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PHARMACOGNOSTIC POTENTIALS OF THE GARCINIA KOLA NUT SOLVENT EXTRACT IN HEAVY METAL CHELATION THERAPY IN SIERRA LEONE

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Abstract

Pharmacognostic Potentials of Garcinia Kola Nut Solvent Extract on Heavy Metal Chelation Therapy has been investigated in Sierra Leone. During the research work, 86 g of the dried powdered samples of Garcinia kola nut (Bitter kola) were subjected to Soxhlet extraction using solvents of increasing polarity. After successive extraction with Petroleum ether (60 -70oC), Chloroform, Acetone, Methanol, Ethanol, and water each of solvent extracts were evaporated to dryness under reduced pressure using ***Buchi Rotary Evaporator*** at 50oC. The dried extracts were weighed, packed into separate air-tight containers for phytochemical screening and heavy metal sequestering potentials.

The masses of the extracts obtained were 3.133g (3.64%), 3.409g (3.96%), 6.318g (7.35%), 2.319 (2.70%), 2.864g (3.33%) and 4.353g (5.06%) for Petroleum ether (60 -70oC), Chloroform, Acetone, Methanol, Ethanol and distilled water respectively. The Acetone and Aqueous extracts were found to be the most extracted fractions of the nut 6.318g (7.35%) and 4.355g (5.06%) respectively.

Standard procedures were used during phytochemical screening of the various solvent extracts with results indicating the presence of sterols, carbohydrates, saponins, alkaloids, flavonoids and tannins thus supporting the use of the plant in traditional medicine.

Standard solutions of the metal ions, Cu2+, Pb2+, Zn2+and Fe2+ prepared from reagent-grade trioxonitrate (V) and tetraoxosulphate (VI) salts using distilled water were treated with the solvent extracts and Na2EDTA solution as standard. Spectrophotometric determination of each of the metal ions in the various solvent extracts and Na2EDTA solution using spectrophotometer model ***PerkinElmer Analyst 800***, showed that the extracts were more effective in sequestering Pb2+ and Fe2+ ions than Cu2+ and Zn2+ ions. The aqueous extract was found to be a better heavy metal sequestering fraction for the metals under investigation when compared with the other extracts including the Na2EDTA.

Synthetic drugs such as ethylene diammine tetra acetic acid (EDTA) and dimercaptosuccinic acid (DMSA) used in chelation therapy have been reported to be dangerous, have side-effects and also remove vital nutrients such as vitamins C and E from the human body. From the results of this study, it has been shown that the aqueous extract of Garcinia kola nut (Bitter kola) has the potential to provide a more readily available, natural and better alternative agent for chelation therapy.

Keywords: heavy metal, sequestering, ethylene diammine tetra-acetic acid, phytochemical, dimercaptosuccinic acid, chelation therapy.

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INTRODUCTION

Garcinia kola (*Bitter kola*) belongs to a family of tropical flowering plants known as ***Guttiferae*** (Plowden, 1972), found in Western and Central Africa. It is a dicotyledonous plant that exists in moist rain forests and swamps and grows as a medium sized tree up to a height of about 12 m. The plant is cultivated through the seedlings or with cuttings. It however grows more easily using the cuttings. The seed is generally known as ***Bitter kola*** and in Nigeria it is commonly called "***Namijigoro***" in Hausa, "***Agbilu***" in Igbo (Esemonu et al., 2005) and *"**orogbo**"* in Yoruba (Ndukwe et al.,2005). Common Sierra Leonean names include; Kono "***sagbe****"*, Krio "***bita-kola***", Mende "***ndenyanie***" and Temne "***ta-sagbe***" (Burkill, 1985).

Figure 1: Dried Seeds of Garcinia kola nut

The plant has been referred to as a "wonder plant" because every part of it has been found to be of medicinal importance (Dalziel, 1937). Garcinia kola, exhibit diverse antimicrobial activities and is used in the treatment of cough and sore throat (Madubunyi, 1995; Okunji et al., 1995; Adefule-Ositelu et al., 2004). Its root and seed possesses anti-inflammatory activities (Braide, 1990), are hepatoprotective (Iwu et al., 1990), and exhibit anti-oxidative (Olatunde et al., 2004) and antiviral properties (Hong-xi and Song, 2001).

The medicinal importance of bitter kola lies mainly on the phytochemical components of the plant. From its roots to its leaves, the plant is known to contain a number of phytochemicals noted for their medicinal importance (Iwu *et al*., 1990). The presence of these bioactive components such as ***alkaloids***, ***saponins, tannins***, ***anthraquinones*** and ***cardiac glycosides*** determine the antibacterial activity of the seed and leaf extracts. In addition, the plant possesses ***antidiabetic***, and ***antihepatotoxic*** activities (Iwu, 1993).

Garcinia kola has been used in folklore remedies for the treatment of ailments such as ***liver disorder***s, ***hepatitis***, ***diarrhoea***, ***laryngitis***, ***bronchitis*** and ***gonorrhoea*** (Iwu 1993; Adesina et al., 1995). The use of this plant for the treatment of ***jaundice***, ***high fever***, ***purgative,*** and ***chewing stick*** was also reported by Iwu (1993). The plant also found usefulness in the treatment of ***stomach ache*** and ***gastritis*** (Ajebesone and Aina, 2004). Garcinia kola is believed to be an important source of new chemical substances with potential therapeutic benefits (Eisner, 1990).

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A number of African countries have been noted to use Garcinia kola to treat variety of conditions. In Congo, for example a bark decoction is taken for ***female sterility*** and to ease ***child birth***. In Ivory Coast, a decoction of the bark is taken to induce the ***expulsion of a dead fetus***, while the seed and the bark are taken for ***stomach pain***. In Sierra Leone, the roots and bark are taken as a tonic for ***sexual dysfunction***. The bark is also added into palm wine to improve its ***potency***. In Nigeria, a cold water extract of the roots and bark with salt are administered to cases of ***Ukwala*** (***bronchial asthma or cough***) or ***agbo*** (***vomiting***). (Iwu *et al.*, 1990). Generally, the mechanical cleansing effect and antimicrobial substances in the seed are seen as major beneficial effects of chewing this nut (Han et al., 2005).

In addition to the benefits of Garcinia kola outlined above, extracts of the seed have also been found to be effective in scavenging and eliminating heavy metals such as Pb, Cd, Hg etc from the liver (Nwokocha, et al. 2011).

HEAVY METAL TOXICITY AND CHELATION THERAPY

The human body often contains microscopic pieces of heavy metals, such as mercury (from our fillings and fluorescence bulbs), lead (in water pipes, storage batteries) or even iron as a result of the toxic environment we live in. These heavy metals are so minuscule that you could think of them as almost the size of atoms, but they are tiny pieces of metal that are actually toxic. They can easily become embedded inside some tissue in the body. The metal could be located inside the liver, for instance, the kidneys, the heart, just about anywhere in the body. While generally overlooked by traditional medicine, these traces of heavy metals in our bodies probably cause and aggravate most health conditions, including heart disease and cancer. Heavy metal exposures cause oxidative stress, altered physiological and biochemical characteristics (Flora, Pachauri, 2010 and Valko, Morris, Cronin, 2005) that lead to organ damage (Goyer et al. 1985, Clarkson et al. 2003, Silbergeld, et al. 2005 and Houston, 2007). Heavy metals impart their toxicological effects mainly through molecular interactions with sulfhydryl groups on various molecules, among other factors, (Ercal et al., 2001). Metal-induced toxicity can be effectively treated by ***chelation therapy*** since it enhances the mobilization and excretion of metallic cations (Nordberg, et al., 2009, Nwokocha et al, 2011 and Graziano et al. 1992).

Chelation therapy is the administration of ***chelating*** (***sequestering***) ***agents*** to remove heavy metals from the body by converting them to a chemically inert form that can be excreted without further interaction with the body. This technique is used as a treatment for acute mercury, iron (including in cases of ***thalassemia***), arsenic, lead, uranium, plutonium and other forms of toxic metal poisoning. The chelating agent may be administered ***intravenously***, ***intramuscularly***, or ***orally***, depending on the agent and the type of poisoning (Natural Standard, 2009). Chelation describes a particular way in which ions and molecules bind metal ions. According to the ***International Union of Pure and Applied Chemistry (IUPAC)***, chelation involves the formation of two or more separate coordinate bonds between a ***polydentate*** (multiple bonded) ligand and a ***single*** central atom. These polydentate ligands (usually organic compounds) are called ***chelating***, or ***sequestering*** agents. Chelating agents were introduced into medicine as a result of the use of

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poison gas in World War I. The first widely used chelating agent is ***dimercaprol*** (a ***dithiol*** compound) also called ***British anti-lewisite (BAL)***. It was used as an antidote to the arsenic-based poison gas, ***lewisite***. The sulphur atoms in BAL's mercaptan groups strongly bond to the arsenic in ***lewisite***, forming a water-soluble compound that entered the bloodstream, allowing it to be removed from the body by the kidneys and liver. BAL had severe side-effects and in the 1960s, it was modified into ***dimercaptosuccinic acid (DMSA)***, a related dithiol with far fewer side effects (Kalia, Kiran; Flora, Swaran, 2005). DMSA quickly replaced both BAL and ***ethylenediaminetetraacetic acid (EDTA)***, becoming the US ***standard of care*** for the treatment of lead, arsenic, and mercury poisoning, which it remains today. More recently, esters such as ***monoisoamyl dimercaptosuccinic acid (MiADMSA)*** which is reportedly more effective than DMSA at clearing mercury and cadmium are used (Kalia, Kiran; Flora, Swaran, 2005).

The drugs used for chelation therapy bind to heavy metals in the body and prevent them from binding to other agents. The metal complexes are then excreted from the body. The chelating process also removes vital nutrients such as vitamins C and E from the body, (Bridges, Sarah, 2006).To stop minerals from being removed, scientists developed the concept of chelating a metal ion with ligands that enhance mineral absorption. Amino acids, being effective metal binders, were chosen as prospective ligands. The metal-amino acid chelates were reported to be able to enhance mineral absorption. When this idea was applied to synthetic chelates such as EDTA, the ***metal-synthetic chelates*** were however, too stable and not nutritionally viable. For example if the mineral was taken from the EDTA ligand, the ligand could not be used by the body and would be expelled. During the expulsion process the EDTA ligand will randomly chelate and strip another mineral from the body (Ashmead, DeWayne, 1993). Although they can be beneficial in cases of heavy metal poisoning, chelating agents can also be dangerous. Use of disodium EDTA instead of calcium EDTA for example has resulted in fatalities due to ***hypocalcaemia***, (U.S. Centers for Disease Control, 2006). Thus EDTA should not be used in the treatment of children (Van der Schaar, Peter, 2011). Since the 1970s, more than 30 deaths have been recorded in cases of intravenous-administered disodium EDTA (Atwood, et al., 2008). ***2, 3-dimercapto-1-propane sulfonic acid*** (DMPS) injections may cause ***skin reactions*** at the injection site. Other side effects reported include ***fever***, ***headache***, and ***nausea***. No death has however been linked to the use of ***DMPS***, (Ruprecht, Johann, 2008).

Virtually all biochemicals exhibit the ability to dissolve heavy metal cations. Thus ***proteins***, ***polysaccharides*** and ***polynucleic acids*** are excellent polydentate ligands for many metal ions. Organic compounds such as the ***amino acids***, ***glutamic acids*** and ***histidine***; organic diacids such as ***malate;*** and polypeptides such as ***phytochelatins*** are also typical chelators. In addition to these adventitious chelators, several biomolecules are specifically produced to bind certain metals. (Krämer, et al., 1996).

Garcnia kola nut exhibits both hepatoprotective (Iwu et al., 1990) and heavy metal sequestering properties (Nwokocha, et al. 2011). It is also widely utilized (e.g. chewing), in many communities across Africa, and no known life-threatening side effect has been reported. This

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study therefore aims at determining the extract of Garcinia kola nut that contains the ***chromophore*** with the most heavy metal sequestering characteristic in order to produce a cheap, safe and readily available drug for chelation therapy.

MATERIALS AND METHODS

Collection of Plant Materials

The seeds of Garcinia kola were collected in January, 2014 from the ***Gola Forest*** in the Eastern Province of Sierra Leone. The seeds were identified and authenticated at the Botany Department, ***Fourah Bay College*** **(*University of Sierra Leone)***. Before the start of analysis, the seeds were peeled and then chopped into tiny pieces which were dried under the shade and not in the sun, in order to protect the thermolabile components in the material if present, from being chemically transformed. The dried plant material was pulverized using a laboratory mill and kept in air-tight containers until the time of the extraction.

The analyses described below were carried out using the powdered sample of Garcinia kola nut.

- ***Soxhlet extraction***
- ***Phytochemical screening***
- ***Determination of heavy metal sequestering ability of extracts and Na_2EDTA***

Soxhlet extraction

86 g of the powdered ***Garcinia kola*** nut was used for the extraction in a ***Soxhlet apparatus*** using solvents of increasing polarity at a temperature of 70˚C, i.e. Petroleum ether (60-80^o C) Chloroform, Acetone, Methanol, Ethanol, and water. Each extraction was carried out for a period of about 15 hours and for each solvent the extraction was done twice with 300 ml of the solvent. Each time before extracting with the next solvent, the powdered material in the thimble was air-dried below 50˚C before being subjected to further extraction. After the extractive work all the organic solutions were dried over Na_2SO_4, filtered through a cotton plug and, where necessary, evaporated under reduced pressure on ***Buchi Rotary Evaporator*** at 50^{o}C (Harborne, 1998). The solid extracts were weighed and packed into separate air-tight containers.

The percentage extractive yield was calculated using the formula below:

$$\% \text{ Extractive Yield} = \frac{\text{Weight of dried solvent extract}}{\text{Weight of dried powdered seeds}} \times 100$$

Phytochemical screening

Phytochemical screening involved testing each of the Garcinia kola nut solvent extracts (***GKSE***) for different classes of compounds secondary plant metabolites. The methods used for detection of the various phytochemicals include qualitative chemical tests to give general idea regarding the nature of constituents present in the different solvent extracts of the seed. (Khandelwal, 1995; Trease et el. 1978; Sazada et el. 2009; Kokate et el. 2006; Nayak , 2007).

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Test for Carbohydrates:

A small quantity of each solvent extract (1.2 mg) was dissolved in 5 ml distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates.

- **Fehling's test: -** 1ml of the **e**xtract filtrate was treated with 1ml Fehling's solution A and 1ml Fehling's solution B and the mixture boiled for 5-10 minutes on a water bath. The formation of Reddish brown precipitate (cupper (I) oxide) indicates the presence of reducing sugar.
- **Benedict's test: -** 1ml of the extract filtrate was treated with Benedict's reagent in a test tube. The mixture was boiled for 5-10 minutes on a water bath. A change in colour of the solution from blue to green, to yellow or brick-red precipitate depending on the amount of test sample present indicates the presence of reducing sugar.
- **Barfoed's Test: -**1.5 mg of the solvent extract was placed in a boiling tube and 3ml of Barfoed's reagent added to it. The mixture was heated on a water bath for 7 minutes. The solution changed colour from blue to dirty green to greenish-yellow and then to dark yellow precipitate. A brick-red precipitate was also seen on top of the dark yellow precipitate, indicating the presence of reducing sugar.
- **Iodine Test:** 2 drops of iodine solution was added to 1ml of the Garcinia kola solvent extract (*GKSE*). The formation of blue-black colour indicates the presence of starch.

Test for Saponin Glycosides:

- **Froth test: -** Each Garcinia kola solvent extract (*GKSE*) was treated with water in a semi-micro tube and shaken well. The appearance of a persistent froth on top of the mixture indicates the presence of glycosides.

Tests for Sterols and Triterpenoids:

- **Libermann-Burchard test**
  The Garcinia kola solvent extract (*GKSE*) was treated with few drops of acetic anhydride and then boiled for few minutes. The mixture was cooled and concentrated tetraoxosulphate (VI) acid added down the side of the test tube. The appearance of a brown ring at the junction of the two layers with the upper layer turning green is indicative of the presence of sterols and formation of a deep-red colour indicates the presence of triterpenoids.
- **Salkowski's test**
  The Garcinia kola solvent extract (*GKSE*) was treated with chloroform and few drops of concentrated tetraoxosulphate (VI) acid. The mixture was shaken well and allowed to stand for some time. The appearance of a red colour in the lower layer indicates the presence of sterols while formation of a yellow coloured lower layer indicates the presence of triterpenoids.

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Tests for tannins and phenolic compounds:

- **Ferric chloride test: -** a small amount of the Garcinia kola solvent extract (*GKSE*) was shaken with water and warmed, followed by addition of 2 ml of 5% ferric chloride solution. The formation of green or blue colour indicates the presence of phenols.
- **Gelatin test: -** 1% gelatin solution containing 10% sodium chloride was added to the Garcinia kola solvent extract (*GKSE*). The formation of a precipitate indicates the presence of tannins and phenolic compounds.
- **Iodine test: -** the Garcinia kola solvent extract (*GKSE*) was treated with dilute iodine solution. The appearance of a transient red colour indicates the presence of tannins and phenolic compounds.
- **Nitric acid test: -** dilute nitric acid was added to the Garcinia kola solvent extract (*GKSE*). The formation of reddish to yellowish colour indicates the presence of tannins and phenolic compounds.

Test for alkaloids:

About 0.5 mg of the dried Garcinia kola solvent extract (*GKSE*) was stirred with about 5 ml of dilute hydrochloric acid and filtered. The following tests were conducted on the filtrate:

- **Dragendroff's test: -** few drops of Dragendroff's reagent (solution of potassium bismuth oxonitrate iodide) was added to the filtrate and observed. The formation of orange-yellow precipitate indicates the presence of alkaloids.
- **Mayer's test:-** few drops of Mayer's reagent (Potassium mercuric iodide solution) was added to the filtrate and observed. The formation of white or cream-coloured precipitate indicates the presence of alkaloids.
- **Hager's test:-**few drops of Hager's reagent (saturated aqueous solution of picric acid) was added to the filtrate and observed. The formation of yellow precipitate indicates the presence of alkaloids.
- **Wagner's test:-**few drops of Wagner's reagent (solution of iodine in potassium iodide) was added to the filtrate and observed. The formation of reddish brown precipitate indicates the presence of alkaloids.

Tests for flavonoids:

- **Shinoda's test (Magnesium Hydrochloride reduction test):-**5ml 95% ethanol was added to the Garcinia kola solvent extract (*GKSE*). The mixture was then treated with 0.5g magnesium turnings and few drops of conc. HCl. The formation of pink colour indicates the presence of flavonoids.
- **Alkaline reagent test:-**Lead acetate solution was added to a small quantity of the Garcinia kola solvent extract (*GKSE*) and observed. The appearance of a yellow coloured precipitate after few minutes indicates the presence of flavonoids.

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Determination of heavy metal sequestering ability of Garcinia kola nut extracts and Na_2*EDTA*

Preparation of standard solutions of the heavy metals

Standard solutions of the metal ions, Cu^{2+}, Pb^{2+}, $Zn^{2+}$and Fe^{2+} were prepared from reagent-grade trioxonitrate (V) and tetraoxosulphate (VI) salts using distilled water. For instance copper (II) tetraoxosulphate (VI) for Cu^{2+}, lead (II) nitrate for Pb^{2+}, Iron (II) tetraoxosulphate (VI) for Fe^{2+} and Zinc (II) tetraoxosulphate (VI) for Zn^{2+}. The standard solutions were prepared by dissolving the respective masses in 50 ml distilled water; i.e. Copper (II) tetraoxosulphate (VI) =1.56 mg/50 ml; Lead (II) nitrate =11.71 mg/50 ml; Zinc (II) tetraoxosulphate (VI) =1.00 mg/50 ml; Iron (II) tetraoxosulphate (VI) =1.60 mg/50 ml; (Paradker and Williams, 1994)

Preparation of solutions of Garcinia Kola nut extracts and Na_2*EDTA*

20 mg of each extract was dissolved in 10 ml of the respective solvents and allowed to equilibrate for about five minutes followed by filtration using Whatman No. 41 ashless filter paper to remove undissolved excess extract. A similar quantity (20 mg) of Na_2*EDTA* was dissolved in 10 ml of distilled water and allowed to equilibrate for about five minutes. (Paradker and Williams, 1994)

Spectrophotometric determination of metal ions in extracts and Na_2*EDTA* solutions

5 ml of each standard metal ion solution was placed in a small beaker.10 ml solution of the Garcinia kola extract was added to each of the standard metal ion solutions in the beakers. The mixture in each beaker was allowed to equilibrate for about 5 minutes before carrying out Spectrophotometric determination of the metal concentration in the mixtures using spectrophotometer model ***PerkinElmer AAnalyst 800*.** This procedure was repeated for all the ions and Na_2 EDTA. (Paradker and Williams, 1994)

RESULTS AND DISCUSSION

Soxhlet Extraction

Figure 2: Solvent extracts

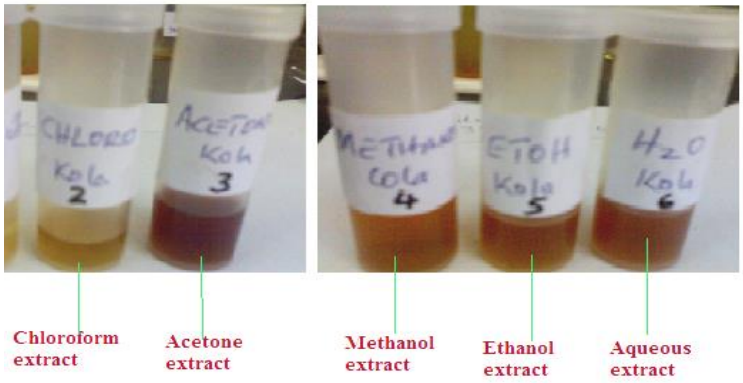

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TABLE 1: Percentage of solvent extractives

No	Solvent used	Mass of powdered plant material (g)	Mass of solvent extract (g)	Percentage of extractives (%)
1	Petroleum ether	86.6	3.133	3.64
2	Chloroform	86.0	3.409	3.96
3	Acetone	86.0	**6.318**	7.35
4	Methanol	86.0	2.319	2.70
5	Ethanol	86.0	2.864	3.33
6	Water (Distilled)	86.0	**4.355**	5.06

Phytochemical Screening

The results of phytochemical screening are reported in the following tables;

TABLE 2: Results of Carbohydrate tests

No	Solvent	Fehling's Test	Benedict's Test	Barfoed's Test
1	Chloroform	+	+	+
2	Acetone	++	++	++
3	Methanol	++	++	++
4	Ethanol	++	++	++
5	Water (Distilled)	+++	+++	+++

TABLE 3: Tests for Saponins

No	Solvent	Froth Test
1	Chloroform	-
2	Acetone	-
3	Methanol	-
4	Ethanol	+++
5	Water (Distilled)	+++

TABLE 4: Test for Alkaloids

No	Solvent	Mayer's Test	Hager's Test	Wagner's Test	Dragendroff's test
1	Chloroform	-	-	-	-
2	Acetone	-	-	-	-
3	Methanol	++	++	++	++
4	Ethanol	+++	+++	+++	+++
5	Water (Distilled)	++	++	++	++

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TABLE 5: Flavonoids Test

No	Solvent	Lead acetate Test	Shinoda's Test
1	Chloroform	-	-
3	Acetone	++	++
3	Methanol	++	++
4	Ethanol	++	++
5	Water (Distilled)	+	+

TABLE 6: Test for Tannins

No	Solvent	Iron (III) Chloride Test	Gelatin Test	Iodine Test	Nitric acid Test
1	Chloroform	-	-	-	-
2	Acetone	+	+	+	+
3	Methanol	+	+	+	+
4	Ethanol	+	+	+	+
5	Water (Distilled)	+	+	+	+

TABLE 7: Test for Sterols

No	Solvent	Libermann-Burchard test		Salkowski's test	
		Sterols	Triterpenoids	Sterols	Triterpenoids
1	Chloroform	++	-	++	-
2	Acetone	++	-	++	-
3	Methanol	++	-	++	-
4	Ethanol	++	-	++	-
5	Water (Distilled)	++	-	++	-

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Heavy metal sequestering capacity of Garcinia kola nut extracts and Na_2EDTA

TABLE 8: Levels of heavy metals in Garcinia kola nut extracts and Na_2EDTA

Metals	Fresh metal (mg/l)	Methanol Extract	Acetone Extract	Ethanol Extract	Aqueous extract	Chloroform Extract	Na_2EDTA Solution
Pb^{2+} (mg/l)	234.1	229.7	218.9	230.4	222.0	233.8	224.1
Fe^{2+} (mg/l)	32.09	27.41	3.802	28.01	25.58	26.18	27.17
Cu^{2+} (mg/l)	31.19	31.09	31.00	30.93	30.62	3.239	30.66
Zn^{2+}(mg/l)	20.00	18.44	18.37	18.46	18.23	18.28	18.20

TABLE 9: Levels of heavy metals removed from standard ion solutions by Garcinia kola nut extracts and Na_2EDTA

Metal	Methanol extract	Acetone extract	Ethanol Extract	Aqueous Extract	Chloroform extract	Na_2EDTA
Pb^{2+} (mg/l)	4.4	**15.2**	3.7	**12.1**	0.3	10
Fe^{2+} (mg/l)	4.68	**28.29**	4.08	**6.51**	5.91	4.92
Cu^{2+} (mg/l)	0.1	0.19	0.26	**0.57**	**27.96**	0.53
Zn^{2+} (mg/l)	1.56	1.63	1.54	**1.77**	1.72	**1.80**

DISCUSSION

The powdered sample of Garcinia kola nut used yielded more of the acetone and aqueous extracts during extraction (table 1) i.e. 6.318 g, about 7.35% (acetone extract) and 4.355 g, about 5.06% (aqueous extract). In addition to carbohydrates, saponins, alkaloids, flavonoids and tannins present in Garcinia kola nut (Iwu, 1993), this study also showed that ***sterols*** are present in the nut (table 7). Plant sterols or phytosterols, are very similar to cholesterol in animals. When plant sterols are ingested, they are not well absorbed by the body but latch on to cholesterol receptors in the intestines. Consequently less cholesterol is able to pass from the intestines into the bloodstream, thereby lowering cholesterol levels in the blood (Tatu A Miettinen, et al. 1995). Spectrophotometric measurements revealed that Pb^{2+} and Fe^{2+} levels are highest in acetone extracts (15.20 mg/l and 28.29 mg/l) followed by the aqueous extracts (12.1 mg/l and 6.51mg/l) respectively (table 9). On the other hand Cu^{2+} level is highest for chloroform extract (27.96 mg/l) followed by aqueous extract (0.57 mg/l) and Zn^{2+} level is highest for Na_2EDTA (1.80 mg/l) followed by aqueous extract (1.77 mg/l). Generally the extracts are found to be more effective in sequestering Pb^{2+} and Fe^{2+} ions than Cu^{2+} and Zn^{2+} ions. On average, the aqueous extract appear to be a better heavy metal sequestering fraction for the metals under investigation than the other extracts including the Na_2EDTA. The aqueous extract of Garcinia kola nut is easier and cheaper

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to prepare locally or otherwise compared to extracts of acetone, chloroform, methanol or ethanol. Also the organic solvents especially acetone, chloroform and methanol are toxic and poisonous. Thus the aqueous extract may be preferable for use as a cheap and safe heavy metal sequestering fraction of Garcinia kola nut; especially when many synthetic chelating agents have been shown to be dangerous, have side-effects and also remove vital nutrients (vitamins C and E) from the human body **(**Bridges, Sarah, 2006**).**

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