

Full Length Research Paper

Physico-chemical quality of some commonly consumed stimulants and spices in Nigeria

John Adekunle Oyedele Oyekunle*, Aderemi Okunola Ogunfowokan, Abolanle Saheed Adekunle, Michael Sola Akanni, Winston Doherty and Damilola Olusanya Abraham

Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.

Accepted 5 November, 2012

Commonly consumed parts of *Tetracarpidium conophorium*, *Garcinia kola*, *Cola acuminata*, *Cola nitida* and *Aframomum melengueta* were investigated for their trace metals, pH, conductivity and total dissolved solid values. Trace metal levels ranged between 42.24 ± 6.23 to 541.51 ± 189.37 $\mu\text{g/g}$ Co and 36500.23 ± 367.01 to 115280.25 ± 397.33 $\mu\text{g/g}$ K. The pH fell within 6.10 ± 0.12 in *Tetracarpidium conophorium* and 9.25 ± 0.22 in *A. melengueta* while conductivity values lied between 0.21 ± 0.05 in *A. melengueta* and 1.92 ± 0.22 in *G. kola*. Total dissolved solid values ranged from 0.14 ± 0.03 in *A. melengueta* to 0.99 ± 0.13 $\mu\text{g/g}$ in *T. conophorium*, *G. kola*, *C. nitida* and *A. melengueta*, and might not constitute much risk since alkaline foods are considered healthy for consumption. A sustained consumption of these stimulants might constitute serious health inharmonies to the consumers because of bioaccumulation of heavy metals.

Key words: Nigeria, stimulants, mineral content, pH, heavy metals, health risk.

INTRODUCTION

The consumption of stimulants and spices for various purposes by humans dated back to the prehistoric era. Several village and city dwellers in Nigeria including the educated ones eat stimulants such as cola nut and bitter cola casually from time to time. Bitter cola is reputed for high medicinal values and snake repellent. People use kola nuts, bitter cola and alligator pepper commonly at such occasions as naming, marriage and graduation ceremonies. In some parts of Africa especially West Africa, kola nuts and bitter cola are offered as a gesture of friendship and hospitality (Gbile, 1984). They are also used as objects of ritual observance at places of traditional worships by traditional healers and priests. The use of cola nuts at ceremonies as stated earlier is similar to the American Indian Peace Pipe of breaking bread in a religion context (Gbile, 1984). In Nigeria, especially among the Yoruba and Ibo, before a marriage is finally contracted, a bag of cola nuts is often given by a groom

to the parents of the bride. However, many consume these stimulants regularly on daily basis for the medicinal benefits (Ajebesone and Aina, 2004). The Tiv and Ibo people (of Nigeria) prepare a blend of alligator pepper with groundnut, and offer it to visitors as appetizers, to be eaten along with garden eggs or cola nuts. Alligator pepper is one of the major spices used for soup making among certain tribes in Nigeria. As such, these stimulants or spices are a common sight in Nigerian markets generally.

So far, a lot of works have been done on the active medicinal components of these stimulants (Ajebesone and Aina, 2004; Crosby and Marshall, 2006), but the same cannot be said of their intrinsic effects such as their ability to negatively impact the blood balance. An acidic food breaks down the normal body function of the biological system resulting in degenerative conditions within the body and providing a receptive environment for the buffering capacity and their heavy metal content whose health effects are well recognized (Ang et al., 1989; Leek et al., 1998; Ecobichon, 2001; Goyer and Clarkson, 2001; Anderson, 2003). It is thus worthwhile to have an idea of what amount of heavy metals that people

*Corresponding author. E-mail: oyekunle@oauife.edu.ng, biola4jaaoo@yahoo.com. Tel: +234 8035673017.

who have become addicted to eating these stimulants may be exposed to through their regular consumption of the stimulants. It is also imperative to know how the stimulants may affect the pH of alimentary canal and the body system as a whole since, according to Wilson (2006), the optimal pH of the body generally is slightly alkaline, between 7.0 and 7.5. Outside this range, the body activity is no longer optimal and the metabolism is out of development of cancer (Wilson, 2006).

The pH of the body is largely influenced by the by-products of metabolism and our diet (Wilson, 2006). Thus, pH is directly affected by the various categories of food that we eat and the internal mechanism involved in their processing. It is quite possible that the body fluid pH and its heavy metal content at a period of time could determine the wellness of the body or how readily pronounced a health attack on the body becomes.

MATERIALS AND METHODS

Sample collection and preparation

The five selected stimulants, namely: *Tetracarpidium conophorium* [Walnut (Yoruba: Asala/Awusa)], *Garcinia kola* [Bitter cola (Yoruba: Orogbo)], *Cola acuminata* [Kola (Yoruba: Obi Abata)], *Cola nitida* [Kola (Yoruba: Obi Gbanja)] and *Afromomum melengueta* [Alligator pepper (Yoruba: Atare)] were purchased from Ile-Ife main market, Osun state, Nigeria. The samples were stored in a polythene bag and kept in a refrigerator prior to further processing. Where necessary, decortications of the samples were manually done. Samples for the determination of pH variation, conductivity and total dissolved solids were ground in the form in which they are usually consumed using an agate mortar and pestle which have been scrupulously cleaned and dried to a constant weight at 105°C. Samples for ED-XRF, having been dried to a constant weight and ground to a fine powder were stored in a self-sealing polythene bag.

Reagents used and treatment of containers

All glassware and crucible containers used were washed in detergent solution, rinsed several times with distilled water and then soaked for 48 h in 10% HNO₃, after which they were rinsed further with distilled water and dried overnight in an oven at a temperature of 120°C (Ogunfowokan and Fakankun, 1998). Freshly obtained doubly distilled water was heated to boiling and allowed to boil for 20 min to ensure that all the dissolved gasses were driven out. The distilled water was then stored in a 1 L pre-washed and acid soaked separating funnel with quick-fit glass cover to minimize further dissolution of atmospheric gasses. It was kept in this condition overnight to bring its temperature down to that of the room temperature ($\approx 25.6^\circ\text{C}$).

pH changes, conductivity and dissolved solids determination

Accurately weighed 10 g of each well ground sample was weighed into a 100 ml beaker containing 50 ml of distilled water previously heated to a temperature of $37\pm 1^\circ\text{C}$ on a thermostated hot plate. The choice of 37°C was informed by the fact that this is the average body temperature of normal human beings. Continuous agitation of the mixture was carried out with the aid of a magnetic stirrer to

obtain a uniformly mixed solution. pH changes were monitored at 30 min interval for 6 h using a hand-held digital pH meter (Microprocessor based pH Testr, OAKLON Instrument, Singapore).

Similarly, conductivity and total dissolved solids were determined simultaneously using a hand-held digital conductivity/total dissolved solid meter [HACH Conductivity/TDS Meter (Cat. No.44600-00), OAKLON Instrument, Singapore]. Triplicate analysis was done in each case to reduce the risk of analytical error. A blank determination was done alongside each measurement.

Determination of mineral content

The method described by Ravisankar et al. (2011) was used for the pelletization procedure. One gram (1 g) of the fine ground sample and 0.5 g of boric acid (H₃B₂O₃) were mixed. The mixture was thoroughly ground and pressed to a pellet of 30 mm diameter using a 15 ton hydraulic press. Mineral content determination was done using Energy Dispersive X-ray Fluorescence (ED-XRF) model 710H, 7300Si (L), 4861BM/PC available at the Centre for Energy and Research Development (CERD) Obafemi Awolowo University, Ile-Ife, Nigeria. The choice of ED-XRF for the analysis was informed by the equipment high sensitivity, its multi-elemental determination capability, lower cost of analysis, availability and the desire to evaluate its potential for foodstuff analysis.

Quality control measures

Standardizing of pH meter

Buffered solutions of pH 4.0 and 9.0 were used to standardize the pH meter.

Recovery analysis

Since no certified heavy metal reference materials were available for this study, recovery analysis was performed in order to ascertain the accuracy of the analytical procedures. A portion (5.0 g) of the powdered duplicate samples (B) in Teflon beakers was spiked with 10 ml of 10 $\mu\text{g/ml}$ standard mixture of Ca²⁺, Cu²⁺, K⁺, Ni²⁺ and Zn²⁺ using doubly distilled water. The spiked sample was gently sonicated for about 30 min to ensure proper dissolution of the dissolved salts. The mixture was put in a Gallenkamp Oven BS (OV-160, England) at 105°C until a constant weight was obtained. A blank (A) was prepared simultaneously to check for background levels of heavy metals in the samples. From these, pelletized samples were prepared for ED-XRF analysis. Percentage recovery (%R) was estimated from:

$$\%R = \frac{B - A}{\text{Amount dissolved in B}} \times 100$$

RESULTS AND DISCUSSION

The pH meter, after standardization gave a pH value of 5.71 ± 0.11 for exposed distilled water while it gave a pH of 6.99 ± 0.21 for the distilled water stored in the separatory funnel. The pH 5.71 was in agreement with O'Neil's observation that exposed distilled water soon acquires a pH of 5.6 (O'Neil, 1993) as a result of the equilibrium reaction:

Table 1. Recovery analysis result.

Element	Amount spiked ($\mu\text{g/g}$)	Amount recovered ($\mu\text{g/g}$)*	Percentage recovery (%R)
Ca	10	8.629 \pm 0.611	86.29 \pm 7.08
Cu	10	9.631 \pm 0.863	96.31 \pm 8.96
K	10	10.102 \pm 0.356	101.02 \pm 3.52
Ni	10	9.122 \pm 0.461	91.22 \pm 5.05
Zn	10	8.342 \pm 0.615	83.42 \pm 7.37

* Values are mean of triplicate analysis \pm s.d.

Table 2. pH variation^a of the stimulants at 37 \pm 1°C.

Time (min)	0	30	60	90	120	150	180	210	240	270	300	330
Sample	pH											
<i>T. conophorium</i>	7.51 \pm 0.12	7.43 \pm 0.10	7.31 \pm 0.35	7.29 \pm 0.11	7.14 \pm 0.29	7.10 \pm 0.15	6.68 \pm 0.13	6.66 \pm 0.26	6.66 \pm 0.15	6.66 \pm 0.00	6.66 \pm 0.03	6.66 \pm 0.00
<i>G. kola</i>	6.10 \pm 0.12	6.29 \pm 0.21	6.58 \pm 0.05	6.41 \pm 0.04	6.66 \pm 0.41	6.69 \pm 0.11	7.02 \pm 0.13	7.13 \pm 0.10	7.15 \pm 0.03	7.16 \pm 0.01	7.15 \pm 0.00	7.16 \pm 0.02
<i>C. acuminata</i>	6.66 \pm 0.12	6.65 \pm 0.21	6.58 \pm 0.05	6.51 \pm 0.11	6.45 \pm 0.15	6.41 \pm 0.01	6.41 \pm 0.06	6.41 \pm 0.17	6.41 \pm 0.21	6.38 \pm 0.13	6.38 \pm 0.01	6.38 \pm 0.00
<i>C. nitida</i>	7.36 \pm 0.23	7.29 \pm 0.05	7.03 \pm 0.03	6.90 \pm 0.12	6.78 \pm 0.22	6.75 \pm 0.16	6.72 \pm 0.15	6.72 \pm 0.11	6.73 \pm 0.05	6.72 \pm 0.00	6.71 \pm 0.11	6.71 \pm 0.11
<i>A. melengueta</i>	9.25 \pm 0.22	9.20 \pm 0.16	8.95 \pm 0.07	8.81 \pm 0.01	8.83 \pm 0.00	8.72 \pm 0.15	8.72 \pm 0.21	8.71 \pm 0.02	8.69 \pm 0.33	8.68 \pm 0.06	8.71 \pm 0.22	8.70 \pm 0.09

^aAverage of triplicate analysis \pm s.d.

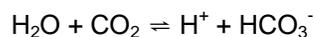


Table 1 represents the results of the recovery analysis, with the percentage recovery (%R) of not less than 83.42 \pm 7.12, the analytical procedures can be adjudged to be reliable. The results of the pH variation of stimulants at 37 \pm 1°C are presented in Table 2. Generally, the pH of the stimulants ranged from 6.10 \pm 0.12 in *T. conophorium* to 9.25 \pm 0.22 in *A. melengueta*. According to the pH variation, the stimulants in this study can be classified into four types: (1) those that are acidic; (2) those that are alkaline, but capable of progressively becoming acidic; (3) those that are acidic, but are capable of acquiring alkaline status and (4) those that are predominantly

alkaline. These features are clearly seen in Table 2.

According to this proposed classifications, *T. conophorium* and *C. nitida* are examples of stimulants that can progressively change from alkaline to acidic status over a period of time in an oxidative environment. Thus, regular consumption of *T. conophorium* and *C. nitida* might constitute some health risks especially when they stay in the body for more than 3 h after which their acidic levels become worrisome from nutrition point of view. If the active ingredients in these stimulants are the components responsible for the observed pH variations and the oxygen from the gut and hemoglobin can bring about the same changes, then those who regularly consume these

stimulants may be in danger of developing chronic inharmonies within their system over a period of time. However, the active ingredients that were probably responsible for the pH changes observed were not the object of the present study. *G. kola* changed from acidic condition to an alkaline condition. This might not constitute any increase in the acidity of the body invariably, more so, that the final pH is only slightly alkaline. *C. acuminata* remained acidic throughout the period of study; it thus might remain acidic throughout in the system of a consumer. As such, it should either be avoided completely or seldomly be eaten as a stimulant. To form a habit of eating it regularly tantamounts to inviting degenerative conditions into the system. On the other hand,

Table 3. Conductivity (ms/cm) ^bvalues of stimulants at 30 min intervals.

Time (min)	0	30	60	90	120	150	180	210
Sample	Conductivity values							
<i>T. conophorium</i>	1.76±0.12	1.84±0.00	1.90±0.11	1.92±0.00	1.92±0.22	1.91±0.01	1.91±0.01	1.92±0.03
<i>G. kola</i>	0.62±0.13	0.64±0.13	0.66±0.25	0.65±0.05	0.67±0.13	0.68±0.21	0.68±0.02	0.69±0.11
<i>C. acuminata</i>	1.75 ±0.21	1.78±0.15	1.82±0.41	1.85±0.14	1.85 ±0.21	1.86 ±0.10	1.87 ±0.05	1.86 ±0.21
<i>C. nitida</i>	1.30 ±0.31	1.34±0.22	1.36±0.15	1.40 ±0.27	1.42 ±0.11	1.42 ±0.13	1.41 ±0.03	1.41±0.31
<i>A. melengueta</i>	0.21±0.13	0.14±0.03	0.15±0.10	0.29 ±0.06	0.29 ±0.08	0.32 ±0.04	0.31 ±0.05	0.32±0.03

^bAverage of triplicate analysis± s.d.

Table 4. Total dissolved solid [TDS] (g/L)^c at 37±1°C.

Time (min)	0	30	60	90	120	150	180	210
Sample	TDS							
<i>T. conophorium</i>	0.91±0.12	0.94±0.16	0.96±0.05	0.99±0.03	0.99±0.13	0.99±0.01	0.99±0.12	0.99±0.12
<i>G. kola</i>	0.26±0.11	0.29±0.04	0.33±0.01	0.35±0.05	0.35±0.05	0.35±0.03	0.35±0.00	0.35±0.00
<i>C. acuminata</i>	0.90±0.11	0.91±0.01	0.93±0.15	0.94±0.08	0.94±0.14	0.95±0.04	0.94±0.02	0.94±0.02
<i>C. nitida</i>	0.65±0.03	0.67±0.02	0.68±0.06	0.69±0.16	0.69±0.05	0.70±0.05	0.71±0.01	0.71±0.01
<i>A. melengueta</i>	0.31±0.13	0.14±0.03	0.15±0.02	0.16±0.06	0.17±0.07	0.17±0.01	0.17±0.02	0.17±0.02

^cAverage of triplicate analysis± s.d.

A. melengueta is a slightly alkaline stimulant predominantly. Although, both acidic and alkaline foods may cause degenerative effects to the consumers, acidic foods are more culpable. Hence, the consumption of *A. melengueta* might be considered safe enough since slightly alkaline foods are considered healthy for consumption (Wilson, 2006). Those who eat two or three different types of stimulants are likely to be more prone to deleterious effects of pH variations especially if the stimulants of choice are those belonging to the acidic class. It might be safer if an alkaline stimulant is interchanged with acidic stimulants for example, *A. melengueta* with *C. nitida* or *T. conophorium*, if stimulants must be

taken at all.

The conductivity and total dissolved solids (TDS) values are presented in Tables 3 and 4. Conductivity values varied between 0.62 ± 0.13 in *G. kola* and 1.92 ± 0.003 in *T. conophorium* while TDS values ranged from 0.26 ± 0.11 in *G. kola* to 0.99 ± 0.13 in *T. conophorium*. The results further established the acidic nature of *G. kola* as compared with the alkaline nature of *T. conophorium* which can be attributed to possibly the oxide form of the dissolved metals present in the stimulant. One other obvious deduction is that the higher the total dissolved solids, the higher the conductivity value, although the variation pattern is not exactly predictable. Apart from this, if the

dissolved solids are soluble salts of some or all the metals present in the stimulants (as the case may be), then, these metals may have high absorptivity values within the alimentary canals and thus get to body organs such as the liver or kidney that may readily store or redistribute them to other parts of the body. The implication is that the health effects of the metals could be felt more readily in such organs depending on whether their threshold levels have been attained or not as a result of the bioaccumulation capacity of the consumers.

Table 5 gives the mineral contents of the stimulants in µg/g. In agreement with the values normally obtained for plant food products

Table 5. Mineral content^d of stimulants in µg/g.

Sample	K	Ca	Mn	Fe	Ti	V	Cr	Co	Ni	Cu	Zn	Br
<i>T. conophorium</i>	106860.23±367.01	51610.12±77.30	375900.15±131.53	1241.46±45.77	ND	ND	ND	ND	147.22±52.30	178.16±61.27	238.58±83.69	ND
<i>G. kola</i>	36500.11±131.91	6485.80±87.81	432.61±52.04	889.80±86.38	ND	ND	ND	541.51±89.38	707.02±52.30	675.74±231.67	583.21±72.08	ND
<i>C. acuminata</i>	103680.35±357.91	10610.28±37.21	ND	274.79±27.50	163.53±75.31	134.87±57.05	139.72±34.65	33.33±2.45	106.53±37.41	112.81±40.62	82.82±31.99	ND
<i>C. nitida</i>	87290.29±301.92	11370.56±39.92	63.61±4.43	352.41±23.27	ND	ND	83.10±5.45	42.24±6.23	141.23±49.15	171.60±59.74	133.46±46.54	ND
<i>A. melengueta</i>	115280.25±397.33	100503.15±56.57	255.53±29.79	900.29±39.57	ND	ND	ND	ND	109.90±38.79	139.91±48.38	145.08±50.65	706.63±51.99

^dAverage of triplicate analysis± s.d.

(Greenwood and Earnshaw, 1984; Adeyeye and Ajewole, 1991), K was the most abundant mineral in all the samples with values ranging from 36500.11 ± 131.97 µg/g in *G. kola* to 115280.25 ± 397.33 µg/g in *A. melengueta*. Similarly, all the samples contained relatively high content of Ca with values between 6485.80 ± 87.81 in *G. kola* to 100503.15 ± 56.56 µg/g in *A. melengueta*. Since K and Ca are alkaline and alkaline earth metals respectively, the high K and Ca value in *A. melengueta* convincingly agreed with the persistent alkaline pH reported for the stimulant over the pH period monitored. Assuming the Ca in these stimulants is quite bioavailable, then there are two implications: it is possible that the stimulants might be reliable sources of Ca for bone calcification or formation; or there may be the risk of calcium oxalate stone formation. According to Von Unruh et al. (2004), calcium is the most potent modifier of the oxalate absorption. Low calcium diet (< 200 mg Ca per day) increases the risk of calcium oxalate stone formation while high calcium diet (>1200 mg Ca per day) decreases it. In none of the stimulants is the level of Ca > 52 mg. Thus, people who eat items with low Ca contents and also eat less of the stimulants are more prone to bladder stone problems. However, large consumption per day of the stimulant may amount to reduction of the risk.

Manganese (Mn) was detected only in four samples with *C. acuminata* containing a non-detectable (ND) amount. Mn levels in the four samples ranged between 63.61 ± 4.43 µg/g in *C. nitida* to 375900.51 ± 131.53 µg/g in *T. conophorium*. While the levels may be within its essential role limits in other stimulants studied, the same cannot be said of *T. conophorium*. Heavy consumption of *T. conophorium* might lead to bioaccumulation of Mn in the system with its attendant health effects like gastrointestinal irritation, pneumonitis, neuropsychiatric disorder which is characterized by irritability and difficulty in walking (Goyer and Clarkson, 2001). Iron (Fe) was detected in all the samples with concentrations ranging from 274.79 ± 27.50 µg/g in *C. acuminata* to 1241.46 ± 45.77 µg/g in *T. conophorium*. The levels of Fe in these samples are not enough to be relied upon for the daily need, but may serve as supplementary sources. If the stimulants should be the source of Fe, the amount consumed may be so high that serious health effects may arise since there are other effects that may arise from heavy consumption of stimulants. Ti and V are minor toxic metals and they occurred only in *C. acuminata* to an extent of 163.53 ± 75.31 and 134.87 ± 57.05 µg/g respectively. Thus, constant consumption of *C. acuminata* can lead to the manifestations of health

effects associated with Ti and V such as slight fibrosis of lung tissue, pulmonary overload, bronchitis and bronchopneumonia (Johnson and Tasell, 1991).

Chromium (Cr) which is an essential metal was detected in *C. nitida* at 83.10 ± 5.45 µg/g levels and 139.72 ± 34.65 µg/g levels in *C. acuminata*. The levels of Cr in these samples are not adequate for daily dietary needs, but may serve as supplementary sources. Large consumption may increase the levels thereby causing cancer of the respiratory tract. Cobalt (Co), Copper (Cu) and Zinc (Zn) are other essential metals with potential for toxicity. Cu and Zn were detected in all the samples while Co was detected only in *G. kola*, *C. acuminata* and *C. nitida*. In none of these stimulants is the level of Co, Cu or Zn greater than the normal body levels. However, large consumption of these stimulants per day can cause bioaccumulation of the elements and may increase the health risks of these metals such as formation of goiter (erythropoietic effect), vomiting, diarrhea, and sensation of warmth, cardiomyopathy, hyperglycemia, Wilson's disease and Indian childhood cirrhosis (Frydman et al., 1985; Tanzi et al., 1993; Schilsky and Sternlieb, 1994; Anttila et al., 1998; Herbert, 1996; WHO, 1996; Chan et al., 1998).

Ni is a major toxic metal. It occurs in all the

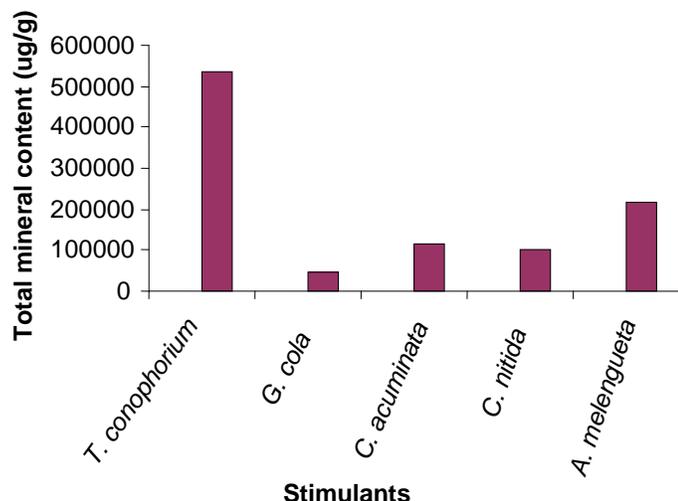


Figure 1. Total mineral content per stimulant.

samples ranging from $106.53 \pm 37.41 \mu\text{g/g}$ in *C. acuminata* to $707.02 \pm 52.30 \mu\text{g/g}$ in *G. kola*. Generally, the levels of Ni in all the sample fall within the range of body level which is 100 to 300 $\mu\text{g/g}$ except in *G. kola*. However, it is evident that people taking *G. kola* are more susceptible to ill effects of Nickel (Ni) toxicity such as lung and nasal cancers, nausea, vomiting and epigastric or chest pain, followed by cough, hyperpnoea, cyanosis, gastrointestinal symptoms, weakness, cerebral edema and death invariably (Dunick et al., 1989; ATSDR, 1997; IARC, 2003).

The total mineral content of the samples (Figure 1) varied in the order *T. conophorium* ($536175.92 \mu\text{g/g}$) > *A. melengueta* ($218040.74 \mu\text{g/g}$) > *C. acuminata* ($115339.03 \mu\text{g/g}$) > *C. nitida* ($99628.68 \mu\text{g/g}$) > *G. kola* ($46815.80 \mu\text{g/g}$). By implication, regular consumption of any two or more of these stimulants as particularly practiced by cigarette smokers and other addicted people in Nigeria may constitute serious health risk considering the possible pH variations, total dissolved solids, conductivity and the mineral contents of the stimulants.

Conclusion

The stimulants may not constitute serious health problems and may serve "good" purposes to humans at low levels of consumption, but when one gets addicted to eating any or a combination of them, the risk of ill-health increases because of their potential for pH variations and possible health problems. Most of the stimulants are acidic and their acidity increases with time. The more these stimulants are consumed, the more they seem to have capacity for breaking down the normal functioning of the biological or immune systems within our body resulting in a more conducive environment for chronic diseases. The lower the immune system of the body, the

more the attack of the chronic degenerative diseases caused by acute toxicity of each of the heavy metals present. There should be an aggressive awareness campaign to reduce the consumption of these stimulants to the barest minimum.

ACKNOWLEDGEMENT

Authors thank the Department of Chemistry, Obafemi Awolowo University Ile Ife, for providing the platform for the successful completion of the study.

REFERENCES

- Adeyeye A, Ajewole K (1991). Characterization of Nigerian Citrus Seed Oils. *Food Chem.* 47:77-81.
- Ajebesone PE, Aina JO (2004). Potential African Substitutes for Hops in Tropical Beer Brewing. *J. Food Technol. Afr.* 9:13-16.
- Anderson D (2003). Introduction to Heavy Metal Monitoring. *Nat. Environ. Res. Council* 3:1-9.
- Ang KP, Tay BT, Gunasingham H, Kohl SB, Koh CH (1989). Determination of Heavy Metal Content in the coastal sea waters of Singapore. *Int. J. Environ. Stud.* 32:261-268.
- Anttila A, Pukkala E, Aitio A, Rantanen T, Karjalainen S (1998). Update of cancer incidence among workers at a copper/nickel smelter and refinery. *Int. Arch. Occup. Environ. Health* 71:245-250.
- Agency for Toxic Substances and Diseases Registry (ATSDR) (1997). Toxicological Profile for Nickel, Public Health Service, Atlanta GA p. 262.
- Aiyelaja AA, Bello OA (2006). Ethnobotanical potentials of common herbs in Nigeria: A case study of Enugu State. *Acad. J.* 1(1):16-22.
- Chan S, Gerson B, Subramanian S (1998). The Role of Copper, Molybdenum, Selenium and Zinc in Nutrition and Health. *Clin. Lab. Med.* 18:673-685.
- Dunick JK, Elwell MR, Benson JM (1989). Lung toxicity after 13-weeks inhalation exposure to nickel oxide, nickel subsulfide or nickel sulfate hexahydrate in rats and mice. *Fundam. Appl. Toxicol.* 12:584-594.
- Ecobichon DJ (2001). Toxic Effects of Pesticides. In: C.D. Klaassen (ed) Casarett and Doull's Toxicology: The Basic Science of Poisons, 6th Edition, McGraw-Hill Medical Pub. Division pp. 763-810.
- Frydman M, Borne-Tamiur B, Farrer LA (1985). Assignment of the gene for Wilson Disease to Chromosome 13: Linkage to the esterase D vauss. *Proc. Natl. Acad. Sci. USA.* 82:1819-1821.
- Gbile ZO (1984). Vernacular Names of Nigerian Plants. Publication of Forestry Research Institute of Nigeria p. 97.
- Goyer RA, Clarkson WT (2001). Toxic Effects of Metals. In: Klaassen CD (Ed) Casarett and Doull's Toxicology: The Basic Science of Poisons, 6th Edition, McGraw-Hill Medical Pub. Division.
- Greenwood NN, Earnshaw A (1984). Chemistry of the Elements. Pergamon Press, Oxford p. 1542.
- Herbert V (1996). Vitamin B₁₂. In: Ziegler, E.E. and Filer, L.J. (eds): Present Knowledge in Nutrition, Washington, D.C. pp. 191-205.
- International Agency for Research on Cancer (IARC) (2003). Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 66. Lyons, France.
- Johnson PN, Tasell PD (1991). Chemistry of elements, their effects and their importance. *Fundam. Appl. Toxicol.* 15:3-45.
- Leek J, Keen CL, Vogler JP (1998). Long Term Marginal Zinc deprivation in Rhesus Monkeys: IV Effects on Skeletal growth and mineralization. *Am. J. Clin. Nutr.* 47:890-895.
- O'Neil P (1993). Environmental Chemistry, 2nd Edition. Chapman and Hall, p. 268.
- Ogunfowokan AO, Fakankun OA (1998). Physicochemical Characterization of Effluents from Beverage Processing Plants in Ibadan, Nigeria. *Int. J. Environ. Stud.* 54:145-152.
- Ravisankar R, Chandrasekarab A, Kirubac S, Raghud Y, Prasade

- MVR, Satpathye KK, Maheswaranf C (2011). Energy dispersive X-Ray fluorescence (EDXRF) analysis of some ancient potteries of Tamilnadu. Arch. Appl. Sci. Res. 3(1):289-295.
- Schilsky ML, Sternlieb I (1994). Animal Models of Copper Toxicosis. Adv. Vet. Sci. Comp. Med. 37:357-377.
- Tanzi RE, Pertukhin K, Cherov I (1993). The Wilson gene is a copper transporting ATPase with homology to the Menkes disease gene. Nat. Gene 5:344-350.
- von Unruh GE, Voss S, Sauerbruch T, Hesse A (2004). Dependence of oxalate absorption on the daily calcium intake. J. Am. Soc. Nephrol. 15:1567-1573.
- World Health Organization (WHO) (1996). IPCS Environmental Health Criteria. Copper, Geneva.
- Wilson ED (2006). Importance of Healthy pH in the body. BL. Publication Ministries. 5:1-10. www.sciencedirect.com.