Investigation of possible heavy metal contaminants of *Zingiber officinale* rhizomes and *Allium sativum* bulbs sourced from a market in Enugu State of Nigeria using rat models

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**Abstract**

Natural products such as plants, animals, microorganisms, and aquatic organisms have been employed by humans from ancient time for treatment and prophylaxis of diseases. In the last decade, attention is being diverted towards traditional medicine partly due to high cost of synthetic and semi-synthetic drugs, increase in multidrug resistant disease causing microorganisms, and unavailability of certain drugs. A major limitation is heavy metal contamination. This study is aimed at investigating the possible heavy metal contaminants of *Zingiber officinale* rhizomes and *Allium sativum* bulbs sourced from a market in Enugu State of Nigeria using rat models. Phytochemical analysis and acute toxicity studies were done according to standard methods with slight modifications. The Atomic Absorption Spectroscopy (AAS) technique was used for extraction and assay of the possible heavy metal contaminants. The phytochemicals in *Zingiber officinale* were alkaloids, tannins, flavonoids, steroids and terpenoids and those of *Allium sativum* were alkaloids, saponins, flavonoids, and glycosides. The actual lethal doses (actual LD50) of *Zingiber officinale*, *Allium sativum* and combination of the two were 8,660, 4,472, and 5,477 mg/kg body weight respectively. The respective amounts of the tested heavy metals (lead, cadmium, mercury, chromium and arsenic) present in the samples were 0.041, 0.082, 0.084, 0.061 and 0.041 ppm in *Zingiber officinale* and 0.012, 0.018, 0.039, 0.045 and 0.030 ppm in *Allium sativum*. These were below the WHO permissible limits. This study therefore concluded that heavy metals contamination of herbal products are inevitable but can be controlled and minimized to WHO acceptable limit.

**Keywords:** *Allium sativum* bulbs; Ethanol extracts; Heavy metal contaminants; *Zingiber officinale* rhizomes

1. Introduction

Natural products such as plants, animals, microorganisms, and aquatic organisms have been employed by humans from ancient time for treatment and prophylaxis of diseases. A research done on this by means of fossil records showed that human use of plants as medicines may have begun as far back as 60,000 years ago (Yuan *et al*., 2016). Such use of natural products was classified under a more general term known as traditional medicine. Traditional medicine was defined as health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied individually or in combination to treat, diagnose and prevent illnesses or maintain well-being (Fokunang *et al*., 2011). In the last decade, attention is being diverted towards...
The concentrations of Pb, Cd, As, and Hg were all under acceptable limits (Luo et al., 2016). African traditional medicine is a form of holistic health care system organized into three levels of specialty, namely divination, spiritualism, and herbalism in which the traditional healer provides health care services based on culture, religious background, knowledge, attitudes, and beliefs that are prevalent in his community (Ezekwesili-Ofili and Okika, 2019). Previous researches had revealed high rates of traditional medicine usage in Nigeria. In a study done on adult women in Ibadan Nigeria, the overall proportion of traditional medicine use was 81.6%. Women from the Igbo and Hausa ethnic groups were significantly less likely to use traditional medicine than the majority Yoruba group (Li et al., 2020).

Despite the fact that plants are rich in a variety of compounds many of which are secondary metabolites and include aromatic substances such as phenols or their oxygen-substituted derivatives such as tannins among others with immeasurable pharmacological activities, heavy metal contamination in herbal medicines is a global threat to human beings especially at levels above known threshold concentrations. Herbal medicines have been reported to contain many contaminants that are potentially harmful to health. These include heavy metals, such as lead (Pb), cadmium (Cd), arsenic (As), copper (Cu), chromium (Cr) and mercury (Hg). According to an earlier study, heavy metal toxicities related to the use of traditional medicines have been reported globally. These heavy metals may be introduced into medicinal plant products through contaminated agricultural resources and/or poor production practices and deliberate addition of heavy metals for alleged medicinal value (Street, 2012). A study aimed at measuring the levels of Pb, Cd, As, and Hg in numerous orally administered herbal products available in the Iranian market was done. Fifteen products labeled (A-O) of different brands from three different production batches (n = 45) were procured from traditional herbal medicine manufacturers in Iran. Each sample was digested with nitric acid by the wet digestion method, and the resultant solutions were used to determine the concentrations of Pb, Cd, As, and Hg. The lead, cadmium, arsenic, and mercury contents in the investigated samples did not show significant levels that may be associated with toxicity. All four metals were present at concentrations below the limits recommended by the WHO (World Health Organization), American Herbal Products Association (AHPA), and Canadian standard, but in several herbal products, the concentrations of these trace elements exceeded the Agency for Toxic Substances and Disease Registry (ATSDR). The concentrations of Pb, Cd, As, and Hg in commercially available herbal remedies were well below the acceptable intake recommended by global recommendations. The researchers concluded that at present, the amount of heavy metals in medicinal herbs processed at the level of supply by licensed pharmacies is favorable (Keshvari et al., 2021). In a similar study, the concentrations of five heavy metals cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg) and copper (Cu) were investigated using Inductively Coupled Plasma Optical Mass Spectrometry (ICP-MS) with 1773 samples around the world. According to Chinese Pharmacopoeia, 30.51% (541) samples were detected with at least one over-limit metal. The over-limit ratio for Pb was 5.75% (102), Cd at 4.96% (88), As at 4.17% (74), Hg at 3.78% (67), and of Cu, 1.75% (31). For exposure assessment, Pb, Cd, As, and Hg have resulted in higher than acceptable risks in 25 kinds of herbs. The maximal Estimated Daily Intake of Pb in seven herbs, of Cd in five, of Hg in four, and As in three exceeded their corresponding Provisional Tolerable Daily Intakes. In total, 25 kinds of herbs present an unacceptable risk as assessed with the Hazard Quotient or Hazard Index. Additionally, the carcinogenic risks were all under acceptable limits (Luo et al., 2021). A more recent study reiterated that people are becoming poisoned through the consumption of herbal remedies that comprise heavy metals (HMs) worldwide and that it is possible for HMs to be present in pharmaceutical herb materials coming from anthropogenic activities like agriculture, industrial waste, and natural sources. In the study, the researchers focused on the assessment of the impacts of HMs on plants, sources of HMs, herbal sample collection, and identification techniques, especially in medicinal plant samples. At the same time, it focuses on the sociocultural applications of HMs as well as the dangers associated with their usage in conventional therapies. They concluded that due to the prevalence of heavy metals and the hazards that go with them, it is necessary to implement appropriate regulation and monitoring systems for natural supplements (Vinogradova et al., 2023).

### 1.1 Pharmacological implications of herbal heavy metal contamination

Contamination of medicinal plants by heavy metals is hazardous to both animal and human health due to the fact that heavy metals have low rate of elimination from the body and they accumulate in the soft tissues (Briffa et al., 2020). According to the researchers, heavy metals interfere with the normal biochemical and metabolic processes of the body, thus resulting in toxicity such as cancer. In another study, several health problems were linked to excessive uptake of dietary heavy metals, including decreased immunity, cardiac dysfunction, fetal malformation, impaired psychosocial and neurological behavior (Luo et al., 2021). Furthermore, despite the fact that Cu is an essential component of many enzymes, its excessive intake can cause dermatitis, irritation of the upper respiratory tract, abdominal pain, nausea, diarrhea, vomiting, and liver damage. On the other hand, As and Hg can damage pulmonary, nervous, renal and respiratory systems, as well as causing skin pathology. They may also induce disorders in the central nervous system.
liver, lungs, heart, kidney and brain (Balal-Mood et al., 2021). Therefore, the need to carry out a comprehensive risk assessment of heavy metal contamination in herbal medicines cannot be over emphasized and a study of accurately quantified heavy metal contents in herbal medicines appears necessary to further assess and justify the dosages of herbal formulas (Lu et al., 2020). Heavy metal toxicities depend upon the absorbed dose, the route of exposure and duration of exposure which may be acute or chronic. These toxicities can be as a result of excessive damage due to oxidative stress induced by free radical formation. A number of public health measures have been embarked on to control, prevent and treat metal toxicities occurring at various levels, such as occupational exposure, accidents and environmental factors. However, in addition to the natural sources of these heavy metals, African natural plant-based products have evolved to incorporate various synthetic products such as heavy metals for alleged medicinal properties (Street et al., 2012).

1.2. Mechanisms of heavy metal toxicity

Arsenic is one of the most important heavy metals causing health problems to humans. Mechanism of arsenic toxicity can be ascertained from its biotransformation in which harmful inorganic arsenic compounds get methylated by bacteria, algae, fungi and humans to give monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). In this biotransformation process, these inorganic arsenic species (iAs) are converted enzymatically to methylated arsenicals which are the end metabolites and the biomarker of chronic arsenic exposure. Bio methylation is a detoxification process and end products are methylated inorganic arsenic such as MMA (V) and DMA (V), which excreted through urine are bio indication of chronic arsenic exposure. However MMA (III) is not excreted and remains inside the cell as an intermediate product and it’s found to be highly toxic compared to other arsenicals, potentially accountable for arsenic-induced carcinogenesis (Khairul et al., 2017). Lead is an extremely toxic metal whose widespread use has caused extensive environmental contamination and health problems globally. Lead metal causes toxicity in living cells by following ionic mechanism and that of oxidative stress. Many researchers have shown that oxidative stress in living cells is caused by the imbalance between the production of free radicals and the generation of antioxidants to detoxify the reactive intermediates or to repair the resulting damage. Antioxidants such as glutathione, protect the cell from free radicals such as H₂O₂. Under the influence of lead, however, the level of the reactive oxygen species (ROS) increases and the level of antioxidants decreases (Jaishankar et al., 2014). The ionic mechanism of lead toxicity occurs mainly due to the ability of lead metal ions to replace other bivalent cations like Ca²⁺, Mg²⁺, Fe²⁺ and monovalent cations like Na⁺, which ultimately disturbs the biological metabolism of the cell. The ionic mechanism of lead toxicity causes significant changes in various biological processes such as cell adhesion, intra- and inter-cellular signaling, protein folding, maturation, apoptosis, ionic transportation, enzyme regulation, and release of neurotransmitters. The researchers also restated that lead also affects the sodium ion concentration, which is responsible for numerous vital biological activities like generation of action potentials in the excitatory tissues for the purpose of cell to cell communication, uptake of neurotransmitters (choline, dopamine and GABA) and regulation of uptake and retention of calcium by synaptosomes (Flora et al., 2012). Mercury is very toxic and exceedingly accumulate in the body. Its presence adversely affects the marine environment. Mechanism of mercury toxicity lies on methylmercury which is a neurotoxic compound responsible for microtubule destruction, mitochondrial damage, lipid peroxidation and accumulation of neurotoxic molecules such as serotonin, aspartate, and glutamate. In addition, methylmercury exposure induces several immune-related chemokines, specifically in the brain, and may cause neurotoxicity (Lee et al., 2020). The brain remains the target organ for mercury, yet it can impair any organ and lead to malfunctioning of nerves, kidneys and muscles. It can cause disruption to the membrane potential and interrupt with intracellular calcium homeostasis. In a certain study, the researchers reported that mercury binds to freely available thiol as the stability constants are high (Ajusuvakova et al., 2020). Cadmium is the seventh most toxic heavy metal as per Agency for Toxic Substances and Disease Registry (ATSDR) ranking and in its inorganic form, has been ranked as the number one most significant substance present in the environment to pose a threat to human health (Bair, 2022). The mechanism of cadmium toxicity is not understood clearly but its effects on cells are known. Cadmium concentration increases 3,000 fold when it binds to cystein-rich protein such as metallothionein. In the liver, the cystein-metallothionein complex causes hepatotoxicity and then it circulates to the kidney and gets accumulated in the renal tissue causing nephrotoxicity. Cadmium has the capability to bind with cystein, glutamate, histidine and aspartate ligands and can lead to the deficiency of iron (Jaishankar et al., 2014). Cadmium and zinc have the same oxidation states and hence cadmium can replace zinc present in metallothionein, thereby inhibiting it from acting as a free radical scavenger within the cell. The most commonly occurring forms of Cr are trivalent (Cr³⁺) and hexavalent (Cr⁶⁺) with both states being toxic to animals, humans and plants. In the environment, trivalent chromium (Cr³⁺) is generally harmless due to its weak membrane permeability. Hexavalent chromium (Cr⁶⁺), on the other hand, is more active in penetrating the cell membrane through passages for isoelectric and isostructural anions such as SO₄²⁻ and HPO₄²⁻ and these chromates are taken up through phagocytosis. Hexavalent chromium (Cr⁶⁺) is a strong oxidizing agent and can be reduced to give ephemeral species of pentavalent and tetravalent chromium that are different from that of (Cr³⁺). The reactions between (Cr³⁺) and biological reductants like thiols and ascorbate result in the production of reactive oxygen species such as superoxide ion, hydrogen...
peroxide, and hydroxyl radical, ultimately leading to oxidative stress in the cell causing damage to DNA and proteins (Collin et al., 2019). Taking into cognizance the prevalence of some of these heavy metals as outlined above, we aimed this current study at investigating the possible heavy metal contaminants in *Zingiber officinale* rhizomes and *Allium sativum* bulbs sourced from a market in Enugu State of Nigeria using rat models. This is justified by the increase in the use of the two herbs both as food spices and as medicinal herbs such as in the treatment of cancers among other ailments.

2. Material and methods

2.1. Materials

2.1.1. Animals

Swiss female Albino rats (135 – 140 g) were used for acute toxicity studies. All the animals were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria. The animals were housed in standard laboratory conditions of 12 hours light, room temperature, and 40 - 60% relative humidity and fed with rodent feed (Guinea Feeds Nigeria Ltd). They were allowed free access to food and water. Maintenance and care of all animals were carried out in accordance with EU Directive 2010/63/EU for animal experiments. Guide for the care and use of Laboratory Animals, DHHS Publ. # (NIH 86-123 were strictly adhered to. Ethical approval was obtained from the Animal Ethical Committee of the Enugu State University of Science and Technology. There was additional approval by the Nnamdi Azikiwe University’s Ethical Committee for the use of Laboratory Animals for Research Purposes (Approval number is NAU/AREC/2023/00021).

2.1.2. Chemicals and Reagents

Hydrochloric acid (Prime laboratories, India); Dragendoff reagent (Sigma Aldrich, United States of America); Ammonia (Shakki Industrial Gases, India), sodium hydroxide (Treveni Chemical Pvt., India); Ferric chloride (AkashPurochem. Pvt., India); Fehling's solution (Lab care Diagnostics, India); Million reagent (Interlab Chemical Pvt., India); Ethanol (TAJ Pharmaceutical Ltd., India); Acetic anhydride (Ashok Organics Industries, India); Concentrated sulfuric acid (Navin Chemical Pvt., India), Acetic acid (Kayla Africa Suppliers, South Africa); Molisch reagent (Interlab Chemical Pvt., India); alcoholic alpha naphatol (Prat Industry Corcopation, India), concentrated nitric acid, perchloric acid, concentrated sulphuric acid.

2.1.3. Equipment

Glass column, flasks, beakers, test tubes, measuring cylinders, rotary evaporator, Analytical Weighing Balance (Metler H30, Switzerland), Electric Oven (Gallenkamp, England), Water Bath (Techmel & Techmel, Texas, USA), National Blender (Japan), Micropipette (Finnipipette® Labsystems, Finland), Plethysmometer (Biodevices, New Delhi, India) and Intubation tubes. Precision pipettes (25, 50, 100 and 300 μL, 1,000 μL) (Labcompare, USA); Disposable pipette tips (Labcompare, USA); Distilled or deionized water (SnowPure Water Technologies USA); Stop watch (Avi Scientific India); disposable hand gloves (Supermax, Malaysia), Atomic absorption spectrometer (Drawell, China), sample digestion flask (Thomas Scientific, USA), fume cupboard, electric hot plate (Zhongshan Gemeer, China), reagent bottles.

2.1.4. Plant materials

Fresh *Zingiber officinale* and *Allium sativum* were purchased from the market in Enugu state of Nigeria.

2.1.5. Extraction of plant material

The *Zingiber officinale* and *Allium sativum* were scraped, sliced, washed and dried away from sunlight at room temperature for 48 hours. The dried materials were pulverized to powder using an electronic blender and kept in clean airtight amber colored bottles separately. Then, 750 g of each of the powdered plant material was cold macerated in 95% ethanol. The mixture was allowed to stand for two days (48 hours) with intermittent agitation. It was filtered and the filtrate concentrated to dryness using water bath at 40 °C for 72 hours. The extract was stored in a refrigerator until used.
2.2. Methods

2.2.1. Phytochemical analysis of Zingiber officinale and Allium sativum ethanol extracts

The qualitative phytochemical analysis of Zingiber officinale and Allium sativum ethanol extracts were carried out using standard methods described by Odoh et al., (2019a) and reported by Ihekwereme et al., (2023).

2.2.2. Acute toxicity studies (LD\textsubscript{50}) of Zingiber officinale and Allium sativum ethanol extract

The actual median lethal doses (actual LD\textsubscript{50}) estimation of Zingiber officinale and Allium sativum ethanol extract were conducted with the method described by Lorke, (1983) which was modified as reported by Ihekwereme et al., 2023.

2.2.3. Investigation of Zingiber officinale and Allium sativum ethanol extracts possible heavy metal contaminants; cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr) and mercury (Hg)

The Atomic Absorption Spectroscopy (AAS) technique was used for extraction and assay of the possible heavy metal contaminants of the Zingiber officinale and Allium sativum ethanol extracts. This was achieved by firstly carrying out samples digestion for the heavy metals. Sample digestion for heavy metals was done by weighing 2 g of each sample into a digestion flask and adding 20 ml of aqua regia acid mixture (65 ml conc HNO\textsubscript{3}; 8 ml perchloric acid; 2 ml conc H\textsubscript{2}SO\textsubscript{4}) into it. The flask was sealed with a cork. The flask was placed under fume cupboard and heated with the aid of electric hot plate at temperature of 60 °C and allowed to heat until clear digest was obtained. The clear digest was diluted to 100 ml with distilled water and filtered into reagent bottle for analysis of heavy metals using Atomic adsorption spectrophotometer (AAS).

2.2.4. Working Principle of Atomic Absorption Spectrophotometer (AAS)

Atomic absorption spectrometer's working principle was based on the sample being aspirated into the flame and atomized when the AAS's light beam from the monochromator was directed through the flame onto the detector that measures the amount of light absorbed by the atomized element in the flame when ignited. Metal elements on their own have distinct characteristic wavelength on the source hollow cathode lamp composed of the element/metals to be analyzed. The amount of energy of the characteristic wavelength absorbed in the flame was directly proportional to the concentration of the element in the tested sample. The sample was aspirated into the oxidizing air-acetylene flame. When the aqueous sample was aspirated, the sensitivity for 1% absorption was observed. The percentages of the different metals in these samples were determined by the corresponding standard calibration curves obtained by using standard AR grade solutions of the elements (Khathi et al., 2016).

3. Results

3.1. Results of phytochemical analysis

The phytochemical compounds present in Zingiber officinale included: alkaloids, tannins, flavonoids, steroids and terpenoids while saponins and glycosides were absent. Furthermore, the phytochemical compounds present in Allium sativum included: alkaloids, saponins, flavonoids, and glycosides while tannins, steroids and terpenoids were absent (Ihekwereme et al., 2023).

3.2. Results of acute toxicity studies

Zingiber officinale, Allium sativum and equal amount of Zingiber officinale and Allium sativum administered in combination had actual median lethal doses (actual LD\textsubscript{50}) of 8,660, 4,472 and 5,477 mg/kg body weight respectively (Ihekwereme et al., 2023).

Table 1 Results of possible heavy metal contamination of Zingiber officinale ethanol extract

<table>
<thead>
<tr>
<th>S/n</th>
<th>Heavy metals</th>
<th>Concentration (ppm)</th>
<th>Maximum acceptable limit (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lead</td>
<td>0.041</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Cadmium</td>
<td>0.082</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>Mercury</td>
<td>0.084</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>Chromium</td>
<td>0.061</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Arsenic</td>
<td>0.041</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 2 Results of possible heavy metal contamination of *Allium sativum* ethanol extract

<table>
<thead>
<tr>
<th>S/n</th>
<th>Heavy metals</th>
<th>Concentration (ppm)</th>
<th>Maximum acceptable limit (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lead</td>
<td>0.012</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Cadmium</td>
<td>0.018</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>Mercury</td>
<td>0.039</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>Chromium</td>
<td>0.045</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Arsenic</td>
<td>0.030</td>
<td>10</td>
</tr>
</tbody>
</table>

4. Discussion

The phytochemicals present in *Zingiber officinale* and *Allium sativum* are of importance due to the reputable anti-oxidative potentials of phytochemicals. This will be comprehensible if we consider the fact that heavy metals cause most of their toxicities by eliciting oxidative stress. A certain study reported that the mechanism by which the heavy metals induce neurotoxicity follows free radicals production pathway(s) especially the generation of reactive oxygen species and reactive nitrogen species. According to the researchers, these free radicals produced in excess have been shown to create an imbalance between the oxidative and anti-oxidative systems leading to emergence of oxidative stress, which may cause necrosis, DNA damage, and many neurodegenerative disorders (Gupta *et al*., 2015). Also, presence of heavy metals can influence the acute toxicities and actual LD$_{50}$ of herbal products. In another study, heavy metals were documented to be well-known environmental pollutants owing to their toxicity, longevity in the atmosphere, and ability to accumulate in the human body, terrestrial and aquatic ecosystems. Heavy metals can become strongly toxic by mixing with different environmental elements, such as water, soil, and air, and humans and other living organisms can be exposed to them through the food chain (Saikat *et al*., 2022). The results of the possible heavy metal contaminants showed that trace amounts of the tested heavy metals (lead, cadmium, mercury, chromium and arsenic) were present in both samples of *Zingiber officinale* and *Allium sativum*. However, these heavy metals were present at a level much below the WHO permissible limits of Pb (10 ppm), Cd (0.3 ppm), Hg (0.1 ppm), Chromium (2 ppm), and As (10 ppm) (Tehseen *et al*., 2021; Ssempijja *et al*., 2020). It is also obvious that these heavy metals were more in *Zingiber officinale* than in *Allium sativum*. According to a certain study, this differential accumulation may be attributed to the following facts: variable exposure to environmental pollution including industrial encroachment, contaminated soil or atmosphere; the physicochemical properties of soil including pH, temperature, redox potential, translocation exchange capacity and organic matter which may influence the availability of heavy metal to plants; the phytological characteristics of medical plants themselves such as reduced biomass, root length and shoot length; the interactions of soil-plant roots-microbes which play vital roles in regulating heavy metal movement from the soil to edible plant parts; herbal plants could be contaminated during manufacturing and agronomic processes such as growing, harvesting, transportation, processing and storage, due to pesticide formulations, chemical fertilizers and irrigation with poor-quality water; fumigants containing heavy metals may also be applied for preventing pests and rodents; difference in plant metal uptake and translocation capabilities; and the bioavailability of heavy metals which could have an impact on their concentrations (Luo *et al*., 2021).

5. Conclusion

Although the popularity of herbal medicine is rapidly increasing universally, the impact of heavy metal toxicity and the consequent effects on the quality of herbal raw materials, herbal extracts, and herbal medicine formulations should not be over emphasized. Effective control of heavy metal content of herbal plants used in pharmaceutical and food industries has become indispensable. However, this study concluded that heavy metal contamination of herbal products are inevitable but can be controlled as to minimize it to WHO permissible limit. The five heavy metals (Pb, Cd, Hg, Cr, and As) tested were below the acceptable limits and herbs with this and similar status can be used to formulate safe medicines.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest
No conflict of interest to be disclosed.

Statement of ethical approval
Maintenance and care of all animals were carried out in accordance with EU Directive 2010/63/EU for animal experiments. Guide for the care and use of Laboratory Animals, DHHS Publ. # (NIH 86-123) were strictly adhered to. Ethical approval was obtained from the Animal Ethical Committee of the Enugu State University of Science and Technology. There was additional approval by the Nnamdi Azikiwe University’s Ethical Committee for the use of Laboratory Animals for Research Purposes; (Approval number is NAU/AREC/2023/00021)

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[20] Ihekwereme P C 1, Asomugha R N 1, Mbogwu S I 1, Oraekei D I 1, * and Ajaghaku D L 2. Phytochemicals, acute toxicities and actual median lethal doses (actual LD50) of Zingiber officinale and Allium sativum given singly and in combination via mice models. GSC Biological and Pharmaceutical Sciences, 2023, 25(01), 008–018.


