This implies that the consumption of these products will not pose long-term non-carcinogenic risk to consumers[18]. Further analysis was made using Hazard Index to understand the effects of combined exposure to these heavy metals. This revealed over limit level only for *Fura da Nono* (1.414). Even though it is unclear how toxicity is influenced by combined exposure to heavy metals, present risk assessment models assume that when an individual is exposed to more than one metal that has the same or similar organ toxicity, the toxins are additive in their effects[43].

The HI index of Ciklavit®, Katoka Mixtures® and Yoyo Bitters® were within the acceptable threshold level of less than one while *Fura da Nono* was above the threshold specification. However, more stringent measures of quality control should be adopted for all the herbal products, given that consumers may also be exposed to these heavy metals by other means such as inhalation, dermal absorption and drinking water, among others.

## 5. Conclusion

The phytochemical screening of *Fura da Nono*, Ciklavit®, Katoka Mixtures® and Yoyo Bitters® showed that the commonly consumed food drink and herbal products, are rich in phytochemicals. The heavy metal screening also revealed that all the products were within the limits for heavy metals in herbal products. Even though the heavy metal screening indicates a positive safety margin for three of the products (Ciklavit®, Katoka Mixtures® and Yoyo Bitters®), a more comprehensive Hazard Index study of these herbal products, incorporating other channels of exposure to arsenic, cadmium, lead and mercury, is highly recommended. This will provide a more broad-based picture of the non-carcinogenic effects of the long-term use of these products

**Conflict of interest**

The authors hereby declare that there are no competing interests in this work.

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