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Heavy metal composition and phytochemical constituents of selected herbal remedies sold in open markets in Owerri metropolis, south east Nigeria

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Abstract
Heavy metal composition and Phytochemical constituents of selected herbal remedies sold in Owerri, South East Nigeria, were studied using standard laboratory procedures. Results obtained revealed that apart from proteins, all the phytochemicals studied were present in all the samples. High levels of phenol, flavonoids, glycosides, steroids and terpenoids were present in most of the samples. Total phenol values as high as 22900.88±1409.19 µg/ml and flavonoids 2644.89±6.94 µg/ml were recorded. Heavy metals identified in these samples included Zn, Ni, Cr, Cd, Pb and Hg. The concentration of Pb (0.081-0.124 ppm), Zn (0.045 -0.223 ppm), Cd (0.003 to 0.045 ppm) and the other heavy metals analyzed did not exceed the approved permissible limits. High levels of these heavy metals when consumed by humans can cause serious health issues. Also, the presence of high levels of phenolic acid, flavonoids, tannins, and alkaloids in samples is a strong indication of the therapeutic capacity of the samples. Therefore, it is important to ensure that this natural resource continues to be beneficial to the global health sector, it is important to set uniform standards and to monitor the quality of herbal treatments for dangerous ingredients.

Keywords: Phytochemicals, Heavy metals, permissible limits, therapeutic capacity, hazardous

Introduction
Herbs are readily available, inexpensive, and easy to access, herbs are currently used extensively in both developed and developing countries for healthcare (Sato, 2012) [58]. The use of herbs as medicine has expanded, primarily because they are safer than modern medication because they come more likely to be secure because they come from natural sources. Present-day medicine comes from manufactured compounds (Inamdar *et al*., 2008) [3]. Due to this, traditional medicine, particularly herbal medicines, has seen tremendous development in popularity worldwide in the latter half of the 20th century (Jayaraj, 2010) [4]. It's typical for herbal remedies to contain heavy metals (Roberts *et al*., 2008; Martena *et al*., 2010; Mohammed *et al*., 2010; Bushra *et al*., 2011; Kiran *et al*., 2011) [11, 7, 8, 1, 5]. This could be caused by a number of factors, including purposeful inclusion for purported therapeutic and medicinal benefits, manufacturing processes, and/or incidental contamination when cultivated in soil contaminated with sewage and industrial waste (Garvey *et al.*, 2001; Sayyed & Sayadi, 2011; Nkansah *et al.*, 2016) [2, 12, 9]. Heavy metals typically bioaccumulate in food chains as a result of domestic, commercial, industrial, and agricultural operations that release them into the environment. Concerns and anxiety have grown as a result of the unsupervised usage, effectiveness, toxicity, and quality of these herbs and natural products. Trace metal toxicity on human health and the environment has received a lot of attention recently. Due to their sensitivity to environmental factors and their capacity to accumulate heavy metals in their usable sections, plants have the ability to alter their overall elemental makeup. They achieve this by root uptake, foliar adsorption, and the deposit of certain components on their leaves (Schreck & Foucault, 2012). Arsenic, lead, cadmium, and mercury are a few examples of heavy metals that are often harmful and not required for plant growth. Herbs grown in contaminated places may cause the transmission of harmful heavy metals to people and other animals, which is a worry for traditional and herbal treatment because plants are the primary conduit for the transmission of heavy metals to people from contaminated soil (Yap *et al*., 2010) [13]. Consuming herbal items made from medicinal plants that were produced in polluted environments might have major negative effects on a person's health. Because of their propensity to bioaccumulate in food chains and their slow kidney excretion rates, heavy metals may harm people even at extremely low quantities.

Metals are vital nutrients that the body needs for its physiological and biochemical functions. Metals like zinc, copper, iron, manganese, and chromium are examples of important metals. However, if its intake is increased beyond what is permitted, it could become harmful (Korfali *et al.*, 2013) [6]. A number of journals devoted to Asian, South American, and African herbal goods have previously reported on the contamination of herbal treatments [12–16]. The safety of conventional herbal treatments offered in Nigerian markets, however, is mostly unknown. In order to evaluate certain commonly used herbal products' relative safety and potential health concerns in accordance with the objective of this study was to determine the level of contamination in each product in relation to the World Health Organization's (WHO) regulatory standards.

2. Materials and Method

2.1 Procurement of herbal remedies

Samples for this study were obtained in open markets (Owerri North, Owerri West, and Owerri Municipal) in the city of Owerri, Imo State, Nigeria.

Thirty (30) samples of each of the ready-to-use herbal remedies were obtained randomly from selected sale outlets, using standard methods as described by Cheesebrough (2006). Samples collected included; Sample A (used for malaria), Sample B (used for typhoid), Sample C (used for Candidiasisdasis), and Sample D (used for sex enhancement) herbal remedies.

2.2 Preliminary qualitative screening for the presence of phytochemicals

The herbal treatments were examined for the content of steroids, terpenoids, phenols, tannins, flavonoids, saponins, glycosides, alkaloids, and carbohydrates. Using techniques developed by Sofowora (1993) [15], Evans & Trease (1989), Harborne (1973) [16], and Yadav & Agarwala (2011) [14].

For each test detailed below, a volume of 1 ml of each sample was utilized. Each test used one milliliter of water as the control.

2.3 Quantitative Phytochemical Screening

Total phenolic content determination: The samples' phenol content was assessed using the Folin-Ciocalteu reagent method, as reported by Yadav & Agarwala (2011) [14].

Total flavonoid content was calculated using quercetin as a reference and the aluminum chloride colorimetric method as published by Muthukumaran *et al.* (2016).

Total tannin content determination: The Folin and Ciocalteu method was used to determine the tannin content of the samples with minimal modification.

Calculation of total alkaloids present

According to Mathew G., the total alkaloids present were expressed as atropine equivalents (g AE/mg of dry extract) (2017).

2.4 Heavy metal content of samples

Using a Varian AA240 Atomic Absorption Spectrophotometer (AAS) and the APHA technique, the concentrations of Zn, Cd, Hg, Cr, Ni, and Pb in the filtrate of herbal treatments were measured (1998)

3. Result

Tables 1 and 2 represent the results of the qualitative and quantitative analysis of phytochemicals Present in the samples studied. Apart from proteins, all the phytochemicals studied were present in all the samples except sample D which lacked saponins and glycosides, and sample A which lacked Steroids. High levels of phenol, flavonoids, glycosides, steroids, and terpenoids were present in abundance in sample C, moderate levels in sample B, and trace levels in samples A and D. Total phenol values as high as 22900.88±1409.19 (µg/ml) and 4013.14±245.60 (µg/ml) were recorded for samples B and C respectively. The same pattern was observed for flavonoids 2294.89±8.39 (µg/ml) and 2684.89±6.94 (µg/ml) in samples B and C respectively Table 3 represents the heavy metal concentration of the samples studied. Sample B had the highest Zn (Zinc), Hg (mercury), and Cr (Chromium) concentration values when compared with other samples. Sample C had the lowest metal concentration n Cd, Hg, and Cr, whereas, sample A and D indicated moderate metal concentration across all parameters.

Table 1: Qualitative phytochemistry results

S/no	Phytochemical	Test	Sample A	Sample B	Sample C	Sample D
1	Proteins	Millon's test	-	-	-	-
2	Carbohydrates	Iodine test	+	++	+	++
3	Phenolic acid	Ferric chloride test	+	++	+++	+
4	Tannins	Gelatin test	+	++	++	++
5	Flavonoids	Alkaline reagent	+	++	++	+
6	Saponins	Frothing test	+	++	++	-
7	Glycosides	Keller-kilani test	+	++	+++	-
8	Steroids	Liebermann-Burchard's test	-	+	+++	+
9	Terpenoids	Salkowski's test	+	++	+++	+++
10	Alkaloids	Wagner's test	+	++	+	+

Key: "-" (Absent), "+" (Slightly Present), "++" (Moderately Present), and "+++" (Slightly Present in Abundant Level).

Table 2: Results for quantitative analysis of the phytochemicals

	A	B	C	D
Total Phenol (µg/ml)	242.47±2.03	22900.88±1409.19	4013.14±245.60	1555.35±17.71
Total Flavonoids (µg/ml)	519.33±3.33	2294.89±8.39	2684.89±6.94	852.67±20.28
Tannins (µg/ml)	246.05±0.48	588.11±0.73	603.03±0.73	454.14±2.08
Alkaloids (µg/ml)	18.51±0.23	1778.09±61.96	17.10±0.25	24.91±0.14

Values are shown as the mean and standard deviation of three sets of data.

Table 3: Heavy metal content of herbal remedies

Samples	Zinc (ppm)	Cadmium (ppm)	Lead (ppm)	Mercury (ppm)	Chromium (ppm)	Nickel (ppm)
A	0.097±0.001	0.008±0.002	0.081±0.003	0.031±0.003	0.007±0.002	0.008±0.003
B	0.223±0.001	0.038±0.001	0.109±0.001	0.063±0.004	0.019±0.001	0.012±0.001
C	0.128±0.004	0.003±0.001	0.124±0.001	0.007±0.001	0.003±0.003	0.009±0.001
D	0.045±0.002	0.045±0.003	0.088±0.004	0.041±0.002	0.012±0.002	0.019±0.004

4. Discussion

Phytochemical Analysis (Quantitative and Qualitative)

Traditional medicine has been practiced and accepted by people for a very long time and has been there since the beginning of time. Herbal medicines have gained popularity as alternative/complementary medicine and nutritional supplement in recent years. The basis for using plants as medicines is the presence of mixtures of different biologically active plant constituents or phytochemicals (secondary metabolites), such as alkaloids, glycosides, terpenoids, and so on, that may act singly, additively, or synergistically to produce an effect that may be beneficial or harmful to health. The majority of medicinal plants have been used to treat a range of illnesses and may have pharmacological effects such as antibacterial, antioxidant, and anti-inflammatory qualities, analgesic, anti-diabetic, anti-hypertensive, and antidiarrheal. 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) [30]. All throughout the world, a large range of herbal medications are easily accessible on the market. As the use of herbal medicines has risen, public health concerns about the safety and efficacy of herbal therapy have emerged. Phenolic acid, flavonoids, tannins, and alkaloids at high concentrations in samples B and C is a reliable indicators of the samples' medicinal potential. It has long been established that flavonoids and phenolic chemicals are beneficial to human health, particularly in the treatment and prevention of various diseases. They frequently appear in foods as nutrients Flavonoids and a variety of other phenolic compounds found in herbal medicines have been shown to be powerful antioxidants, anticancer, antibacterial, cardioprotective, anti-inflammatories, immune system boosters, skin protectors from UV rays, and intriguing candidates for pharmaceutical and medical use. These substances are known as plant secondary metabolites. They possess a (Kumar & Pandey 2013; Chen *et al*. 2014; Dziao *et al*. 2016; Andreu *et al.* 2018; Meng *et al*. 2018) [19, 21] aromatic ring with at least one hydroxyl group. A class of significant polyphenols known as flavonoids is found frequently in plants. They function as antioxidants or free radical scavengers because they have more than one benzene ring in their structures (Kar 2007) [33]. According to Rice-Evans *et al.* (1996) [34], flavonoids possess anticoagulant, anti-inflammatory, and aphrodisiac effects and function to lower the incidence of coronary heart disorders (Houghton *et al*., 2005) [35]. Phenols are antioxidants that help prevent a number of degenerative disorders (Rice-Evans *et al.,* 1996) [34]. They function as anti-inflammatory and anticancer agents and guard against heart conditions. The management of human illnesses brought on by pathogens is one of the many uses of phenols, which also serve as a plant's defense against pathogens and predators of herbivores (Puupponen-Pimiä *et al.,* 2008) [36]. Other glycosides are known to have pharmacological actions with exceptional therapeutic potential. Many glycosides are used therapeutically. Glycosides are organic substances that come from plants or animals. Analgesic, anti-inflammatory, cardiotonic, antibacterial, antifungal, antiviral, and anticancer properties are some of their pharmacological activity. Particularly in Asian (Japanese and Chinese) medicine, tannin-containing plant extracts are used as astringents, against diarrhea, as diuretics, against stomach and duodenal cancers, and as pharmaceuticals for anti-inflammatory, antibacterial, antioxidant, and hemostatic purposes. Tannins are used in the dyestuff industry to make cationic dyes (tannin dyes) as well as in the production of inks (iron gallate ink). Scientists' interest in tannins has lately increased due to the rising incidence of deadly diseases including AIDS and various cancers.

Heavy Metal Analysis

Human health has been threatened by heavy metal pollution in herbal remedies, especially at concentrations exceeding recognized threshold values. Numerous studies on Nigerian herbal treatments have revealed that the majority of the samples had high levels of heavy metals (Obi *et al*., 2006) [10]. This is a clear sign that using Nigerian herbs frequently, even in the prescribed doses, is likely to have negative consequences on one's health. All of the heavy metals that were evaluated in this investigation were discovered in varied amounts in the herbal beverages proportions. These heavy metals' prevalence is consistent with past research that found contaminants in soft drinks, beverages, and herbal goods in Nigeria, including heavy metals like cadmium, copper, iron, nickel, selenium, zinc, lead, and mercury (Adepoju-Bello, *et al*., 2012) [48-49]. The range of lead (Pb) levels in the examined samples was 0.081-0.124 ppm. (WHO 2006, WHO 2005) [38, 39] The FAO/WHO maximum permitted value of lead in eaten medicinal plants is 10 ppm. The findings made it very evident that none of the examined samples had ever exceeded the allowable limit. Previous investigations have revealed high concentrations of lead in medicinal plants and herbs that are above acceptable levels. The typical mean lead concentration is according to Martin and Griswold (2009), the greatest lead concentrations found in both Spices and medicinal herbs were 14.4 ppm and 21.7 ppm in Egypt and Iran, respectively. According to reports, Jordan's common medicinal herbs have 13.9 ppm lead on a dry weight basis. (Abou-Arab & Abou Donia, 2000; Ziarati, 2012) [40, 41]. A very dangerous environmental contaminant is lead. It can interact negatively with different biomolecules by forming complexes with them. According to Johnson (1998) [45] and the Agency for Toxic Substances and Disease Registry (ATSDR), lead overexposure typically has negative effects on the blood, nervous, immune, renal, skeletal, muscular, reproductive, and cardiovascular systems, leading to poor muscle coordination, gastrointestinal symptoms, damage to the brain and kidneys, hearing and vision impairments, and reproductive defects. Additionally, lead exposure during fetal and early infancy is linked to a number of negative outcomes, including learning disabilities and slower cognitive development (Johnson 1998

[45]; Agency for Toxic Substances and Disease Registry) (ATSDR, 2007) [46]. No sample showed a zinc concentration greater than 50 ppm, the FAO/WHO permissible limit (PL) specified for zinc in herbal medicines. The zinc concentration in the examined samples ranged from 0.045 to 0.223 ppm (WHO 2005, WHO 2006) [38, 39]. Zinc is a important trace element required for thyroid function, blood coagulation, healthy growth, and the synthesis of proteins and DNA. Zinc use in excess has detrimental consequences on copper, blood lipoprotein levels, and the immunological system levels (Fosmire 1990). Every sample tested positive for cadmium. Overall, none of the samples exhibited high levels of cadmium above 0.3 ppm, the permitted limit (PL) established by FAO/WHO for therapeutic herbs and plants in various nations. The Cadmium (Cd) concentration ranged from 0.003 to 0.045 ppm (WHO 2005, WHO 2006) [38, 39]. However, other investigations (Ziarati, 2012; Abou-Arab & Abou Donia, 2000) [40, 41] have discovered significant cadmium concentrations in Egyptian and Iranian medicinal plants and herbs.

5. Conclusion

The study's findings on flavonoids and numerous other phenolic components qualify herbal medicines as potent antioxidants, anticancer, antibacterial, cardio protective, and anti-inflammatory agents as well as intriguing prospects for pharmaceutical and medical use. The obtained results also showed that all of the herbal treatments had concentrations of all six potentially hazardous heavy metals that were examined that were far lower than allowed. When discussing herbal medicines for human consumption, the implications of the current findings may be taken into account. To assess the dosage of herbal medicines made from these plants, toxic metal analysis can be helpful (Parveen *et al*., 2013). Establishing worldwide standards and quality criteria for dangerous ingredients in herbal medicines is therefore highly advantageous so that this natural resource can continue to grow and improve global health (Luo *et al.*, 2019).

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