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CHEMICAL AND TOXICOLOGICAL EVALUATION OF EDIBLE CLAY (*ULO*) SOURCED FROM SOUTHERN NIGERIA

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ABSTRACT

The study was aimed at the pharmaco-chemical analysis and toxicological evaluation of three edible clay *(ulo)* samples from parts of southern Nigeria. The elemental constituents of the samples were determined by Atomic Absorption Spectroscopy (AAS) while the moisture and ash contents were determined using standard procedures. The acute toxicity assay was carried out according to Lorke's method using albino mice. The chloroform, butanol and aqueous fractions of the methanol extract were assayed for the presence of phytochemicals. There was presence of calcium, magnesium, nickel, cobalt, chromium, manganese, iron, lead and cadmium in all three samples. The mean concentration of some of the elements varied significantly ($p<0.05$). The lead (Pb) content of the samples was within the standard approved limits except sample B whose content (0.84ppm) was above the specified limit (0.3ppm). Only sample B contained mercury. The mean moisture contents did not vary significantly among the samples ($p=0.498$) while the mean ash contents varied significantly among the samples ($p=0.010$). No deaths were recorded from the acute toxicity assay. Phytochemical screening showed the presence of reducing sugars and steroidal nucleus. In conclusion, edible clay was found to be mostly inorganic but contained some traces of organic deposits with low nutritive value. The high inorganic content, low moisture content and low toxicity might be indicative of its relative safety for human consumption as a source of some minerals but caution should be applied in its sourcing to avoid ingestion of heavy metals such as lead and mercury.

KEYWORDS: Edible clay, toxicity, phytochemicals, minerals, heavy metals, pharmaco-chemical analysis.

INTRODUCTION

An eating disorder characterized by continuous intake of substances which are of little or no nutritional benefits is known as pica. It is characterized by a craving for substances that are to a large extent, non-nutritive. Such substances as ice, hair, paper, metal, stones, earth, glass and feces which are considered non-food may be consumed and the act of ingesting such pica is known as pagophagia, trichophagia, papyrophagia, metallophagia, litophagia, geophagia, hyalophagia and coprophagia respectively.[1] Geophagia is a type of pica. It is the pica of pottery, clay, earth or dirt.[2] The act is observed mainly in pregnancy, as a remedy for morning sickness.[3] Edible clay is one of the geophagic substances widely consumed by people from different localities for diverse reasons.

Edible clay is also known as calabash chalk and depending on the locality, it is known by different names such as 'Ulo', 'Ndom', 'Mabele', 'Nzu', 'Ebumba', 'Argile', 'Poto', 'Argile', 'Lacraie'.[4] The sample, *ulo (*as called in Igbo language*)*, best fits into the description of bentonite clays having extremely flat card-like crystals that are stacked together with weak electronic bonds. They adsorb positively charged substances because they are also negatively charged, and may be obtained naturally from springs.[5] The chemical constituents of clay show the presence of certain toxic trace elements such as Pb, Cr, Hg and Cd which are biologically toxic even in minute quantities.[6] Also reported in clay, in addition to heavy metals, are radioactive gases and organic chemicals.[7] However, the presence of iron, copper and manganese which play essential biological roles are also evident.[6] The toxic elements over time accumulate in biological systems causing toxicity of various kinds because they are not excreted easily.[8] Clay has equally been reported to contain both organic and inorganic constituents.[9] Certain plant chemicals may find their way into clay as their roots burrow deep into the soil. Beligh *et al* has shown that rhizopheric soil samples contain certain plant chemicals.[10] This study was, therefore, aimed at the chemical and toxicological evaluation of three edible clay *(ulo)* samples from parts of southern Nigeria to determine the safety and nutritional relevance of its wide local consumption.

MATERIALS AND METHODS

Sample collection and preparation

Three samples of edible clay were obtained between October and November, 2016 from different markets within South East and South South Nigeria. They were labeled accordingly: Sample A from Oil mill market Port Harcourt, sample B from Choba market Port Harcourt Rivers State and sample C from Ekwulobia market, Anambra State. The samples were properly identified by a taxonomist, dried, milled into powder and stored in air-tight containers at room temperature.

Reagents and solvents

The reagents and solvents used in carrying out this research include: Distilled water, Methanol, 2% H_2SO_4, chloroform, Butanol, Mayer's reagent, Dragendorff's Reagent, Picric acid, 5% Ferric Chloride, 10% Ammonia solution, Hydrochloric Acid,10% alcoholic solution of ∝-Napthol, Glacial acetic acid, Bennedict's reagent, Magnesium ribbon, Conc. Sulphuric acid.

Some Apparatus and Equipment Used

Analytical weighing balance, rotary evaporator, desiccator, water bath, amber glass macerating jars, beakers, conical flasks, ceramic crucible, filter paper, glass funnels, intra-gastric cannula, syringes (100 IU), test tubes, oven, muffle furnace, retort stand, hot plates, atomic absorption spectrophotometer (Varian AA240), volumetric flasks, separating funnels.

METHODS

Extraction

500 g of each sample was macerated for 48 h in 1000 ml of methanol and then filtered. The filtrate was concentrated using a rotary evaporator and the concentrate transferred to a crucible whose weight was initially determined. This was then placed in a desiccator until a constant weight was attained.

Atomic Absorption Spectroscopy

Sample Digestion

Metal analysis was conducted using Varian AA240 Atomic Absorption Spectrophotometer according to the method described by APHA.[11] Dry digestion method was carried out as described by Adrian.[12] The digested samples were analyzed for the presence of Hg, Cd, Pb, Co, Cr, Fe, Ca, Mg, Ni and Mn. Determination was carried out in triplicates for each metal.

Acute Toxicity Assay

Albino mice (of both sexes) weighing between 20 - 40 kg were used for the experiment. They were purchased and kept in the Animal House of Faculty of Pharmaceutical Sciences, University of Port Harcourt. The animals were cared for according to international regulations governing the use and care of laboratory animals. They were housed in cages and maintained on standard feeds pellets. Drinking water was allowed *ad libitum*. The animals were allowed to acclimatize for 1 week. Each animal was weighed prior to the commencement of the experiment.[13] Weighed quantities of each sample were suspended in distilled water and various concentrations prepared for LD_{50} determination using Lorke's method.[14]

Moisture Content Determination

2 g of each sample was weighed into a clean, dry crucible and the weight of the crucible + sample was noted before drying. The crucible containing the sample was put in the oven and heated at 100^0C for 3 h. The weight of the dried sample was determined and its percentage moisture content was calculated using the formula:

$$\% \text{ moisture content} = \frac{W_1 - W_2}{W_0} \times 100$$

Where

W_0= weight of sample

W_1 = weight of crucible + sample before drying

W_2= weight of crucible + sample after drying.

The determination was done in triplicates for the 3 samples.

Ash content determination

The ash content was determined according to the methods described by AOAC.[15]

Liquid-liquid Partition

Weighed quantity of the dry sample extracts were reconstituted in 5 ml of methanol and then made up to 75 ml with distilled water. Fractionation was done by liquid/liquid partitioning with chloroform (200 ml) and, subsequently, butanol (200 ml) using separating funnel. The chloroform, butanol and aqueous fractions were collected and concentrated *in vacuo*. The same method was repeated for the 3 samples.

Phytochemical Screening

Phytochemical screening was carried out on the chloroform, butanol and aqueous fractions of the methanol extract. The method used was according to Harbourne[16] and Sofowora[17] with some modifications.

Statistical Analysis

The results obtained from the metal analysis, moisture and ash contents were presented as mean ± SEM (n=3). Data obtained were subjected to One-way ANOVA using statistical package for social sciences (SPSS) software for windows version 16. $P \leq 0.05$ was considered significant.

RESULTS AND DSCUSSION

Table 1: Mean Concentrations (± SEM) of Metallic Constituents of *Ulo* in Samples A, B and C Compared with FAO/WHO Safe Limits.

Metal	Sample A (PPM)	Sample B (PPM)	Sample C (PPM)	FAO/WHO safe limit (PPM)	P- value
Iron	23.066±0.001	23.284±0.001	23.00±0.600	425.5	0.825
Cobalt	0.040±0.011	0.050±0.006	0.050±0.006	50	0.630
Manganese	3.287±0.091	4.650±0.108	6.343±0.075	500	0.000
Chromium	0.127±0.008	0.137±0.007	0.083±0.008	2.3	0.008
Calcium	56.053±0.001	56.481±0.001	64.305±0.001	250	0.000
Mercury	0.100±0.023	0.000±0.000	0.000±0.000	0.3	0.002
Magnesium	21.425±0.001	21.583±0.001	21.419±0.001	150	0.000
Cadmium	0.020±0.006	0.013±0.003	0.020±0.000	0.2	0.422
Lead	0.153±0.039	0.840±0.010	0.193±0145	0.3	0.000
Nickel	0.837±0.022	1.276±0.054	1.228±0.028	67	0.000

The FAO/WHO limits for calcium and magnesium are the maximum daily limits.

Table 2: Mean Percentage Moisture contents (± SEM) of samples A, B and C.

Sample	Percentage Moisture Content (%)
A	8.832±0.611
B	8.715±1.21
C	7.281±1.01

P value: 0.498.

Table 3: Mean Percentage Ash contents (± Standard Error of Mean) of samples A, B and C .

Sample	Percentage ASH Content (%)
A	77.955±0.245
B	79.143±0.449
C	84.442±1.723

P value: 0.01.

Table 4: Results of phytochemical screening.

	Sample A			Sample B			Sample C		
Phytochemical	***AQ***	***CHLOR***	***BUT***	***AQ***	***CHLOR***	***BUT***	***AQ***	***CHLOR***	***BUT***
Alkaloid	−	−	−	−	−	−	−	−	−
Saponin	−	−	−	−	−	−	−	−	−
Tannins	−	−	−	−	−	−	−	−	−
Phlobatannins	−	−	−	−	−	−	−	−	−
Combined Anthraquinone	−	−	−	−	−	−	−	−	−
Free Anthraquinone	−	−	−	−	−	−	−	−	−
Flavonoid	−	−	−	−	−	−	−	−	−
Reducing Sugars	−	+	−	+	+	−	−	+	−
Salkowski	−	+	−	−	+	−	+	+	−
Molisch	+	+	−	+	+	−	−	+	−
Leiberman-Buchard	−	+	−	−	+	−	+	+	−

KEY: + = present; −= absent; Aq: aqueous fraction; Chlor: chloroform fraction; But: butanol fraction.

Table 5: Result of 24 hour acute toxicity test.

Groups	Behavioural Observations	Deaths
A (10mg/kg)	Normal	0
B (100mg/kg)	Normal	0
C (1000mg/kg)	Normal	0
D (1600mg/kg)	Normal	0
(2900mg/kg)	Normal	0
(5000mg/kg)	Normal	0

NB: Groups A, B and C contained 3 animals each.

DISCUSSION

The elemental analysis results showed the presence of calcium, magnesium, nickel, cobalt, chromium, manganese, iron, lead and cadmium in all three samples. Mean concentration of Mg, Ca, Pb, Cr, Mn, Ni and Hg varied significantly among the samples ($p<0.05$), while that of Co, Fe, and Cd did not differ significantly among the samples ($p>0.05$). The mean concentrations of most of the metals were below the safe limits recommended by FAO and WHO. Out of the ten metals analyzed, sample B recorded the highest mean concentrations in about six of them (Table 1) viz: Pb, Ni, Mg, Cr, Co and Fe. Sample B also had a mean lead concentration exceeding the officially recommended limit. This slightly high lead content could be as a result of its natural source or degree of exposure on storage. Lead contamination comes from improper industrial waste disposal (lead batteries and other devices) or gaseous pollutants from fuel combustion settling on the soil and getting leached into the soil which over time become part of clay matrix. On ingestion, inorganic lead is absorbed depending on the age, health and nutritional status of the individual. Lead is not metabolized in the liver. Nearly all ingested organic lead is absorbed and exchanged primarily among

three compartments namely: blood (where its half-life is estimated to be 28-36 days),[18,19] mineralizing tissues (bones and teeth) which contain the majority of the lead burden and soft tissues (liver, kidney, lungs, brain, spleen etc.).[20] Lead has the tendency to displace calcium in mineralizing tissues because they both have the same oxidation state of +2. It therefore overwhelms the physiological roles of calcium, especially in children, accumulates and get stored in the bones and could be released slowly over time resulting to neuronal, hepatic and renal damages. With lead, there is also a likelihood of congenital intoxication.

On the other hand, iron, magnesium and calcium are macro minerals occurring naturally on the earth crust. Iron is a component of haemoglobin, the oxygen carrying pigment in blood while calcium is a major component of bone and teeth. Magnesium is required for energy production, oxidative phosphorylation and glycolysis. Cobalt and nickel are trace elements essential for human health. Nickel is also an important factor in hormone, lipid and cell membrane metabolism as it improves insulin function by activating enzymes associated with glucose breakdown and utilization. Cobalt is an integral part of vitamin B12 (cobalamin) and is essential in myelin formation, erythrocyte production, carbohydrate and protein synthesis, including bioactivation of foliate.

From the results, only sample A contained mercury. Mercury has no health benefits. The absorption of elemental mercury occurs rapidly and diffusion occurs into the blood stream and throughout the body (including the blood brain barrier and placenta because it is lipophilic), which could result to neuronal damage and congenital malformations. It occurs naturally in nature and can also find its way into the soil through fuel combustion, improper disposal of mercury-containing wastes such as mercury-containing devices. However, the level contained in sample A is within the permissible limit. Manganese plays some metabolic roles, assists enzyme functions and contributes to healthy bone structure. Cadmium contents of samples A and C were the highest. Cadmium in the soil could be from natural sources, sewage sludge and phosphate fertilizers. Most cadmium ingestions are of terrestrial origin with the aforementioned sources constituting the major routes through which the earth gets laden with cadmium. It has no health benefits and can accumulate as it is not easily metabolized. Cadmium accumulates throughout life time especially in the liver and kidney.

With reference to the results obtained (Table 2), Sample A had the highest moisture content of 8.832%, but the values among the 3 samples did not vary significantly ($p=0.498$). Moisture content above 15% is considered conducive for microbial growth.[21] The three samples, however, had mean moisture contents below the stated value, hence, too dry to support microbial growth. It could also be observed from the results in Table 3 that Sample C had the highest ash content of 84.442%, with the values varying significantly among the samples ($p=0.01$). Thus, *ulo* has a high ash content indicating high inorganic composition which also might not support heavy microbial contamination. The toxicity results in Table 5 when compared with the classification criteria for the toxicity of substances indicate that edible clay can be considered to be of relatively low toxicity, though under certain circumstances may present some dangers to vulnerable population (children and pregnant women). With edible clay, at a dose of 5000mg/kg, no deaths were recorded.

Phytochemical analysis of the samples revealed the presence of some plant metabolites in the organic component of the edible clay. The chloroform, as well as the aqueous fractions of some of the samples, contained reducing sugar and also tested positive for Salkowski's and Leiberman-Buchard tests, indicating the presence of tritepenoids and steroids respectively. They equally tested positive for Molisch's test indicating the presence of simple sugars. Other phytochemicals such as flavonoids, tannins, phlobatannins, anthraquinones, saponins, and alkaloids were found to be absent. Thus, the chloroform fractions of the three samples contained the highest amount of phytochemicals. Previous reports have shown that the presence of phytochemicals varies among various plant parts and also depends on the fractions evaluated as the ability of a solvent to extract a compound depends on the solubility of the compound in the solvent.[22] Since chemical tests showed the presence of both sugars and steroids, this could also suggest the presence of inositol,[10] as the compound has been isolated from soil samples. Inositol or its phosphates and associated lipids are found in many fruits.[23] The presence of phytochemicals in the samples could be from the natural sources: fallen leaves of plants, dead animals, insects, fungi, etc. which could have been leached into the soil and gotten embedded into clay over a long period.

CONCLUSION

Edible clay (*ulo*) can serve as a source of mineral (although its nutritive value is relatively low). It has low toxicity and could be considered relatively safe for consumption. It is not susceptible to heavy microbial contamination, but depending on its source, it might have high heavy metal composition, hence, caution should be applied in its sourcing and indiscriminate consumption.

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