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Organophosphate pesticides in herbal mixtures from Bayelsa State, Nigeria: implication for human exposure and risks

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Abstract

This study investigated the presence of organophosphate pesticides (OPPs) in fifty herbal mixture samples obtained from major towns in Bayelsa, Nigeria, to evaluate their safety. OPPs were quantified using a gas chromatograph (GC) coupled with a mass-selective detector after solvent extraction. The results showed that all fifty herbal mixtures contained detectable levels of OPPs, with detection frequencies for individual OPP congeners ranging from 52% for pyraclofos to 90% for diazinon, the most frequently detected congener. At least three OPPs were detected in each sample. Total OPP concentrations varied from 3.80 to 48.0 ng·L^{-1}, 4.50 to 51.6 ng·g^{-1}, and 2.96 to 18.1 ng·g^{-1} in liquid, powder, and capsule herbal mixtures, respectively. These concentrations were below the maximum residue limits (MRLs) set by the European Pharmacopeia. Computed hazard index (HI) values were generally < 1, indicating no significant non-carcinogenic risk associated with the ingestion of these herbal mixtures.

The contribution of individual OPP congeners to the HI followed the order: O-ethyl O-4-nitrophenyl phenylphosphonothioate (EPN) > diazinon > pirimiphos-methyl > quinalphos > chlorpyrifos > chlorpyrifos-methyl. This study underscores the need for continuous monitoring and the application of rigorous scientific standards to herbal mixtures to ensure consumer safety.

Keywords: OPPs, hazard index, daily intake, non-carcinogenic risk, GC-MSD

INTRODUCTION

Pesticides are broadly defined under the United States Federal Insecticides, Fungicides and Rodenticides Act (FIFRA) as substances or mixtures intended to prevent, destroy, repel, or mitigate pests, including insects, rodents, and weeds[1]. In agricultural activities, a range of pesticides are widely employed to control or eradicate undesired insects. Among these, organophosphate pesticides (OPPs) are particularly notable. OPPs are characterized by the presence of phosphate (or thio- or dithiophosphate) groups and organic components. Because of their potent insecticidal and herbicidal properties, combined with their ability to degrade rapidly in the environment, OPPs have become the most commonly used pesticides and herbicides in agricultural applications[2,3]. Globally, it is estimated that OPPs are responsible for 750,000 to 3 million cases of human poisoning annually. Exposure to OPPs can cause a range of acute symptoms, including headache, nausea, dizziness, excessive salivation, muscle twitching, weakness, tremors, abdominal cramps, and diarrhea, which are often early signs of toxicity[4,5]. Furthermore, both short-term and long-term exposure to OPPs has been linked to various neurotoxic conditions, such as intermediate syndrome, cholinergic syndromes, organophosphate-induced neuropsychiatric disorders, as well as immunotoxic, genotoxic, and carcinogenic effects[2,6].

Herbal medicines or medicinal plants have been widely used for centuries in many countries as both curative agents and dietary supplements due to their remarkable therapeutic benefits and minimal side effects[7-9]. It is estimated that over 80% of people in developing countries, including low- and middle-income nations, rely on plant-based materials for primary healthcare. Additionally, approximately 51% of all pharmaceutical formulations in developed nations are, in some way, derived from medicinal plants[10]. In Nigeria, herbal medicine has a long-standing tradition and is deeply embedded in cultural and traditional practices. Nigerian traditional medicine encompasses the use of plants, animals, and minerals for the prevention, treatment, and management of various health conditions. It plays a significant role in the healthcare system, especially in rural and underserved communities where access to conventional medical services may be limited[11].

Nonetheless, the growing use of herbal mixtures and the expansion of the global market have raised safety concerns among healthcare professionals and regulatory authorities. Reports from various regions of the world indicate that herbal medications sometimes contain undisclosed or undesirable ingredients. To address this, numerous international organizations and nations have established guidelines regarding pesticide levels in herbal plants and related products[12]. Studies on pesticide residues in herbal medicine and medicinal plants have identified OPP residues[13-16]. However, in Nigeria, there is a notable lack of studies investigating the presence and associated risks of pesticide residues, particularly OPPs, in herbal medicines. The limited research available on pesticide contamination in Nigerian herbal mixtures[17,18] has primarily focused on organochlorines. Thus, this study aims to assess the occurrence and potential risks of OPPs in herbal mixtures sold in Bayelsa, Nigeria.

MATERIALS AND METHODS

Description of the study area

The study area is Bayelsa State, situated in the south-south zone of Nigeria, at the heart of the Niger Delta [Figure 1]. The state is located at latitude 4.7719° N and longitude 6.0699° E, with Yenagoa city as its capital. It shares borders with Delta State to the north, the Atlantic Ocean to the southwest, and Rivers State to the east. Bayelsa State spans a total area of 10,773 square kilometers and has a population of over 2.7 million (https://bayelsastate.gov.ng/our-history/). This region experiences tropical weather, characterized by an annual mean temperature ranging from 25.4 to 28.0 °C, placing it just north of the equator[19]. Relative humidity fluctuates between 70% and 90% during the wet season and drops to 50% in the dry season[20]. Despite hosting one of the biggest natural gas and crude oil reservoirs in Nigeria, Bayelsa State is still beset by extreme poverty and environmental pollution from oil spillage.

Sample collection

Fifty herbal mixtures were collected, comprising 35 liquids, 11 powders, and 4 capsules, from retailers and vendors in major towns across Bayelsa State, Nigeria, including Odi, Ogbia, Ekeremor, Brass, Nembe, Kaiama, Amassoma, Sagbama, Toru-Orua, Otuoke, Igbogene, and Yenagoa. For each brand, three samples were obtained and combined into a single mixture. An aliquot of the composite sample was taken for analysis. Details regarding the brand names, physical forms, ingredients, indications, origins, and other characteristics of the sampled herbal mixtures are given in Supplementary Table 1.

Reagents

All reagents used were of high purity (99.9%) and of analytical grade. A standard solution containing fourteen OPP congeners (diazinon, isazophos, chlorpyriphos-methyl, pirimiphos-methyl, fenitrothion, pirimiphos-ethyl, quinalphos, chlorpyrifos, triphenyl phosphate, O-ethyl O-4-nitrophenyl phenylphosphonothioate (EPN), phosalone, pyrazophos, azinphos-ethyl, and pyraclofos) was obtained from Accu Standard, USA. A stock solution of the OPPs (100 μg·mL^{-1}) was prepared in n-hexane, and subsequent working standards were prepared by serial dilution in n-hexane. Deuterated chrysene, used as a surrogate standard, was procured from GFS Chemicals, Columbus, Ohio, USA. Additional reagents, including acetone, hexane, petroleum ether, Florisil, and anhydrous Na_2SO_4, were purchased from Labtech Chemicals, Italy.

Extraction of OPPs from herbal mixtures

The extraction procedure previously described by Pan *et al.* was followed[21]. For liquid herbal mixtures, 50 mL of the sample was measured into a separating funnel, and 20 ng of ^{2}D-labelled chrysene was added as an internal standard. Then, 50 mL of a 1:4 v/v petroleum ether/acetone mixture was introduced, and the sample was extracted for 60 min. The resulting extracts were transferred to a flask, and the extraction process was repeated twice. The combined extract was concentrated, and the solvent was replaced with hexane using a rotary evaporator. Next, the concentrated extract was eluted through a column containing Na_2SO_4 and activated Florisil to remove impurities. The OPPs were recovered in 30 mL of 1:4 v/v acetone/hexane. The final elution with OPPs was further concentrated, the solvent was exchanged with hexane, and the volume was reduced to 0.5 mL using a rotary evaporator before analysis. For powdered and capsulated herbal mixture, 10 g of homogenized sample was mixed with 20 ng of ^{2}D-labeled chrysene in an extraction thimble. Then, the same procedure described above for liquid mixtures was followed.

Chromatographic analysis

The extracted samples were analyzed for OPPs using an Agilent 6890A gas chromatograph (GC) coupled with an Agilent 5973 mass spectrometry detector (MSD) (Agilent Technologies, Santa Clara, USA). Separation was achieved using an HP-5 5% phenyl methyl Siloxane capillary column (30 m × 0.25 μm ×

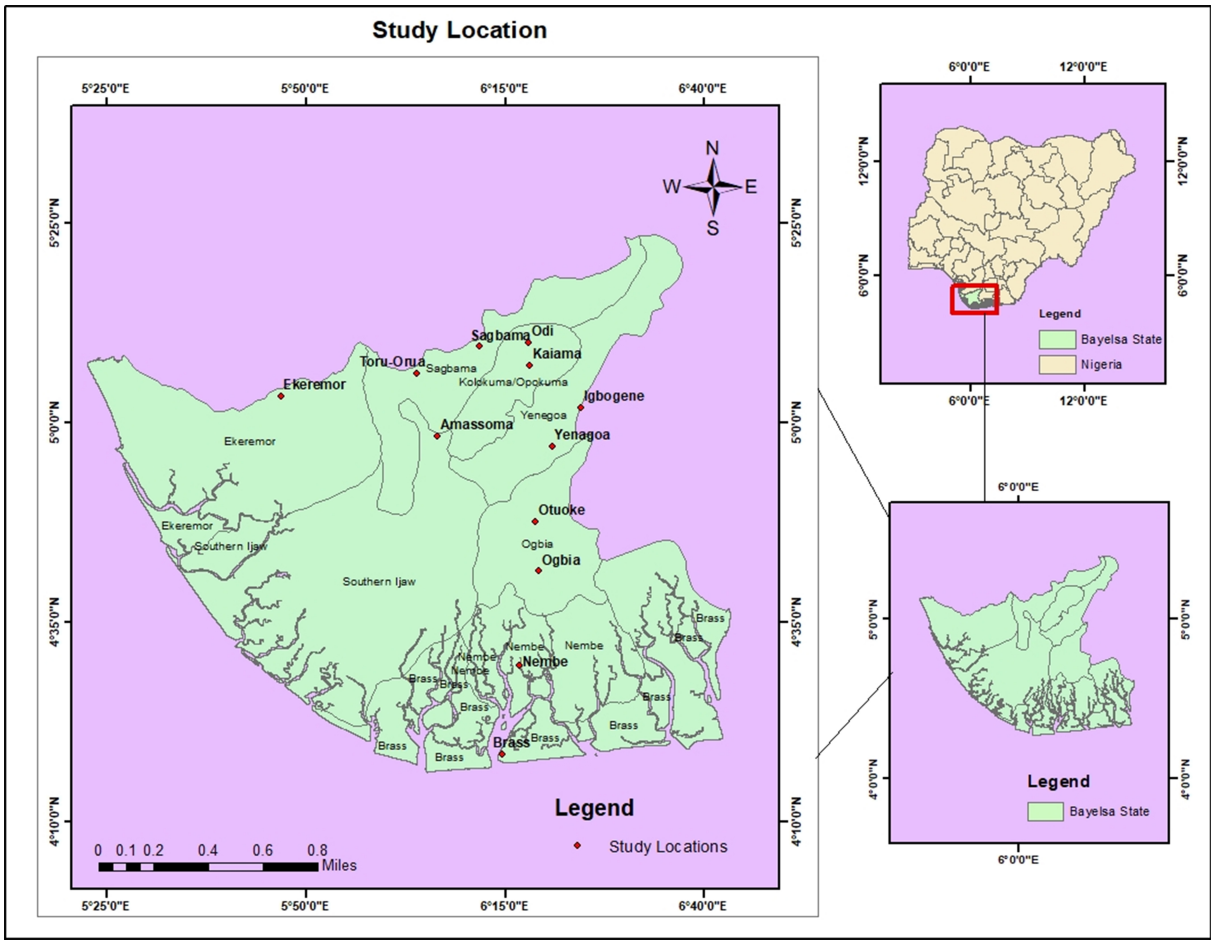

Figure 1. Map of study area.

0.25 mm). Helium gas, at a flow rate of 1.8 mL/min, was used as the carrier gas. The GC column was initially heated to 150 °C and held for 1.3 min, then increased at a rate of 15 °C·min^{-1} to 200 °C and held for 2 min, and finally raised to 300 °C at 15 °C·min^{-1} and held for 4 min. The transfer line, quadrupole, ion source, and injection port temperatures were set to 280, 150, 230, and 250 °C, respectively. A 1 μL sample was injected into the instrument in splitless mode. The MSD was operated with an electron impact energy of 918 EmVolts, and data were collected in ion monitoring mode. The OPPs were identified by comparing their retention times to those of real OPP standards.

Method validation and quality control/assurance

This study followed the European Commission[22,23] guidelines for method validation. In addition to the recovery study, blank determinations were performed. Previously analyzed herbal mixtures were spiked with standard OPP solutions, and the spiked samples were subsequently analyzed. The percentage recoveries were then calculated. The herbal mixtures were spiked with OPP concentrations of 5, 10 and 20 ng·mL^{-1}. OPP concentrations in the blank samples were below the limit of quantifications (LOQs). The recovery of OPPs ranged from 92.2% to 101%. The limit of detection (LOD) was determined based on the OPP concentration that produced a signal-to-noise ratio of 3, while the LOQ was taken as three times the LOD. The LODs and LOQs for the OPPs varied from 0.003 to 0.02 ng·g^{-1} and 0.01 to 0.06 ng·g^{-1}, respectively. The relative standard deviation (RSD) for replicate analysis (n = 3) was less than 8%, and the R^2 values from the calibration curves were > 0.9995.

Statistical analysis

The statistical analysis of the data obtained was conducted using IBM-SPSS software, version 23. Kruskal-Wallis test was utilized to assess whether OPP concentrations varied significantly within the liquid, powdered, and capsulated herbal mixtures, as well as among the three groups, at a 95% confidence limit. OPP concentrations below LOQ were treated as zero in all statistical analyses and data computations.

Health risks assessment

Assessment of dietary intake of OPPs

The dietary intake (DI) of OPPs from herbal mixtures was assessed using the following Equation (1);

$$\text{Estimated Dietary Intake (ng·kg}^{-1}\text{ bw·day}^{-1}) = \frac{Concentration\ of\ OPPs \times Ingestion\ Rate\ (IR)}{Body\ Weight\ (BW)} \quad (1)$$

Ingestion rates (IRs) of 0.25 and 0.75 L·day^{-1} and corresponding body weight (BW) of 15 and 60 kg were used for children and adults, respectively[24].

Assessment of non-carcinogenic risk

The non-carcinogenic risk related to OPPs via the intake of these herbal mixtures was evaluated as hazard quotients (HQ) and hazard index (HI) using Equations (2) and (3)[2,18,25]. The HQ for each OPP was calculated and the HI was obtained by summing the HQs of individual OPP based on dose additivity[2,26]. In this study, only the six OPPs with established oral reference dose (RfD) were used for the risk assessment.

$$HQ = \left[\frac{OPPs\ Concentration \times IR \times EF \times ED}{BW \times AT_{nc}} \times 10^{-6}\right] / RfD \quad (2)$$

$$HI = HQ1 + HQ2 + HQ3 + \ldots + HQ6 \quad (3)$$

The definition and values of variables in equation 2 are given in Supplementary Materials and our previous study[2,18]. The RfD values used were chlorpyrifos (1×10^{-3}), chlorpyrifos-methyl (1×10^{-2}), diazinon (7×10^{-4}), pirimiphos-methyl (7.3×10^{-4}), quinalphos (5×10^{-4}), azinphos-ethyl (3×10^{-3}), and EPN (1×10^{-5})[27]. A HI value below 1 indicates that non-carcinogenic risk is absent[27].

RESULTS AND DISCUSSION

Occurrence of OPPs in herbal mixtures

The results of OPPs in the herbal mixtures are displayed in Table 1, Figures 2 and 3, and Supplementary Table 2. The results showed that the detection frequency of OPP congeners in the herbal mixtures varied from 52% for pyraclofos to 90% for diazinon [Figure 2]. This variation suggests that the contamination of herbal mixtures by OPPs is pervasive, likely resulting from the use of OPPs in cultivating the plants used to prepare these mixtures. OPPs were detected in all 50 herbal mixtures, with at least three different OPPs detected in each sample [Figure 3]. The high prevalence of OPP contamination in the herbal mixtures in our study agrees with the findings reported by other researchers. For instance, Kowalska[28] reported a detection frequency of 72% in a survey of pesticide residues in 104 herbal samples in Poland. Similarly, pesticides were found in 54.6% of analyzed herbs, vegetables, and fruits from Egypt[29]. The findings of this study are further corroborated by another investigation[30], where 59% of the samples were found to contain pesticides. Additionally, pesticides were reported in 63% of herbal materials examined in Italy[31] and 55% in

Table 1. Summary of OPP results in the herbal mixtures

OPPs	Detection frequency (%)	Liquid samples (n = 35) ng·L^{-1}		Powder samples (n = 11) ng·g^{-1}		Capsule samples (n = 4) ng·g^{-1}		European Pharmacopeia MRLs*
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
Diazinon	90.0	3.15 ± 3.22	< LOQ-14.9	4.26 ± 5.59	< LOQ-18.3	3.51 ± 1.08	2.57-4.85	500
Isazophos	88.0	1.73 ± 2.05	< LOQ-8.88	2.85 ± 3.85	< LOQ-13.8	3.12 ± 5.50	< LOQ-11.3	-
Chloropyriphos methyl	86.0	2.72 ± 2.99	< LOQ-10.5	2.28 ± 2.17	< LOQ-6.89	0.64 ± 0.87	< LOQ-1.85	100
Pirimiphos-methyl	80.0	1.30 ± 1.65	< LOQ-6.41	2.30 ± 5.38	< LOQ-18.3	0.27 ± 0.47	< LOQ-0.98	4000
Fenitrothion	76.0	1.30 ± 1.77	< LOQ-5.99	1.16 ± 1.69	< LOQ-5.46	0.29 ± 0.57	< LOQ-1.14	500
Pirimiphos-ethyl	66.0	1.06 ± 1.69	< LOQ-7.28	0.91 ± 1.17	< LOQ-3.22	< LOQ	< LOQ	50
Quinalphos	64.0	0.99 ± 1.35	< LOQ-3.95	0.89 ± 2.04	< LOQ-6.51	0.04 ± 0.08	< LOQ-0.16	50
Chlorpyrifos	68.0	1.03 ± 1.33	< LOQ-5.83	0.55 ± 0.96	< LOQ-3.03	0.04 ± 0.08	< LOQ-0.16	-
Triphenyl phosphate	74.0	1.28 ± 1.74	< LOQ-7.98	0.81 ± 1.63	< LOQ-5.32	0.01 ± 0.02	< LOQ-0.03	-
EPN	70.0	1.29 ± 1.76	< LOQ-8.71	1.41 ± 3.95	< LOQ-13.3	0.21 ± 0.29	< LOQ-0.62	-
Phosalone	60.0	0.38 ± 0.66	< LOQ-3.15	0.87 ± 1.98	< LOQ-6.40	0.11 ± 0.17	< LOQ-0.36	100
Pyrazophos	78.0	1.87 ± 3.07	< LOQ-12.6	0.21 ± 0.38	< LOQ-1.09	0.03 ± 0.03	< LOQ-0.07	-
Azinphos-ethyl	58.0	0.52 ± 0.97	< LOQ-5.17	0.23 ± 0.40	< LOQ-1.18	0.03 ± 0.06	< LOQ-0.12	100
Pyraclofos	52.0	0.54 ± 1.15	< LOQ-6.14	0.46 ± 0.90	< LOQ-2.53	0.20 ± 0.39	< LOQ-0.78	-
TOTAL	**100**	**17.4 ± 10.5**	**3.80-48.0**	**19.2 ± 12.8**	**4.50-51.6**	**8.49 ± 6.62**	**2.96-18.1**	

*Klier *et al.*[13]. OPP: Organophosphate pesticide; MRLs: maximum residue limits; LOQ: limit of quantification.

Tunisia[32]. In studies analyzing Chinese medicines made from herbal materials, pesticide residues were detected in 36.73% to 100% of the herb samples[33-36].

Numerous factors, including the biological characteristics of plants and the physicochemical characteristics of OPPs, influence the concentration of OPP residues in plants. These factors may exert either positive or negative synergistic effects on the half-lives of OPPs. The total OPP concentrations in the herbal mixtures ranged from 3.80 to 48.0 ng·L^{-1}, 4.50 to 51.6 ng·g^{-1}, and 2.96 to 18.1 ng·g^{-1} in liquid, powdered, and capsulated mixtures, respectively. The lowest and highest concentrations of total OPPs were observed in LHM32 and LHM9 for liquid samples, PHM5 and PHM11 for powder samples, and CHM3 and CHM4 for capsulated samples, respectively. Significant variations ($P < 0.05$) in OPP levels were noted both within each category of herbal mixtures and among the different categories. The occurrence of diazinon followed the order: powder > capsules > liquids. The diazinon levels in our study were comparable to those reported in the literature: the 5 ng·g^{-1}[37] in Chinese herbal medicines and ranging from not detected to 98.2 ng·g^{-1}[12] in medicinal plants consumed in Iran. Isazophos was present in the order of capsules > powder > liquids. For pirimiphos-methyl, EPN, and phosalone, the order of occurrence was: powder > liquids > capsules. The pirimiphos-methyl levels in our study were similar to those documented in previous studies. Specifically, Sarkhail *et al.* reported pirimiphos-

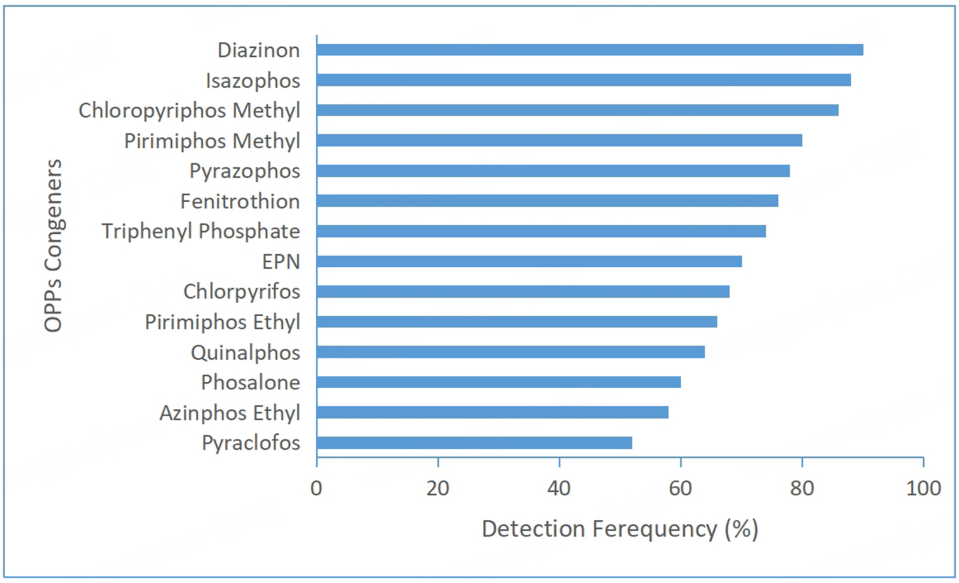

Figure 2. Detection frequency of OPPs in herbal mixtures. OPPs: Organophosphate pesticides.

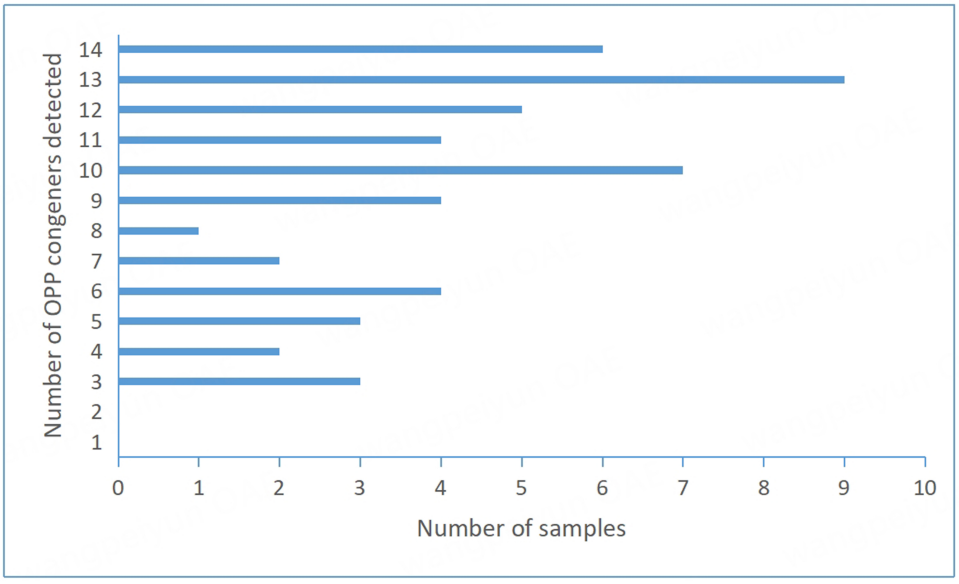

Figure 3. Distribution of herbal mixtures with detected OPP residues. OPP: Organophosphate pesticide.

methyl concentrations of < 0.5 ng·g^{-1} in Iranian medicinal plants, while Adusei-Mensah *et al.* reported concentrations ranging from 8.0 to 82 ng·g^{-1} in herbal medicinal products from Kumasi, Ghana[12,14]. Other OPPs, including chlorpyrifos-methyl, fenitrothion, pirimiphos-ethyl, quinalphos, chlorpyrifos, triphenyl phosphate, pyrazophos, azinphos-ethyl, and pyraclofos, appeared in the order of liquids > powder > capsules. The level of fenitrothion in our study was lower than the 50 ng·g^{-1}[14] reported for Ghanaian herbal medicinal products. Similarly, the chlorpyrifos levels in our study were lower than the 5.0 to 42.0 ng·g^{-1} range documented for Ghanaian herbal products[14]. On average, the total OPP concentration in the herbal mixtures followed the order: powder > liquids > capsules. This trend was also seen for organochlorine pesticides (OCPs) in herbal medicines[18]. The higher OPP concentrations in powdered herbal mixtures

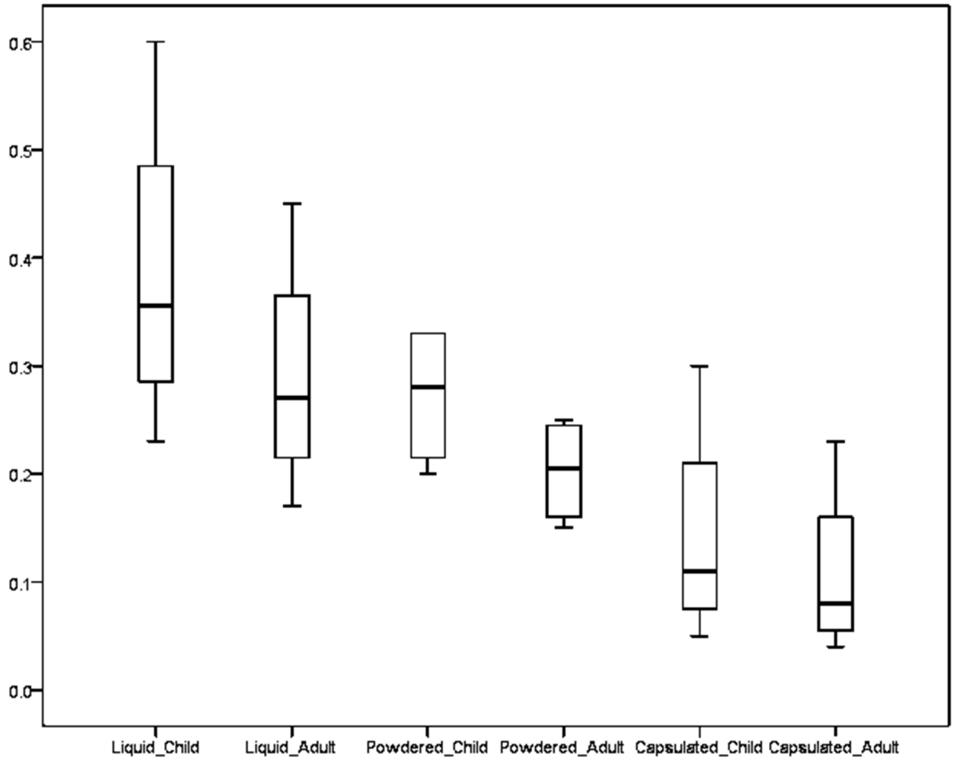

Figure 4. Box plot of EDI of OPPs in the herbal mixtures. EDI: Estimated daily intake; OPPs: organophosphate pesticides.

might be due to inadequate quality control and non-uniform application of active ingredients, in addition to uneven contamination during the formulation of herbal mixtures. The OPP concentrations obtained in our study were below their MRLs stipulated by the European Pharmacopeia[13].

Estimated daily intake and health risk of OPPs in the herbal mixture

The estimated daily intake (EDI) of OPPs from the consumption of the studied herbal mixtures by children and adults is shown in Figure 4 and Supplementary Table 3. The daily intake of OPPs by adults varied from 0.05 to 0.60, 0.06 to 0.64, and 0.04 to 0.23 ng·kg^{-1} bw·day^{-1} for liquid, powdered, and capsulated herbal mixtures, respectively. For children, the daily intake varied from 0.06 to 0.80, 0.08 to 0.86, and 0.05 to 0.30 ng·kg^{-1} bw·day^{-1} for liquid, powdered, and capsulated herbal mixtures, respectively. The highest daily intake values of OPPs were observed in LHM9, PHM11, and CHM4.

The non-carcinogenic risk, represented by the HI, associated with OPP exposure through the ingestion of these herbal mixtures by children and adults is shown in Figure 5 and Supplementary Tables 4 and 5. The HI values ranged from 1.95×10^{-5} to 1.62×10^{-2} for adults and 2.61×10^{-5} to 2.16×10^{-2} for children. The HI values were generally < 1, indicating no significant non-carcinogenic risk from ingesting these herbal mixtures. The contribution of each OPP congener to the HI followed this order: EPN > diazinon > pirimiphos-methyl > quinalphos > chlorpyrifos > chlorpyrifos-methyl.

CONCLUSION

This study provides the first comprehensive data on the occurrence, distribution, and risks of OPPs in herbal mixtures in Bayelsa State, Nigeria. All fourteen analyzed OPPs were detected across the herbal mixtures, with detection frequencies ranging from 52% for pyraclofos to 90% for diazinon, the dominant

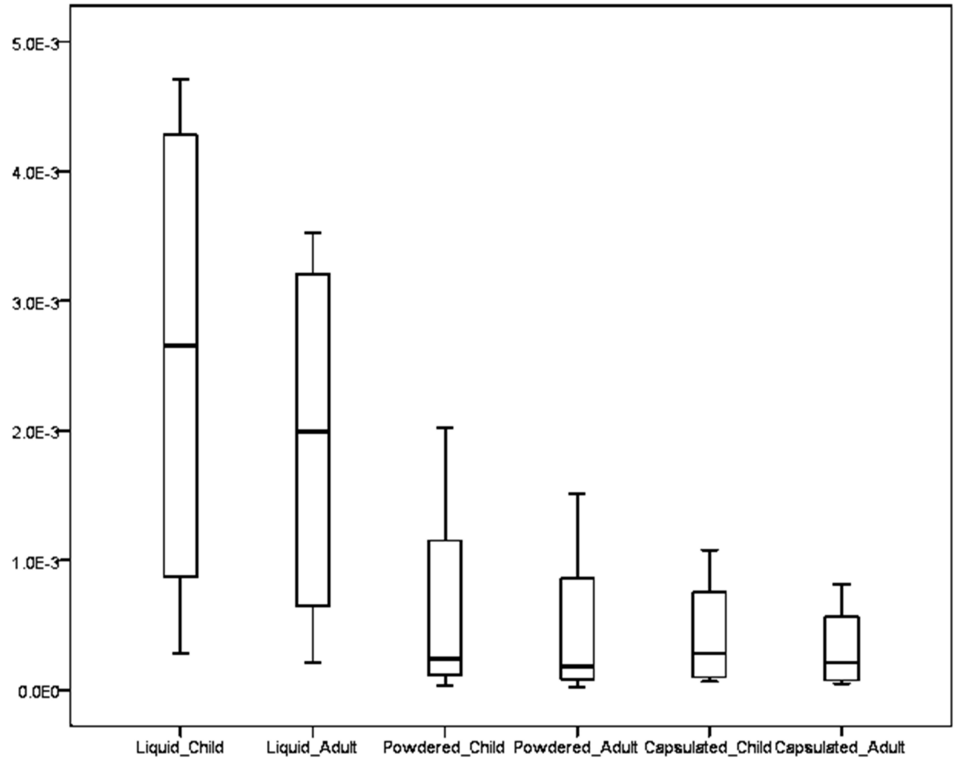

Figure 5. Box plot of HI of OPPs in the herbal mixtures. HI: Hazard index; OPPs: organophosphate pesticides.

congener. At least three OPPs were presented in each of the fifty herbal mixtures, and the OPP concentrations were below their respective MRLs stipulated by the European Pharmacopeia. The computed HI values confirmed that ingestion of these herbal mixtures poses no significant non-carcinogenic risk, with EPN contributing the most to the HI values. Although the findings suggest minimal health risks, the study emphasizes the importance of continuous monitoring of herbal products. It also recommends applying the same scientific rigor required for conventional medicines to herbal mixtures to ensure their safety and reliability for consumers.

DECLARATIONS

Authors' contributions

Conceptualization, visualization, methodology, writing - original draft, and funding acquisition: Tesi GO
Methodology, investigation, and resources: Lari B
Investigation, data curation, and formal analysis: Ogbuta AA
Investigation, methodology, and writing - original draft: Felagha I
Investigation, data curation, formal analysis, and project administration: Obodoka GC
Data curation, formal analysis, and visualization: Ogbomade WE
Methodology, formal analysis, resources, and writing - review and editing: Okpara KE
Resources, visualization, and writing - original draft: Osioma E
Validation, writing - review and editing, and supervision: Agbozu IE

Availability of data and materials

The data used and/or analyzed in this study are given in the work and Supplementary Materials.

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Conflicts of interest
All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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