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# Phytochemical Analysis and Heavy Metal Level in Selected Commercial Herbs Sold in Iperu-Remo, Ogun State, Nigeria

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**Abstract**
*Herbal drinks are popular drinks in Nigeria nevertheless; most herbal drinks do not adhere to WHO standards. This shows the need to examine the concentration of phytochemicals and specific heavy metals in some of the commercially sold herbs in Iperu-Remo was the objective of the study. Four (4) herbs were purchased from vendors. The major aim of the study was to estimate phytochemicals using standard methods while an atomic absorption spectrometer was used for heavy metals estimations. The findings revealed that the highest concentration of Alkaloid was recorded in Agbo Iba (4.11 mg/L), Phenolic compound in Agbo Iba (3.66 mg/L), Tannin was in Agbo Iba (0.411 mg/L), and Terpenoids was in Jedi Jedi (0.931 mg/L). The highest Arsenic (As) concentration was recorded in Opa Eyin (1.02 mg/L), followed by Agbo Iba (0.581 mg/L), Jedi Jedi (0.351 mg/L) while the lowest concentration was recorded in FIJK Flusher (0.0046 mg/L). The highest Cadmium (Cd) concentration was recorded in Opa Eyin (0.273 mg/L), followed by Agbo Iba (0.112mg/L), Jedi Jedi (0.087 mg/L) while the lowest was recorded in FIJK Flusher (0.002 mg/L). As and Cd concentration in the samples were above the WHO standard except for FIJK Flusher. Nigeria as well as other countries with similar practices should incorporate these herbs into the National Healthcare System because there is a need for quality assurance, safety, awareness and standardization of these herbs for the benefit of the consumers.*

**Keywords: Commercial Herbs, Heavy Metals, Phytochemicals, Iperu-Remo, Ogun State**

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## INTRODUCTION

Beverages provide the nutrients required for our wholesome development, growth, and maintenance (Izah *et al.,* 2015). Nigerians consume a wide variety of beverages (Idumah *et al.,* 2020). These beverages are classified into alcoholic and non-alcoholic categories, with the non-alcoholics being in the herbal categories (such as bitter concoctions that are anti-pile, anti-malarial, and anti-jaundice) (Taiwo *et al.,* 2020; Aletan *et al*., 2024).

Herbal medicine, which involves the medicinal use of herbs, shrubs, and plant-based products, existed before the development of modern pharmaceuticals and is still a widely utilized and successful treatment option for a variety of disorders (Umoren *et al.,* 2022). Commercial Medicinal plant species,' secondary metabolites and biologically active phytochemicals, as well as narcotic elements found in tropical and subtropical plants, spices, herb and root extracts, are used to prepare herbal concoctions (Chandrasekara and Shahidi, 2018; Ajani *et al.,* 2024).

Herbal concoction contains a variety of naturally occurring phytochemicals (e.g. alkaloids, tannins, flavonoids, and soluble starches), some of which have medicinal properties (Umoren, 2021). In poly-herbal liquid treatments, herbs are widely utilized as digestive aids, immune system boosters, anti-infection agents, aperitifs, and carminatives (Showande and Amokeodo, 2015).

Due to its low price, accessibility and claims to treat a variety of conditions, including pain, cough, malaria, HIV, infection, and other infectious diseases (Taiwo *et al.,* 2020; Umoren *et al.,* 2022), it is widely embraced by the general public. Many people around the world believe that herbal formulations are safe despite several researches on the harmful consequences of herbal medicine (Okareh *et al.,* 2018).

The negative impact of heavy metals, which enter herbal plants through absorption from water and soil, may be one method for this negative effect to manifest itself (Bolawa *et al.,* 2020; Malik *et al.,* 2024). Because of their high density and/or mass number, heavy metals are sometimes referred to as potentially hazardous elements (Rehman *et al.,* 2020). Drinks contaminated with heavy metals can seriously affect the quality and consumer health (Taiwo *et al.,* 2020).

In Nigeria, herbal drinks are marketed as a panacea, yet the majority have never undergone scientific testing (Anionye *et al.,* 2017). Additionally, because of their well-known health benefits, they are in high demand and are widely used in many Nigerian homes (Nwachuku

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and Elekima, 2019; Aletan *et al*., 2024). The vegetation growing alongside roads and around historic dump sites, and contaminated soils are frequently a source of heavy metals (Malik *et al*., 2024). After being processed into herbal concoctions, heavy metals found in herbal plants will eventually enter the human body and may contribute to the development of biological illnesses such as cancer, diabetes, hypertension, and ulcers (Bolawa *et al*., 2020). The fact that the bulk of these products have not been deemed safe for use by patients is also a big cause for concern (Ajani *et al*., 2024; Aletan *et al*., 2024).

Researchers have reported a variety of phytochemicals and heavy metals in herbs that are sold in Nigerian marketplaces. For instance, Ngumah *et al.* (2023) found that the majority of the samples they examined had significant concentrations of phenol, flavonoids, glycosides, steroids, and terpenoids. Furthermore, even though they are within the authorized allowable levels, heavy metals like Zn, Ni, Cr, Cd, Pb, and Hg was also found in Owerri's open markets. Furthermore, excessive consumption of anti-malarial herbal mixtures may be generally beneficial to health but dangerous due to heavy metals that could expose users to probable cancer risks and other biological process distractions. This information was reported by a study conducted in Kano, Nigeria, on the determination of heavy metals contamination, risk prediction, and antioxidant properties of anti-malarial herbal mixtures sold in the market (Babandi *et al*., 2024).

The inadequacy in regulation by authorized agencies in third-world countries, result in the arrival of herbs in the marketplace with little to no details regarding their usefulness and safety. Therefore, it is essential to estimate the level of heavy metal in commercially sold herbs to provide information for pivotal intervention and policy development. This study hereby focuses on the phytochemical analysis and heavy metal level in selected commercial herbs compared to the W.H.O permissible limit sold in Iperu-Remo, Ogun State, Nigeria.

## MATERIALS AND METHOD

### Study Area

The research was carried out in Iperu-Remo (coordinates: 6.9149°N 3.6649°E), a town located in Ogun State, southwest, Nigeria in December 2023. It is situated close to the Ibu River in the Remo Region of the Ikenne Local Government Area, it is the town with the highest population. The entire LGA is 137.13 $km^2$ in size, and the 2006 census found 178,412 people living there. Large reserves of limestone, which are used in the area's main industry, the making of cement, are found beneath the town of Iperu-Remo in the Remo region. Cocoa and kola nuts are among

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the region's agricultural products. One of the biggest kola nut gathering areas in the nation is Iperu-Remo. The production of baskets and rope, which are used to store kola nuts, is one of the many ancillary industries that the kola nut industry supports.

**Sample Collection**

Four (4) selected commercial herbs samples (Opa-Eyin, Agbo- Jedi, Agbo- Iba and Fijk - flusher) were purchased and transported to the laboratory for phytochemical (alkaloid, total flavonoid, saponin, total phenol and tannin) and heavy metal (Arsenic ($As^{2+}$), Cadmium ($Cd^{2+}$), Copper ($Cu^{2+}$), and Lead (Pb)) estimation.

**Determination of Phytochemicals**

- **Alkaloids Content**

Alkaloids were quantitatively estimated using the alkaline precipitation gravimetric method (Okareh *et al.*, 2018), and the results were represented as mg of alkaloids per mL of the sample. By adding concentrated $NH_4OH$ drop by drop until the solution was fully turbid, the alkaloid in the herbs was precipitated. To recover the precipitate, it was filtered, cleaned with a solution of 1% $NH_4OH$, dried at 100 °C for an hour, cooled in a desiccator, and reweighed. The weight of the alkaloid was calculated and reported as mg alkaloid per mL of the sample by difference.

- **Total Flavonoid**

After 30 minutes of incubation, the total flavonoid content and absorbance at 415 nm were determined using the aluminium chloride technique (Magomya *et al.*, 2015). 20 mL of the herb sample was made up with methanol, and then 0.1 mL Na-K tartrate and 2.8 mL distilled water were added in turn. After 30 minutes of incubation, the test solution was forcefully agitated, and absorbance at 415 nm was measured. A known quercetin concentration was used to create a standard calibration plot at 415 nm. The calibration plot was used to compute the flavonoid concentrations in the test samples, which will then be reported as mg quercetin equivalent/mL of the sample.

- **Total Phenolic Content**

By using a Folin-Ciocalteau assay, the total phenolic content of the sample extract was determined. A 25 mL flask was filled with 5 mL of distilled water, 5 mL of sample, and 1.0 mL of Folin-Caiocalteau reagent. The content was blended and left to stand at room temperature for 5-8 minutes. Then 10 mL of a 7% sodium carbonate solution and distilled water was added to the mark. After mixing the solution and letting it stand at room temperature for 15 minutes, the absorbance at 750 nm was measured (Okareh *et al.*, 2018).

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- **Saponin Content**

Using the methods of Edeoga *et al*. (2005) saponin content was determined. 20 mL of diethyl ether was added after 100 mL of sample was placed in a 250 mL separating funnel. While the ether layer was discarded, the aqueous layer was retrieved. 60 mL of n-butanol was added when the purification procedure was repeated. There were two washes of the combined n-butanol sample using 10 mL of 5% aqueous sodium chloride. In a water bath, the residual solution was warmed. Following evaporation, the samples were oven-dried to a consistent weight so that the saponin concentration could be determined.

- **Tannin Content**

By utilizing the butanol-HCl reagent, the amount of tannin was calculated and represented in mg per mL (Okareh*et al.*, 2018). 3.0 mL of the butanol-HCl reagent, 0.1 mL of the ferric reagent, and 0.5 mL of the sample were added to a glass test tube that has been diluted with 70% acetone. Each tube's mouth was covered with a glass marble before the tubes were placed in a heating block and heated to 97 to 100 °C (or submerged in boiling water) for 60 minutes. After the tubes had been cooled, an absorbance reading at 550 nm was taken.

**Sample Digestion and Heavy Metals estimation**

A quantity of 20 ml of each sample was measured into a flask of 100 ml with 10 ml of Nitric acid added and gently heated on a hot plate inside a fume cupboard. Then, the heating was maintained until the brown vapours became colourless. The beaker was lowered to allow it to reach ambient temperature. The combination was filtered using Whatman no. 42 filter paper, rinsed with 20 ml of deionized water, into a standard 250 ml volumetric flask, and ready for metal estimation. Heavy metals such as $As^{2+}$, $Cd^{2+}$, $Cu^{2+}$ and $Pb^{2+}$ in samples were analyzed using an Atomic Absorption Spectrometer (AAS). Obtained data was computed using Microsoft Excel 2013 version

## RESULT AND DISCUSSION

### Results

### Phytochemicals analysis

The concentration of phytochemicals in the selected commercial herbs is presented in Table 1, it revealed that the highest concentration of Alkaloid was recorded in Agbo Iba (4.11 mg/L), while the lowest was recorded in Jedi Jedi (0.284 mg/L). The highest concentration of phenolic compound was recorded in Agbo Iba (3.66 mg/L) while the lowest was recorded in Opa Eyin (1.13 mg/L) with phenolic compound not detected in FIJK Flusher. The highest concentration

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of Saponin was recorded in Agbo Iba (0.17 mg/L), while the lowest was recorded in Opa Eyin (0.035 mg/L) with Saponin not detected in Jedi Jedi. The highest concentration of Tannin was recorded in Agbo Iba (0.411 mg/L) while the lowest was recorded in Opa Eyin (1.93 mg/L). The highest concentration of Terpenoids was recorded in Jedi Jedi (0.931 mg/L) while the lowest was recorded in Agbo Iba (0.112 mg/L).

Table 2: Phytochemicals in selected commercial herbs

| Heavy Metals (mg/L) | Agbo Iba | Opa Eyin | Jedi Jedi | FIJK Flusher |
|---|---|---|---|---|
| Alkaloid | 4.11 | 1.93 | 0.284 | 0.891 |
| Phenolic Compound | 3.66 | 1.13 | 1.58 | nd |
| Saponin | 0.17 | 0.035 | nd | 0.067 |
| Tannin | 0.411 | 0.027 | 0.122 | 0.089 |
| Terpenoids | 0.112 | 0.243 | 0.931 | 0.323 |

Nd=not detected

**Heavy metal concentration**

Heavy metal concentration in the selected commercial herbs is presented in Table 2, the result revealed that the highest Arsenic (As) concentration was recorded in Opa Eyin (1.02 mg/L), while the lowest was recorded in FIJK Flusher (0.0046 mg/L). The highest Cadmium (Cd) concentration was recorded in Opa Eyin (0.273mg/L) while the lowest was recorded in FIJK Flusher (0.002 mg/L). The highest Copper (Cu) concentration was recorded in Opa Eyin (0.121 mg/L), while the lowest was recorded in FIJK Flusher (0.003 mg/L). The highest Lead (Pb) concentration was recorded in Agbo Iba (0.001mg/L), while the lowest was recorded in FIJK Flusher (0.0004 mg/L) with Pb not detected in Opa Eyin and Jedi Jedi.

Table 3: Heavy metals (mg/L) concentration in selected commercial herbs

| Heavy Metals | Agbo-Iba | Opa-Eyin | Jedi -Jedi | FIJK Flusher | WHO (2011) |
|---|---|---|---|---|---|
| **As** | **0.581** | **1.02** | **0.351** | 0.0046 | 0.01 |
| **Cd** | **0.112** | **0.273** | **0.087** | 0.002 | 0.003 |
| **Cu** | 0.103 | 0.121 | 0.113 | 0.003 | 2.00 |
| **Pb** | 0.001 | nd | nd | 0.0004 | 0.01 |

*NB: Values in bold are higher than the WHO Standard* *Nd=not detected*

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**Discussion**

Herbal beverages are a type of beverage, generally alcoholic, that is flavored with herbal essences and characterized by a bitter or bitter-sweet taste. Herbal drinks are made from secondary metabolites and biologically active phytochemicals from medicinal plants, narcotic components of tropical and subtropical plants, spices, herbs, and root extracts. The individual or combined phytoconstituents of a medicinal plant define its therapeutic value. Some of the significant phytochemicals with a variety of biological activity found in different portions of medicinal plants include alkaloids, flavonoids, phenolic, tannins, saponins, steroids, glycosides, and terpenes (Ezeonu & Ejikeme 2016). Phytochemical screening of the herbs studied showed that all the herbs have Alkaloids, Tannin and Terpenoids. The maximum number of metabolites was found in Agbo Iba and Opa Eyin while the minimum was recorded in Jedi Jedi and FIJK Flusher. The highest concentration of alkaloids was recorded in Agbo Iba which is used in the treatment of malaria. Alkaloid phytochemicals can suppress and act on viruses through a variety of methods. They can impede viral fusion or attachment with the host cell surface receptors preventing entry into host cells. They can also interfere with viral DNA or RNA synthesis (Fikatas, 2015).

Phenolic compounds have been described as exhibiting anticancer activity in vitro and in vivo and their efficacy varies from one compound to another, which is due to the variations in their structures as well as their molecular targets (Shahidi, and Ambigaipalan, 2015; Anantharaju *et al.,* 2016). The phenolic compound was recorded in the studied herbs but not detected in FIJK Flusher. The concentration of Phenolic compounds in the herbs followed the downward order of Agbo Iba>Jedi Jedi>OpaEyin.

Saponins can also be beneficial for hyperlipidaemia and are capable of reducing the risk of heart disease in humans. Saponins may play a major role in protection from cancer. Research on colon cancer cells suggests that it is the lipophilic saponin cores that may be responsible for this biological activity (Mohan *et al,* 2016). The concentration of saponin in the herbs shows a downward order of Agbo-Iba>FIJK Flusher>Opa-Eyin.

Tannins are chelating agents for metals and can form complexes with macromolecules through the process, essential substrates and enzymes of microorganisms are depleted leading to cell death. Tannins are good anti-microbial agent with precipitate protein thereby providing water proof layer on the skin when used externally or protect the underlying layers of the skin and limit the loss of fluid. They are also known to be good anti-viral agents (Okerulu, 2017). The

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concentration of Tannins in the herbs followed the downward order of Agbo Iba> Jedi Jedi>FIJK Flusher>OpaEyin.

Total Terpenoids are a beneficial phytochemical present in all the studied herbs. Terpenoids represent the largest and most diverse class of beneficial chemicals. More than 40,000 individual terpenoids exist. Plants use terpenoid metabolites to support basic functions like growth, repair and development. Among humans, terpenoids have long been valued for medicinal purposes, and they've also been used for food, pharmaceutical and chemical purposes. The cancer drug Taxol and the antimalarial drug artemisinin are both terpenoid-based drugs, (Mercola, 2017). The concentration of Terpenoids in the herbs followed the downward order of Jedi Jedi>FIJK Flusher>OpaEyin>AgboIba

The overall results indicated clearly that As, Cd, Cu are present in all the investigated commercial herbs with an acceptable level. The presence of heavy metals in the herbs can be attributed to contaminated soils where the plant materials used for the production of the herbs were cultivated. Heavy metals are mostly hazardous and carcinogenic as they tend to accumulate in the visual and sensory organs of human beings leading to cancer. Thus, a substance meant for consumption must be free from chemical contaminants for it to be safe for drinking (Bolawa *et al.*, 2020). The concentrations of two of the heavy metals (As and Cd) in the herbs, Opa Eyin, Agbo Iba and Jedi Jedi except FIJK flusher were above the WHO standard for As (0.01mg/L) and Cd (0.003 mg/L) respectively.

Arsenic is one of the non-essential heavy metals found in the environment. Its concentration in ingestible items suggests contamination. However, arsenic has been reported in potable water resources in Nigeria (Izah and Srivastav, 2015). The concentration of Arsenic in three of the studied herbs (Agbo Iba, Opa-Eyin and Jedi Jedi) sold is above the WHO permissible limit of 0.01 mg/L. The As concentration from the herbs was also higher than the As concentration reported in FIJK flusher (0.00578 mg/L) and Darasi bitters (0.00974 mg/L) in Nigeria (Bello *et al.*, 2016). Tchounwou *et al.* (2012) and Izah *et al.* (2017) reported that high arsenic exposure could lead to cardiovascular, haematological, neurological, respiratory, gastrointestinal, and developmental disorders, dermatitis and cancer, diabetes, and hearing loss. As such, high concentrations of arsenic could affect the kidney, liver, bladder, and skin (Izah *et al.*, 2017).

The concentration of cadmium in three quarter (¾) of the samples, Opa Eyin (0.273 mg/L), AgboIba (0.112 mg/L) and Jedi Jedi (0.087 mg/L) were above the WHO standard of 0.003 mg/L. This is also higher than the concentration recorded for bitters (0.012 mg/L), anti-malaria

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(0.025 mg/L) and anti-pile (>0.01 mg/L) in a study in Abeokuta (Taiwo *et al.*, 2020). A high concentration of cadmium causes a hazardous effect on human health, whereby the kidney serves as a target organ for exposure. Based on its mechanism of action, cadmium causes damage to cells primarily through the generation of reactive oxygen species (ROS). The role of Cd in neurodegenerative disorders is further emphasized by its effect on diverse CNS cell types (Branca J. *et al*., 2020)

Copper is an element that is an important component of various enzymes and it plays a significant role in the production of melanin, free radical elimination, iron utilization, etc. However, excessive consumption of copper can cause diarrhoea, vomiting, liver damage, nausea, and abdominal pain (Aletan *et al*., 2024; Ajani *et al*., 2024). The concentration of copper in the entire sample was lower than the WHO standards of 2.0 mg/L. The Cu concentrations in all the samples, Opa Eyin (0.121 mg/L), Jedi Jedi (0.113 mg/L), AgboI ba (0.103 mg/L) and FIJK Flusher (0.003 mg/L) respectively from the study were lower than the report from Alomo bitters (0.14 mg/L) and Origin Bitter (0.61 mg/L) in an investigation form Lagos state (Bolawa *et al*., 2020).

Lead is described by the United States Environmental Protection Agency as potentially hazardous to most forms of life (USEPA, 2015). Pb is an extremely toxic heavy metal that disturb various plant physiological processes and unlike other metals such as Zinc (Zn), Manganese (Mn) and Copper (Cu), it does not play any biological functions. Moreover, it fastens the production of reactive oxygen species (ROS), causing lipid membrane damage that ultimately leads to damage of chlorophyll, photosynthetic processes and suppresses the overall growth of the plant (Najeeb U, *et al*., 2017). The concentration of Lead was observed in only Agbo-Iba and FIJK Flusher at a concentration lower than the W.H.O standard (0.01 mg/L) indicating that it is free from lead contamination. The recorded Pb concentration was also lower than the report made from Origin Bitter (0.001 mg/L) and Alomo bitters (0.07 mg/L) in Lagos state (Bolawa *et al.,* 2020) and anti-malaria (0.08 mg/L) in Abeokuta (Taiwo *et al.*, 2020).

## CONCLUSION

In Iperu-Remo, plants and various herbal remedies are peddled by the side of the road and sold in shops, and the majority of them are not approved for human consumption by the appropriate drug agencies. The study investigates the concentrations of some phytochemical and heavy metals in four selected herbs sold commercially. Alkaloids, Tannin and Terpenoids were detected in all the samples. The concentration of As and Cd in the herbs (Opa Eyin, Agbo Iba

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and Jedi Jedi) were above the WHO standards. Therefore, Nigeria as well as other developing countries with similar practices should incorporate these herbs into the National Healthcare System because there is an urgent need for quality assurance, safety and standardization of these herbs for the benefit of the consumers.

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