ORIGINAL PAPER

Nig. J. Pharm. Res. 2016, 12 (2) pp 95-103
ISSN 0189-8434 **Available online at http://www.nigjpharmres.com**

Investigation of the Antioxidant Activity and Quantification of the Amount of Heavy Metals and some Vitamins in Carrot

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Carrot is a root vegetable from the Umbelliferae family. It is a biennial plant grown for their edible root. Carrots are a good source of carbohydrates and minerals like Calcium, Phosphorus, Iron and Magnesium and may contain toxic amounts of metals as a result of run off effects. It is also rich in carotene, niacin, riboflavin, thiamine and vitamin C.

Objectives: To determine the amounts of toxic heavy metals, quantify the amounts of vitamins A and E and investigate the antioxidant activities of Carrot.

Method: The research investigated the antioxidant properties of carrot on the basis of the radical scavenging activity on DPPH (1,1-diphenyl-2-picryl hydrazyl), heavy metal analysis were carried out using Flame Atomic Absorption Spectrophotometer while analysis of vitamins was done using HPLC (High Performance Liquid Chromatography).

Result: The carrot sample analyzed contained considerable amount of some toxic metals of interest (Cr 0.024, 0.105ppm; Cu 15.76, 30.95ppm; Fe 66.94, 103.95ppm; Zn 16.57, 44.22ppm; Pb 0.018, 0.021ppm) in the leaves and root respectively. The samples also contain a very good amount of the vitamin A (12.863, 44.977ppm) and Vitamin E (0.087, 0.22ppm) in leaves and root respectively. It also showed some antioxidant activity and test positive for most phytochemicals.

Conclusion: The actual concentrations of the respective heavy metals found in two parts of the *D. carota* samples were within the threshold limit but there was slight variation in the amount present in the root *D. carota* as compared to its leaf. The root had more concentrations of the metals and this could be due to the fact that the root is more exposed to these metals during plant uptake.

Keywords: Heavy metals, Vitamin, DPPH, Antioxidant, *Daucus carota*

INTRODUCTION

Carrot *(Daucus carota*) is a root vegetable, usually orange in colour, though purple, red, white, and yellow varieties exist (Banga, 1963). It has a crisp texture when fresh. It is a biennial plant that grows a rosette of leaves in the spring and summer, while building up the stout taproot, which stores large amounts of sugars for the plant to flower in the second year. Carrots belong to the Umbelliferae family (now Apiaceae) and are thought to be of Asian origin (Simon et al., 2008). The nutritional value of the carrot is understated and unfortunately frequently is an under - utilized source of pro-vitamin A, especially in developing parts of the world. Greater consumption of carrots and other Umbelliferae sources of carotenoids can improve the health of

people, especially those deficient in vitamin A, as well as enhance the enjoyment of meals.

The moisture content of carrot varies from 86 to 89% (Anon, 1952; Howard et al., 1962; Gill and Kataria, 1974; Gopalan et al., 1991). Carrots are a good source of carbohydrates and minerals like Ca, P, Fe and Mg. Gopalan et al., (1991) have reported the chemical constituents of carrot as moisture (86%), protein (0.9%), fat (0.2%), carbohydrate (10.6%), crude fiber (1.2%), total ash (1.1%), Ca (80 mg/100 g), Fe (2.2 mg/100 g) and P (53 mg/100 g). The edible portion of carrots contains about 10% carbohydrates having soluble carbohydrates ranging from 6.6 to 7.7 g/100 g and protein from 0.8 to 1.1 g/100 g in 4 carrot cultivars (Howard et al., 1962). Caffeic acid is the predominant phenolic acid in carrots. Thiamin, riboflavin, niacin, folic acid and vitamin C are present in appreciable amounts in carrot roots (Howard et al., 1962). The anthocyanins content in roots may vary from trace amounts in pink cultivars to 1,750 mg/kg in black carrots (Mazza and Minizte, 1993). The major anthocyanins have been identified as cyanidin 3- (2-xylosylgalactoside), cyanidin 3-xylosylglucosylgalactoside and cyanidin 3-ferulylxyloglucosyl galactoside (Harborne, 1976).

Plant components, primarily secondary metabolites that have health promoting properties are called phytonutrients. The importance of antioxidant constituents in the maintenance of health and protection from coronary heart disease and cancer is raising considerable interest among scientists, food manufacturers and consumers as the trend of the future is moving toward functional food with specific health effects (Velioglu et al., 1998).

Heavy metals are metals which have specific gravity that is five times greater than that of water. Toxic metals are metals that form poisonous soluble compounds and have no biological role, i.e. are not essential minerals, or are in the wrong form (Dartmouth, 2004). Not all heavy metals are particularly toxic, and some are essential, such as iron. Some heavy metals have been found to be beneficial in optimum quantity while some are known to be extremely toxic. Heavy metals of non-anthropogenic origin are always present at a background level with their occurrence in soils being related to weathering of parent rocks and pedogenesis (Ghiyasi et al., 2010).

The sources of heavy metals include food, water, airborne, medications, and direct skin contact. Food substances that are grown near highways or close industrial plants may contain deadly and other toxic amounts of metals as a result of run off effects which carries these heavy metals through the soil thereby affecting the crops. The excessive and inappropriate use of pesticides can also affect food. The use aluminum coated containers use in food packaging can also affect food products. Heavy toxic metals includes the following; Antimony, Arsenic, Aluminium, Cadmium, Chromium, Copper, Iron, Lead, Mercury, Nickel, Thallium, Barium, Beryllium, Osmium, Vanadium.

Hence this research work is aimed at the determination of the amounts of toxic heavy metals in Carrot, quantification of the amounts of vitamins A and E it contains and also an investigation of the antioxidant activities of Carrot

MATERIALS AND METHODS

Chemicals and Reagents

Ethanol (Analar grade) (Sigma Aldrich®), Ultra-Pure Water 1.0 * 18^{-18} ohms, Metals Reference Standard (1000ppm) (Fishers®), Vitamins Reference Standard (500ppm) (Fishers®), Nitric Acid (0.5 N) (British Drug House®), Sulfuric Acid (1 M) (British Drug House®), Chloroform (British Drug House®), Perchloric Acid, Alkaloidal Reagents, Ultrapure Water, Methanol, Ethanol.

Samples Collection

Large amount of carrot (3kg) was procured from mile 12 market in Lagos Nigeria and it was stored in cool dry place free from sunlight.

METHODS

Preparation of Heavy Metals/Vitamins Standard Stock and Calibration Solutions

Preparation of Heavy Metals Standard Stock and Calibration Solutions

Heavy metals reference standard with 1000 ppm: 2.5 ml of the respective heavy metals were measured into a 25 ml volumetric flask, about 10 ml of ultra-pure water was added and effectively mixed until a uniform mixture was obtained, and the mixture was later made up to 25 ml marked volume with ultra–pure water to obtain 100 ppm working concentration.

From the freshly prepared working concentration, a gradient calibration concentrations range of (0.0–8.0) ppm by measuring (0.00–0.8) ml into 10 ml volumetric flask and made up to the 10 mL marked volume with ultra–pure water. Duplicate absorbance was taken and the mean absorbance was determined.

Preparation of Vitamins Standard Stock and Calibration Solutions

Vitamins reference standard with 500 ppm: 5 ml of the respective vitamins were measured into a 25 ml volumetric flask, about 10 ml of ultra-pure water was added and effectively mixed until a uniform mixture was obtained, and the mixture was later made up to 25 ml marked volume with ultra–pure water to obtain 100 ppm working concentration.

From the freshly prepared working concentration, a gradient calibration concentrations range of (0.0–8.0) ppm by measuring (0.0–0.8) ml into 10 ml volumetric flask and made up to the 10 mL marked volume with ultra–pure water. Duplicate absorbance was taken and the mean absorbance was determined.

Preparation of Carrots (*D. carota*)

D. carota roots and leaves were grated and dried in an oven at 37^{o}C. The sample was digested in accordance with literature (Matil, 2003). 5g of carrot was weighed and pulverized, 0.5g of the pulverized carrot was weighed and transferred into 50 ml digestion tube and 10 ml of digestion mixture (HNO_3:$HClO_4$:H_2SO_4; 3:2:1.5) was measured and transferred into the 50 ml digestion tube. The digestion was carried out at 250^{o}C for two and half hours in a fume cupboard. After the completion of the wet digestion, it was cooled at ambient temperature and it was made up to 50 ml marked volume with ultra-pure water. The mixture was then transferred into 50 ml centrifuge tube and it was shaken on a mechanical shaker for ten minutes and it was later centrifuged at 5000rpm for 5 minutes. The layer containing the metals was transferred into auto cup analyzers cups and ran on the Atomic Absorption Spectrophotometer (AAS) using the respective metal cathodes.

Phytochemical Screening of Carrots (*D. carota*)

Cold marceration was used to extract the *D. carota* sample using methanol, ethanol, water, methanol/ethanol (50:50). Phytochemical screening was carried on the respective four different solvent extracts of the *D. carota* in order to confirm the presence or absence of the following phytoconstituents: alkaloids, flavonoids, phenolics, saponin, steroids, triterpenoids, cardiac glycoside, anthraquinone, coumarin glycoside and reducing sugar and this was carried in accordance with literature methods (Sofowora, 1993; Siddiqui, 1997; Trease and Evans, 2003).

Flame Atomic Absorption Spectrophometric Conditions for Heavy Metals

The assay of the respective heavy metals (Chromium, Nickel, Lead, Mercury, Arsenic, Zinc, Iron, Copper, and Cadmium) was carried out using flame atomic absorption spectrophotometer. Air – acetylene was used as the flame and hollow cathode lamps of the respective heavy metals were used as the resonance line source. A calibration curve of mean absorbance against concentration was plotted and the linear regression equations as well as the correlation coefficient for each heavy metal were obtained (Garcia & Baez, 2012). The actual concentrations of the heavy metals present in the samples were extrapolated from the linear regression equation of the respective heavy metals.

Analysis of Vitamins

The analysis of vitamins A and E was carried out with high performance liquid chromatography (HPLC). The sample, 5.0g was weighed into a beaker. A solution of chloroform, ethanol and ultrapure water (40.0 ml) was prepared in the ratio 1:1:2 (10 ml of chloroform, 10 ml of ethanol and 20 ml of ultrapure water) in a volumetric flask. 10 ml of the mixture was fully measured with a 20 ml measuring cylinder and added to the sample in a beaker. The mixture was transferred to a set of centrifuge tubes, shaken and allowed to stand for one hour for the extraction to take place. The sample was then centrifuged at 5000 rev/min and the supernatant was collected. HPLC eluent (mobile phase: water, 40 ml and ethanol, 20 ml) was prepared (Chotyakul et al., 2014).

Determination of Antioxidant Activity of the *D. carota* extracts against DPPH (Free radical Scavenging Activity).

The Antioxidant activity of the plant extracts was assessed using ultraviolet/visible spectrophotometric

method. Vitamin C reference standard was used on the basis of the radical scavenging effect against 1, 1-diphenyl-1-picryl hydrazyl radical (Sigma-Aldrich), the readings were determined by measuring UV/Visible absorbance at 517 nm. Radical scavenging activity was measured by a slightly modified method of Brand (Williams *et al*., 1995; Braca *et al*., 2002).

The concentrations of Vitamin C reference standard used range between 20 to 100 µg/ml. The following concentrations of extracts were also prepared (0.1 mg/ml to 0.5 mg/ml). Methanol was used as the diluent. Each prepared concentrations, 2ml was placed into plain sample bottles and 0.5 ml of 1 mM DPPH solution in methanol was added. The experiments were carried out in triplicates. The plain sample bottles were incubated for 30minutes and the absorbance was read at 517 nm. A blank solution was prepared and measured containing 2 ml of methanol 0.5 ml of DPPH. The radical scavenging activity was calculated using the formular.

$$\% \text{ Inhibition} = \frac{\{AB - AA\}}{AB} \times 100$$

Where AB is the absorption of blank sample and AA is the absorption of tested extract solution. The experiments were performed under the same condition for the reference standards and the respective extracts.

RESULTS

The result of the phytochemical analysis for *D. carota* extracts with different solvents showed the presence of various constituents such as Alkaloids, phenolics, flavonoids, saponin, coumarin glycoside, reducing sugar, steroids and triterpenoids glycosides.
The antioxidant activity of the 4 different solvent extract of *D. carota* was measured on the basis of its DPPH radical scavenging activity, with one positive control used.

Table 1: Actual Concentrations of the Heavy Metals and Vitamins Present in Each Sample (ppm)

	Pb	Ni	Zn	Fe	Hg	As	Cr	Cu	Cd	Vit. A	Vit. E
MEAN ±	0.021±	0.103	44.210±	103.940±	0.030±	0.018±0.	0.104±	30.942±	0.037±	44.977±	0.224±
s.d (root)	0.000	0.001	1.210	3.020	0.000	000	0.000	0.604	0.011	0.357	0.001
MEAN ±	-0.018	0.050±	6.562±0	66.940±3.8	0.010±	-0.012	0.024±0.	15.755±0.4	0.015±0.	12.863±0.5	0.087±0.0
s.d (leaf)	±0.000	0.000	.659	04	0.000	±0.000	000	74	000	06	05

Table 2: Phytochemical Screening Data of *D. carota*

Test	**MeOH/EtOH**	**EtOH**	**MeOH**	**H_2O**
Reducing sugar	+	+	+	+
Anthraquinone	–	–	–	–
Terpenoids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Tannins	–	–	–	–
Alkaloids	+	+	+	+
Cardiac glycosides	+	+	+	+
Coumarin glycosides	+	+	+	+

Key: (+) present (-) absent

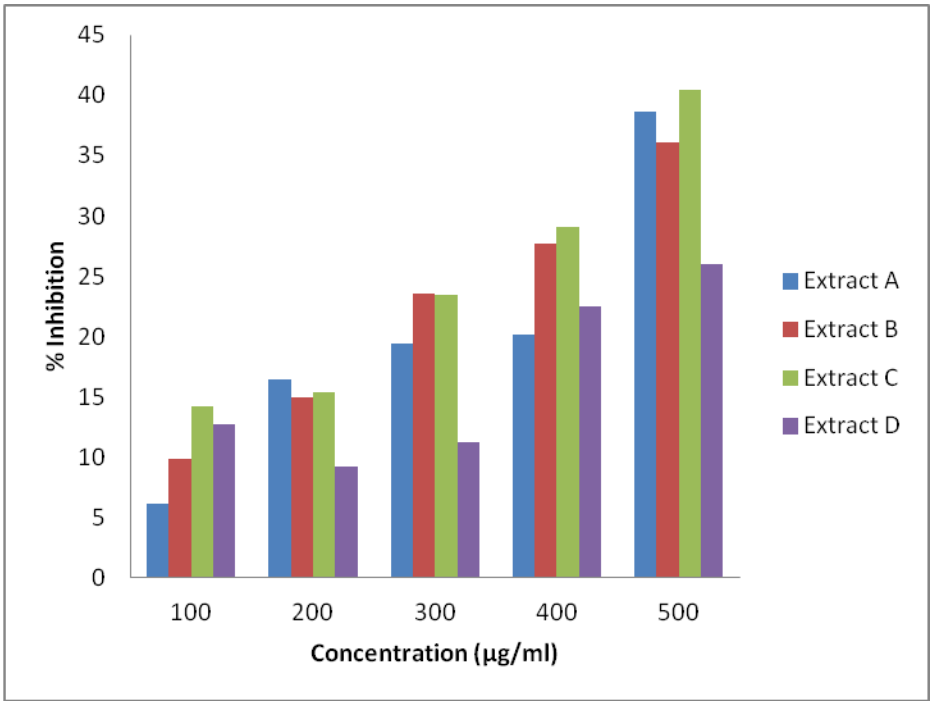

Figure 1: Bar Chart of % Inhibition of Daucus carota Extracts against Concentration

Extract A- Methanolic/Ethanolic Extract
Extract B- Ethanolic Extract
Extract C- Methanolic Extract
Extract D- Water Extract

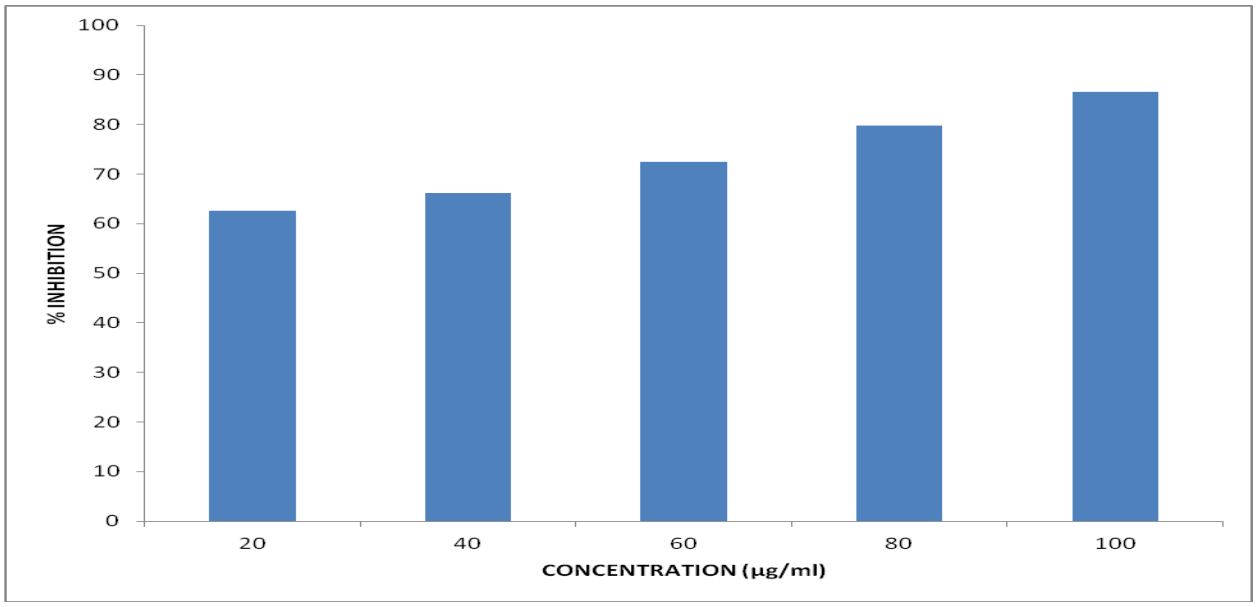

Figure 2: Bar Chart of % Inhibition of Vitamin C against concentration

Table 3: Permitted Daily Exposures (PDE) for Elemental Impurities Guidelines (Destefano, 2010)

Parameters	Oral PDE µg/day	USP Oral Limit µg/g	USP Parenteral limit µg/g
Chromium	150	15	1.5
Mercury	15	1.5	0.15
Lead	10	1	0.1
Nickel	1,000	100	10
Arsenic	15	1.5	0.15
Cadmium	25	2.5	0.25
Zinc	15,000	1,500	150
Copper	500V	50	5
Iron	15,000	1,500	150

Table 4: Dietary Vitamin Intake Guidelines, (Destefano, 2010)

Vitamins	Oral RfD (µg/kg/day)	Recommended daily oral dose*PDE (µg/day)	Oral component limit (OCL)	Parenteral Limit Component (PCL)
Vitamin A	60	3000	300	30
Vitamin E	300	15000	150	150

*recommended daily oral dose on a 50kg person

DISCUSSION

Carrot is an indispensable fruit eaten by a lot of people irrespective of their age and cultural make up. Due to the importance of this fruit, there is need to carry out a quality evaluation of some of its phytoconstituents as well as the elemental analysis in order to ascertain if they are within the threshold limit. Carrots are widely used in many cuisines, especially in the preparation of salads, and carrot salads are a tradition in many regional cuisines. The juice of the carrot when expressed contains crystallizable and uncrystallizable sugar, a little starch, extractine gluten, albumen, volatile oil (on which the medicinal properties of the root depend and which is fragrant, aromatic and stimulating), vegetable jelly or pectin, saline matter, malic acid and carotin (Grieve, 1971). Carrot is highly rich in carotenoid and has been shown to possess high level of antioxidant activities

For safety of human health, various regulatory organizations such as USP, BP, EPA, WHO, USEPA have set up parameters to limit the presence of heavy metals in fruits and vegetable (Nazemi, 2012). Parameters such as permissible daily exposure (PDE), rationale for reference doses (RfD's), oral component limit (OCL) and parenteral component limits (PCL) are guidelines set to regulate elemental contaminations as well as dietary vitamin intake (Adepoju-Bello *et al.*, 2012).

The calibration plots obtained for each metal were used to determine the concentrations of heavy metal contaminants in the samples. The standard deviation for each metal analysis was determined to estimate how far each value was, from the mean. The heavy metal concentrations in the samples, with reference to the information in table 3, have concentrations below the USP oral limit.

The calibration plots for the vitamin A and vitamin E standard solutions were linear with correlation coefficient of 0.9984 and 0.9999, respectively. The standard deviation for each vitamin analysis was determined. With reference to table 4, all samples have concentrations below the USP OCL hence will rarely supply the daily vitamin requirements. However, since these vitamins are fat-soluble and can be stored in biological system over time, it carries a potential health risk to regular consumers who are also on supplementation of the vitamins especially vitamin A leading to hypervitaminosis A (Dietary supplement fact sheet-Vitamin A).

The phytochemical screening conducted on *D. carota* extracts in this research project, revealed the presence of flavonoids and these phytoconstituents are believed to have a strong contribution to the antioxidant activity of *D. carota* leaf. Oxidative stress of free radicals produced in the human system has some conditions associated with various oxidative damage. Hence, antioxidant properties of some plants have also been used as a free radical scavenger to mop up these free radicals.
A DPPH test shows the ability of the test compound to act as a free radical scavenger. DPPH is a free radical and it gives a strong absorption band at 517nm in the visible spectrum (deep violet colour) of the electromagnetic radiation *(Ayoola et al, 2008).*

The antioxidant assay revealed that the flavonoid and phenolic contents present in the *D. carota* extracts analysed, were in low quantity such that their radical scavenging activities were dependent on their increasing concentration as none of the four extracts in the concentration range used yield the concentration that could be used to calculate 50% scavenging activity (IC_{50}), even at 500µg/ml. Hence, none of the extracts at 500µg/ml concentration could be compared to the least Vitamin C concentration 20µg/ml having 62.6% inhibition. The ascorbic acid concentration needed for 50% scavenging activity (IC50) was less than 20µg/ml.

Comparison of the 4 extracts at 500µg/ml concentration indicates that methanolic extract has the highest % inhibition of 40.45%, followed by methanolic/ethanolic extract with 38.67% inhibition, ethanolic extract with 36.14% inhibition, water extract with 26.03% inhibition. The results of the antioxidant assay was analysed statistically using Two-way ANOVA to compare the different extracts.

Methanolic vs ethanolic extract yield a P value of 0.1012, methanolic vs methanolic/ethanolic extract with a P value of 0.0842, water vs methanolic extract with a P value of 0.3413, water vs ethanolic extract with a P value of 0.0794, methanolic/ethanolic vs ethanolic with a P value of 0.2940. All these pairs with P values greater than 0.05 ($P > 0.05$) indicate no statistical significant difference. However the water vs methanolic extract shows a statistically significant difference with a P value of 0.0236.

The calculated Coefficient of Determination, R^2 and % Coefficient of Determination for the antioxidant properties for the four different extract of *D. carota*,

obtained from the graph as shown indicates that the constant of direct proportionality between % inhibition against concentration follows a more linear form for ethanolic extract having R^2 = 0.9898, 98.9% than the rest of the extracts with values as follows; methanolic extract R^2 = 0.9405, 94.1%; methanolic/ethanolic extract R^2 = 0.8531, 85.3% and water extracts R^2 = 0.7175, 71.8%.
For Vitamin C standard, R^2 = 0.9875, percentage of Coefficient of Determination was found to be 98.8%.

CONCLUSION

From the result obtained, it can be inferred that the actual concentrations of the respective heavy metals found in two parts of the *D. carota* samples were within the threshold limit as specified by DeStefano et al., (2010) but there was slight variation in the amount present in the root as compared to the leaf. Hence indicating that the root had more concentrations of the metals and this could be due to the fact that the root is more exposed to these metal during nutrient uptake. The heavy metals might have found their way into the soil where *D. carota* was planted through run off of industrial chemicals. There is a more probability of having elevated heavy metal concentrations in plant materials if planted closed to industrial factories, automobile workshops and pharmaceutical companies.

Hence, adequate care must be ensured when plantation of any plant product is to be established. Government agency should monitor and regulate the quality of food products being supplied into the market in order to ascertain their safety because excess of some of these heavy metals in the human body can lead to metabolic disease conditions which could ultimately leads to death.

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Conflict of Interest: None declared

Received: 20 May, 2016

Accepted: September 20, 2016