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Chemical Composition and Toxicological Risk Assessment of Potentially Toxic Elements (PTEs) in a Commercially Available Alcoholic Bitters

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ABSTRACT

This study aimed at determining the Chemical composition and Toxicological Risk Assessment of Potentially Toxic Elements (PTEs) in a commercially available Alcoholic Bitters. Samples of Confam bitters were purchased and transported to the laboratory for analysis using the standard method of the Association of Official Analytical Chemists (AOAC) and the toxicological risk assessment was estimated using predictive modelling of the United State Environmental Protection Agency (US EPA). The result obtained indicates that the bitters contain compounds such as Bactobolin (2.02%), 3-Methoxyamphetamine (1.10%), Propanamide (3.04%), Methylpent-4-enylamine (1.10%), Diisopropyl phthalate (35.62%), N, N'-Bis (3-aminopropyl) ethylenediamine (1.95%), Didodecyl phthalate (2.29%), n-Hexadecanoic acid (8.17%), Metaraminol (2.98%), Methyl octadec-11-enoate (4.53%), Linoelaidic acid (8.70%), Octadecanoic acid (3.30%) and Octadecyl prop-1-en-2-yl carbonate (1.45%). The result also indicates that the concentration of Zn (1.26 mg/l), Cu (0.22 mg/l) and Ni (90.16 mg/l) were within the WHO (2011) standard (3.00, 2.00 and 0.02 mg/l) respectively, while Pb (0.03 mg/l), Cd (0.005 mg/l), Mn (3.85 mg/l) and Cr (0.66 mg/l) were higher than the WHO (2011) standard (0.01, 0.003, 0.05 and 0.05 mg/l) respectively. Toxicological risk assessment revealed that the children population is more susceptible to PTE exposure in the bitters. Therefore, disapproving children exposure to the bitters. Furthermore, Mn and Zn were the most dosed PTEs of exposure. Hazard Quotient (HQ) revealed that the values for the PTEs are less than 1, while carcinogenic risk values are less than 1×10^{-6}, suggesting a non-significant non-carcinogenic and carcinogenic risk respectively for the human population. In conclusion, the alcoholic bitter contains compounds of excellent pharmacological effects, a potentially hazardous compound and high concentrations of some PTEs of public health concern, more so other brand of bitters should be monitored.

Keywords: Alcohol, Bitters, Health Risk, Potentially Toxic Element

INTRODUCTION

Bitters or herbal drinks are made up of numerous groups of chemical compounds extracted from plant materials that have the common characteristic of a bitter taste (Anionye *et al.*, 2017; Umoren *et al.*, 2022). Herbal is widely accepted among the populace probably due to its low costs and easy accessibility, it has been used for the treatment of various diseases such as cough, parasitic, bacterial and fungal diseases, infertility, and pain among others in children and adults (Taiwo *et al.*, 2020; Umoren *et al.*, 2022). In Nigeria, different types of bitters/herbal drinks are consumed which could be categorized into alcoholic and non-alcoholic (Taiwo *et al.*, 2020). Bitter/herbal drinks are marketed as multi-potent in Nigeria and a majority of them have never been subjected to scientific scrutiny (Anionye *et al.*, 2017). More so, they are much sought after for their acclaimed health benefits (Nwachuku and Elekima, 2019). Bitters/herbal drinks which are plants derived can be contaminated with high concentrations of potentially toxic elements (PTEs) from environmental media (air, water and soil) (Bolawa *et al.*, 2020).

Potentially toxic elements also referred to as heavy metals have high densities and/or nucleon numbers (Kumar and Suman, 2023). The essential PTEs required by humans are found in precise and regulated concentrations (Izah *et al.*, 2016). Studies have shown that exposure to PTEs through general dietary consumption contributes negatively to human health (Wei and Cen, 2020). The consumption of drinks containing these elements can lead to severe health damage. After the transformation of herbal plants into medicinal mixtures (bitters), the toxic elements present in the plants will enter the human body via ingestion and lead to various diseases such as ulcers, diabetes, hypertension, cancer etc. (Bolawa *et al.*, 2020). Particularly as PTEs are known to have low renal clearance rates, potentially resulting in harmful consequences in humans even at extremely low concentration (Dghaim *et al.*, 2015). They produce toxic effects due to their interference with many known normal biochemical and metabolic processes (Shaban *et al.*, 2016).

The importance of plant material in medicine remains even of greater relevance with the current global shift to obtain drugs from plant sources. As a result of this, attention has been given to the medicinal value of herbal remedies for safety, efficacy and economy (Inuwa and Mohammed, 2018). Therefore, this work is set to evaluate the chemical constituents and to determine the concentration of PTEs in compliance with the recommended standards.

MATERIALS AND METHODS

Sample Collection and Chemical Composition

Samples of an alcoholic bitter (Confam bitter) were purchased from different vendors in Lagos state, Nigeria then made into composite, labelled and transported to the chemistry laboratory of the University of Lagos (UNILAG), Nigeria. The sample was percolated in a mixture of methanol/dichloromethane (70:30) for 48 hours. The extracts were shaken vigorously on a mechanical shaker for 2 hours and transferred to an ultrasonic bath to equilibrate for 2 hours. It was further purified using solid phase extraction (SPE) and finally concentrated to 2 ml using a nitrogen concentrator before finally submitted for Gas Chromatography-Mass Spectroscopic (GC/MS) analysis (AOAC, 2019) in the same laboratory.

Sample Digestion and PTE Estimation

The bitter sample (10 mL) was measured into a quartz beaker containing 10 mL of nitric acid (HNO_3) and gently heated on a hot plate inside a fume cupboard until the brown fumes turned colourless. The beaker was brought down to cool at room temperature, then the mixture was diluted with 20 ml of deionized water and filtered using Whatman No. 42 filter paper into a standard 250 ml volumetric flask and was made up to mark in readiness for PTE estimation with Atomic Absorption Spectrometer (AAS) (AOAC, 2019).

Toxicological Risk Assessment

Human health risks of exposure (consumption) to the bitters for both children and adults were evaluated according to the United States Environmental Protection Agency (US EPA) equation (RAGS, 2018). Children's exposure was included in the assessment. Although, it is a regulatory policy that persons below 18 years is discouraged from taking alcohol. However, it has been observed in the study area that they are sometimes given these bitters, either to induce sleep or as a source of medicine for the treatment of certain ailments. The average daily intake (ADI) for PTEs in the sample was calculated using Equation 1.

$$ADI_{ing} = C \times \frac{IngR \times EF \times ED}{BW \times AT} \times 10^{-6} \qquad (1)$$

where

ADIing stands for "average daily intake" per kilogram of body weight (Hadzi *et al.*, 2018).

The PTE concentration (C) in bitters is measured in mg/l,

Ingestion rate (*IngR*) is measured in litres per day,

Exposure duration (ED) is measured in years, which is equal to life expectancy

Exposure frequency (EF) is measured in days per year,

Body weight (BW) is measured in kilograms

Averaging time (AT) is measured in hours (ED x EF).

The hazard quotient (HQ) was calculated by dividing the average daily intake (ADI) of each PTE by their respective reference dose (RfD) (equation 2).

$$HQ = \frac{ADI}{rfD} \qquad (2)$$

Where;

HQ represents the hazard quotient via ingestion

RfD is the daily oral reference dose (mg/L).

The HQ is used to determine if PTEs present a non-carcinogenic risk to consumers after consumption (Naveedullah *et al.*, 2014; Famuyiwa *et al.*, 2023).

If the HQ values is equal to or less than one, it suggests that there will be no adverse health Implication on prolonged consumption, while a value of HQ > 1 indicates that there is a chance that there will be an adverse implication. The assessment of whether the bitters pose a risk of human cancer on prolonged consumption is known as the carcinogenic risks (CRs) of PTEs (Naveedullah *et al.*, 2014). This is calculated by multiplying the ADI with the slope factor (SF) using Equation 3.

$$CR = ADI \times CSF \qquad (3)$$

Where:

CR - cancer risk and ADI- average daily intake via consumption,

CSF - cancer slope factor (mg/kg/ day).

CR values falling between $1 \times 10^{-6} – 1 \times 10^{-4}$ are considered negligible and acceptable while those above 1×10^{-4} are considered significant and unacceptable (US EPA, 2013).

Data Analysis

Microsoft Excel, 2016 was used for data analysis, data visualization, and computing the toxicological risk assessment.

RESULTS AND DISCUSSION

Chemical Composition

The GC/MS total ion chromatogram (TIC) of the Confam bitter sample is presented in Figure 1, the chromatogram reveals the complexity of the system. However, a majority of the peaks appear to be baseline-separated, but some of them possess a different mass spectrum at different positions and are overlapped peaks. There are fourteen (14) single component peaks in the bitters, which can be easily identified and quantified by direct library searches. The compounds identified and their area percentage in the Confam bitters were Bactobolin (2.02%), 3-Methoxyamphetamine (1.10%), Propanamide (3.04%), Methylpent-4-phenylamine (1.10%), diisopropyl phthalate (35.62%), diisopropyl phthalate (1.86%), N, N'-Bis (3-aminopropyl) ethylenediamine (1.95%), Didodecyl phthalate (2.29%), n-Hexadecanoic acid (8.17%), Metaraminol (2.98%), Methyl octadec-11-enoate (4.53%), Linoelaidic acid (8.70%), cis-13-Octadecenoic acid (21.88%), Octadecanoic acid (3.30%), Octadecyl prop-1-en-2-yl carbonate (1.45%) and other compounds (Table 1).

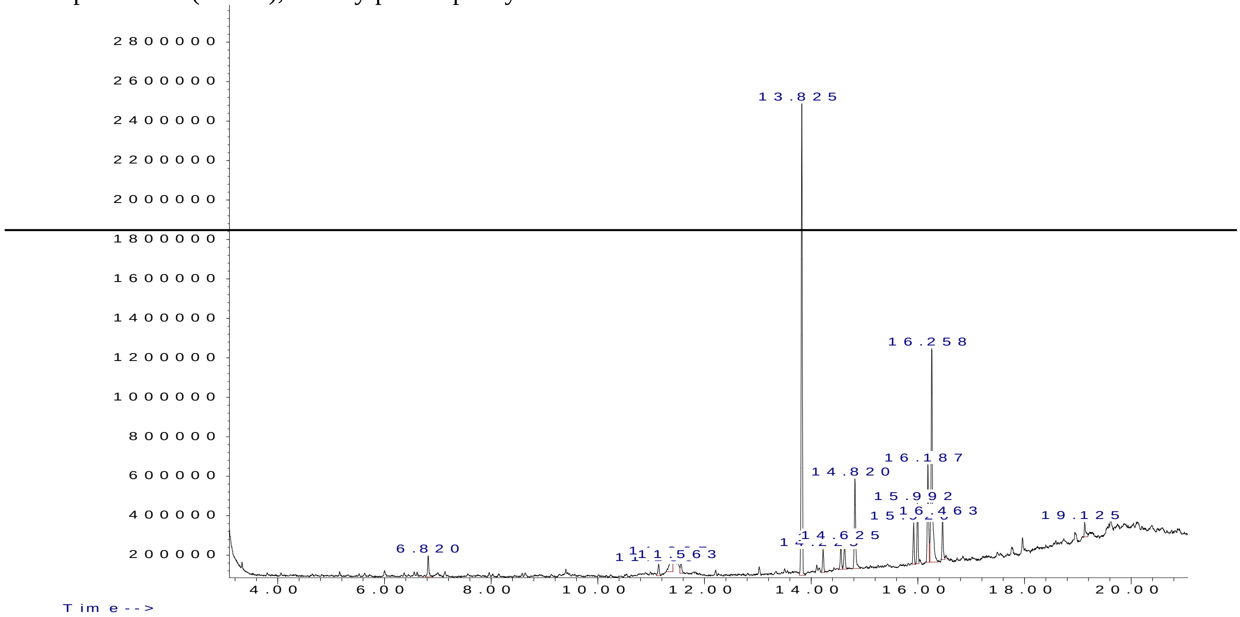

Fig. 1: Chromatogram of the compounds in Confam Bitters

Table 1: Chemical constituents in Confam Bitters

Peak No	Retention time (t_r)	Peak Area %	Chemical Components	Abundance
1	6.820	2.02	Bactobolin	107,059
2	11.139	1.10	3-Methoxyamphetamine	57,736
3	11.397	3.04	Propanamide	71,608
4	11.563	1.10	Methylpent-4-enylamine	60,706
5	13.825	35.62	Diisopropyl phthalate	2,390,084
6	14.558	1.95	N,N'-Bis (3-aminopropyl) ethylenediamine	128,231
7	14.625	2.29	Didodecyl phthalate	135,712
8	14.820	8.17	n-Hexadecanoic acid	454,300
9	15.920	2.98	Metaraminol	209,685
10	15.992	4.53	Methyl octadec-11-enoate	305,687
11	16.187	8.70	Linoelaidic acid	494,831
12	16.258	21.88	cis-13-Octadecenoic acid	1,082,415
13	16.463	3.30	Octadecanoic acid	212,340
14	19.125	1.45	Octadecyl prop-1-en-2-yl carbonate	74,705

Potentially Toxic Elements (PTEs) Concentration

The concentration of potentially toxic elements (PTEs) in bitters compared to the WHO standard (WHO, 2011) is presented in Figure 2, its shows that Zinc (1.26 mg/l), Copper (0.22 mg/l) and Nickel (90.16 mg/l) are within the WHO standard for Zn (3.00 mg/l), Cu (2.00 mg/l) and Ni (0.02 mg/l) respectively while Pb (0.03 mg/l), Cadmium (0.005 mg/l), Manganese (3.85 mg/l) and Chromium (0.66 mg/l) were higher than the standard for Pb (0.01 mg/l), Cd (0.003 mg/l), Mn (0.05 mg/l) and Cr (0.05 mg/l) respectively.

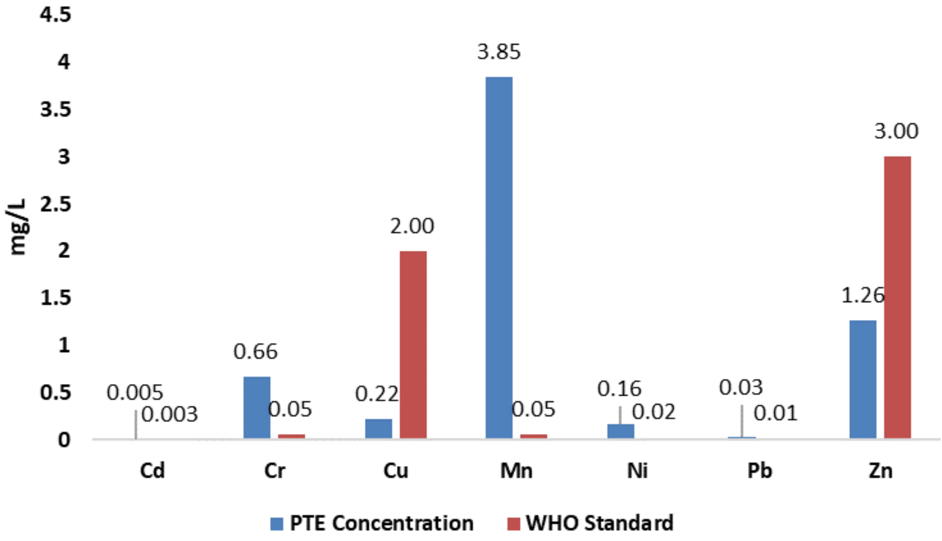

Figure 2: PTE concentration in bitters compared to WHO standard

Toxicological Risk Assessment

The toxicological risk of exposure to PTEs in sampled bitters was evaluated using average daily intake (ADI), non-carcinogenic risk (HQ) which was also used as the HI due to the absence of other exposure routes (inhalation and skin contacts) and carcinogenic risk (CR) over a lifetime exposure by the human population (Tables 3 and 4). The ADI showed that the children population was more susceptible to PTEs in the bitters than the adult population. The PTEs based on the ADI appeared in the descending order of Mn > Zn > Cr > Pb > Cu > Ni > Cd for both children and adults (Table 3). The Hazard Quotient (HQ) value revealed that all estimated PTEs have a value less than 1, with the studied PTEs appearing in descending order of Cr > Mn > Zn > Cd > Ni > Cu > Pb. Carcinogenic risk value revealed that estimated PTEs were less than 1 $\times 10^{-6}$, appearing in the descending order of Cr > Ni > Pb and Ni > Cr > Pb for children and adults respectively (Table 4).

Table 3: Average Daily Intake of PTEs in Confam Bitters

PTE	Children	Adults
Cd	3.29 x 10^{-7}	3.5 x 10^{-8}
Cr	4.34 x 10^{-5}	4.6 x 10^{-6}
Cu	1.45 x 10^{-5}	1.5 x 10^{-6}
Mn	2.53 x 10^{-4}	2.7 x 10^{-5}
Ni	1.05 x 10^{-5}	1.1 x 10^{-6}
Pb	1.97 x 10^{-6}	2.1 x 10^{-7}
Zn	8.28 x 10^{-5}	8.9 x 10^{-6}

Table 4: Non-Cancer and Cancer Risk Assessment

PTE	Oral RfD	Children		Adults	
		HQ	Cancer Risk	HQ	Cancer Risk
Cd	3.52 x 10^{-8}	3.29 x 10^{-4}		3.52 x 10^{-5}	
Cr	3.00 x 10^{-3}	1.45 x 10^{-2}	1.86 x 10^{-6}	1.55 x 10^{-3}	7.95 x 10^{-7}
Cu	4.00 x 10^{-2}	3.63 x 10^{-4}		3.88 x 10^{-5}	
Mn	1.40 x 10^{-1}	1.81 x 10^{-3}		1.94 x 10^{-4}	
Ni	2.00 x 10^{-2}	5.25 x 10^{-4}	3.97 x 10^{-6}	5.65 x 10^{-5}	1.70 x 10^{-6}
Pb	3.50 x 10^{-3}	5.63 x 10^{-4}	1.44 x 10^{-9}	6.03 x 10^{-5}	6.16 x 10^{-10}
Zn	3.00 x 10^{-1}	2.76 x 10^{-4}		2.96 x 10^{-5}	

Chemical Composition

The abundance of the components which is also used to represent its concentration in the Confam bitter sampled ranges from 57736 to 2,390,084. Majority of the compounds shows an excellent pharmacological property while a few have potentially hazardous effects. Bactobolin (107,059) is a natural compound known to possess an antitumor activity (Greenberg *et al.*, 2020). 3-Methoxyamphetamine (3-MA) is a derivative of amphetamine that acts as a central nervous system stimulant by releasing serotonin, dopamine, and norepinephrine (National Center for Biotechnology Information (NCBI, 2024). Methylpent-4-

enylamine (60,706) in an *in vitro* study revealed an anticancer activity against breast cancer (Ram *et al.,* 2020). 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester (2,390,084), was the most abundant compound detected in the Confam bitters, this is a compound with an antimicrobial, α-Glucosidase inhibition and the *in vivo* hypoglycemic effect (Govindappa *et al.,* 2014). N, N'-Bis (3-aminopropyl) ethylenediamine (128,231) is an indirect additives used in food contact substances which has also been reported to activate procaspase 2 (an inactive protease enzyme that is an inactive precursor of caspase) (U.S. Food and Drug Administration (US FDA, 2024). Caspase-2 is considered a pro-apoptotic caspase that has a unique role as a tumor suppressor in multiple tissue types (Brown-Suedel and Bouchier-Hayes, 2020). Didodecyl phthalate (DIDoP) (135,712) is a water-insoluble liquid but soluble in commonly used organic solvents. Didodecyl phthalate is used as a plasticizer in PVC and plastic production (Hahn *et al.,* 2016), with antimicrobial and antifouling properties (Vaithiyanathan and Mirunalini, 2015). n-Hexadecanoic acid (454,300) which is one of the most common saturated fatty acids found in animals, plants, microorganisms and major component of the oil from the fruit of oil palms (Koulman *et al.,* 2019). It pharmacological applications has been reported to antioxidant, hypocholesterolemic nematicide, pesticide, flavor, lubricant, antiandrogenic and hemolytic 5- alpha reductase inhibitor (Vaithiyanathan and Mirunalini, 2015). Metaraminol is a potent sympathomimetic amine used in the prevention and treatment of hypotension, particularly as a complication of anaesthesia (Kee, 2003). In a study by Ram *et al.* (2020), Metaraminol (209,685) among all the studied compounds in a study of *Bacillus subtilis* showed the best docking score towards estrogen receptor alpha and also showed an *in vitro* anticancer activity against breast cancer. Methyl octadec-11-enoate (305,687) are fatty acid with the property of absorption and distribution in human plasma and lipoprotein lipids (Ayoola *et al.,* 2020). Linoelaidic acid (494,831) is an isomer of linoleic acid a derivative of a fatty acid linoleic acid. It has been studied extensively due to its ability to modulate cancer, atherosclerosis, obesity, immune function and diabetes in a variety of studies (Mac Donald, 2002). Cis-13-Octadecenoic acid (1,082,415), an unsaturated fatty acid was the second most abundant compound isolated from the Confam bitters. Octadecanoic acid (212,340) also known as stearic acid belongs to the class of organic compounds known as long-chain fatty acids used to cure asthma, anti-inflammatory, and antiviral (Juliannah, 2023). It is a potentially toxic compound capable of causing schizophrenia, a psychotic disorder that is characterized by the disintegration of thought processes and emotional responsiveness (Xuan *et al.,* 2011; Yang *et al.,* 2011).

Potentially toxic element concentration

The estimated PTES (Zn, Cu, Pb, Ni, Cd, Mn, and Cr) were detected in the samples. The concentration of Zn, Cu and Ni were within the WHO standard (3.00, 2.00 and 0.02 mg/l) respectively while those of Pb, Cd, Mn and Cr were higher than the WHO (2011) standard (0.01, 0.003, 0.05 and 0.05 mg/l) respectively. Zinc is a significant antioxidant, an essential trace element which contributes to proper growth, blood clotting, DNA synthesis, and protein biosynthesis among others. However, excessive intake of zinc produces toxic effects on the blood lipoprotein levels, copper levels and the immune system (*Bolawa et al.*, 2020). The concentration of Zn (1.26 mg/l) in the bitters is lower than the report from Origin Bitter (5.12 mg/l) and Baby Oku (7.42 mg/l) but higher than that of the Alomo bitters (0.89 mg/l) by *Bolawa et al.* (2020). The concentration is, however, within the WHO (2011) standard (3.00 mg/l). Copper is an element which 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 (Ulla *et al.*, 2012). The concentration of copper (0.22 mg/l) from the study is higher than the report by *Bolawa et al.* (2020) from Alomo bitters (0.14 mg/l) and Baby Oku (0.03 mg/l) but lower than that of the Origin Bitter (0.61 mg/l). Although, the concentration is within the WHO (2011) standard (2.00 mg/l). Nickel is a trace element with nutritional function in the human body, although not fully understood (Taiwo *et al.*, 2019). Nickel possesses various mechanisms of toxicity including redox-cycling and inhabitation of DNA repair as well as exhibiting allergenic/sensitizing effects (Hague *et al.,* 2008). Other health issues associated with the toxicity of Nickel include cancer of the nose, larynx and prostate, respiratory failure, lung embolism, asthma, chronic bronchitis, heart disorders and birth defects (Khan *et al.,* 2016). The concentration of Ni (0.16 mg/l) from the study is lower than that reported from Baby Oku (0.18 mg/l) in Abeokuta, Nigeria (Taiwo *et al.,* 2020), but higher than that of the Origin Bitter (0.02 mg/l) (*Bolawa et al.,* 2020). The concentration is also higher than the WHO (2011) standard (0.02 mg/l). Lead (Pb) is described by the United States Environmental Protection Agency as potentially hazardous to most forms of life (US EPA, 2013). Exposure to Pb has been associated with reduced IQ, learning disabilities, slow growth, hyperactivity, anti-social behaviours and impaired hearing, damage of the kidney, liver and reproductive system, basic cellular processes and brain function (Iwegbue *et al.,* 2011). The concentration of Pb (0.03 mg/l) in the bitters is higher than the report from Origin Bitter (0.001 mg/l) but lower than Alomo bitters (0.07 mg/l) by

Bolawa et al. (2020). The concentration of Pb from the study is higher than the WHO standard (0.01 mg/l) which could be a potential hazard to the health of the consumers. The concentration of Cd (0.005 mg/l) from this study is lower than that recorded for Bitters (0.012 mg/l) by Taiwo *et al.* (2020) but higher than the WHO standard (0.003 mg/l). High Cd concentration causes serious effects on human health such as kidney damage which slows excretion and also affects the liver, the immune system and the vascular system (Atarug *et al.,* 2010). Manganese (Mn) as an element serves as an active constituent of various enzymes such as mitochondrial superoxide dismutase among others (Batista *et al.,* 2011). The concentration of Mn (3.85 mg/l) from this study is extremely higher than the WHO standard (0.05 mg/l). The concentration is higher than the report from Baby Oku bitters (0.04 mg/l) by Taiwo *et al.* (2020), Alomo bitters (0.009 mg/l) but lower than that of Origin bitters (7.41 mg/l) *Bolawa et al.* (2020). High levels of Mn in the human body have been linked to neurological disorders (Agency for Toxic Substance and Disease Registry (ATSDR), 2015). Chromium (Cr) toxicity is dependent on the species and oxidation states present, it is normally found in the considerably less toxic trivalent (Cr^{3+}) state and poorly absorbed in the gastrointestinal tract although, it has been reported to have beneficial effects on Type II diabetes (Hague *et al.,* 2008). However, the hexavalent (Cr^{6+}) form is carcinogenic (ATSDR (2015). The concentration of Cr (0.66 mg/l) from the study is higher than the WHO standard (0.05 mg/l) and that reported from Alomo bitters (0.009 mg/l), Baby Oku bitters (0.015 mg/l) (*Bolawa et al.*, 2020) and bitters (0.04 mg/l) (Taiwo *et al.,* 2020). The unacceptable concentration of Cd, Cr, Mn and Pb detected in the alcoholic bitters could be traced to contaminated plant materials from contaminated or polluted soil, and or the machineries and equipment used in the production phases.

Toxicological Risk Assessment

The toxicological assessment revealed that ADI value suggests the children as the primary susceptible group to the PTEs in the bitters, indicating Mn and Zn are the major exposure PTEs. This implies that giving the bitters to children needs to be avoided. Furthermore, the non-carcinogenic and carcinogenic risk assessment suggests a non-significant non-carcinogenic and carcinogenic risk for the human population. However, care should be taken for Cr exposure to children and Ni exposure to both children and adults due to their relatively close value to the standard limit.

CONCLUSION

The study investigated the chemical composition and toxicological risk assessment of PTEs in alcoholic (Confam) bitters. The bitters reveal the presence of several chemicals with a reported biological activity which may be contributing to the pharmacological effect (antimicrobial, anti-tumor, anti-cancer, anti-ulcer, anti-inflammation, anti-diabetics etc.) on the consumers but it also observed the presence of a compound with schizophrenic effect. Furthermore, some of the detected PTEs (Pb, Mn, Cd and Cr) can cause potential health risks due to their incompliance with the WHO standards. Toxicological risk assessment predicted a non-significant non-carcinogenic and carcinogenic risk for the human population.

RECOMMENDATIONS

Based on the study, it is recommended that good and hygienic practices should be followed during the production of the bitter, the sources of pollution should be identified and eliminated, there should be regular monitoring of the quality of bitters, packaging bottle and the plant materials used for production should be analyzed before use.

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