

Phytochemical Screening, Antioxidant activity, Antimicrobial activity and Heavy metal quantification of *Chrysophyllum albidum* Fruit Extract (Sapotaceae)

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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: *Chrysophyllum albidum* (Sapotaceae) is a forest fruit tree described by the Scottish botanist George Don. It is commonly found throughout tropical Africa with the common name “white star apple”. *Chrysophyllum albidum* is popularly called Agbalumo in the Southwestern region of Nigeria and closely related to the African star apple (*Chrysophyllum africanum*).

Objectives: This study aims to determine the phytochemical constituents of the *Chrysophyllum albidum* fruits, quantify its heavy metals composition and also determine the antioxidant and antimicrobial activity of the fruit.

Material and Methods

Phytochemical screening of the fruit extract was carried out using standard methods while the antioxidant activity was done using DPPH (2, 2-Diphenyl-1-picrylhydrazyl). Atomic absorption spectrophotometer was used to detect and quantify the level of some toxic metal (Cadmium, Lead, Iron, Copper, Chromium, Zinc) contamination of the fruit. Antibacterial assay was carried out using disc diffusion method with measured zones of inhibition.

Results: Results obtained reveal the presence of flavonoids, indicative of its antioxidant potentials, tannins and saponin. Heavy metals were also found to exist in varying amount in the fruit with the conspicuous absence of Cadmium. The antimicrobial assay showed increasing activities with increasing concentrations.

Conclusion: The fruit of *C. albidum*, thus possesses antibacterial and antioxidant activities and are so beneficial for consumption. However stringent environmental control needs to be put in place to minimise the level of soil contamination with heavy metals through various human explorative activities.

Keywords: Heavy metal, Antioxidant, Antimicrobial, *Chrysophyllum albidum*

INTRODUCTION

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. These include elements having atomic weights between 63.546 and 200.590 Dalton (Kennish, 1992), and a specific gravity greater than 4.0. Chronic exposure to these metals can have serious health consequences. Humans are exposed to heavy metals through inhalation of air pollutants, consumption of contaminated drinking water, exposure to contaminated soils or industrial waste, or

consumption of contaminated food. Exposure to metals, such as mercury and lead, may also cause development of autoimmunity, in which a person's immune system attacks its own cells. This can lead to joint diseases such as rheumatoid arthritis, diseases of the kidneys, circulatory system, and nervous system (Kennish, 1992).

Toxic metals, including "heavy metals" are individual metals and metal compounds that negatively affect people's health. In very small amounts, many of these metals are necessary to support life. However, in larger amounts, they become toxic. They may build

up in biological systems and become a significant health hazard (OSHA, 2014).

Mercury is a toxic heavy metal and a persistent environmental pollutant. Exposure to mercury is associated with serious adverse health and developmental effects, especially in pregnant women, developing fetuses, and young children. At levels well below World Health Organization limits, it has been shown to affect unborn fetuses and their embryonic nervous systems, leading to learning difficulties, poor memory and shortened attention spans. Low-level exposures also adversely affect male fertility. Common sources of mercury exposure include mining, production, and transportation of mercury, as well as mining and refining of gold and silver ores. High mercury exposure results in permanent nervous system and kidney damage (OSHA, 2014).

Lead is a soft, heavy, blue-gray metal that occurs naturally in the earth's crust. Lead affects almost every organ system in the human body. The central nervous system is particularly vulnerable in infants and children under age six. The effects are the same whether it is breathed or swallowed. Large amounts of lead exposure may lead to blood anaemia, severe stomach ache, muscle weakness, and brain damage. Lower levels of exposure, may affect a child's mental and physical growth leading to learning disabilities and seizures. Lead cannot be destroyed or eradicated, thus lead from past medical and scientific products and from old paints and discarded batteries remains in the environment (Clark et al. 2008).

Arsenic is a steely grey metal that is widely distributed in the Earth's crust and found naturally in the environment. There are two different types of arsenic, organic and inorganic. Organic arsenic compounds consist of arsenic combined with oxygen and hydrogen. Inorganic arsenic compounds consist of arsenic combined with oxygen, chlorine and sulphur. The Department of Health and Human Services (DHHS), The International Agency for Research on Cancer (IARC) and US Environmental Protection Agency have classified inorganic arsenic as a known human carcinogen (ATSDR, 2016). Arsenic has been shown to cross the placenta to the foetus and has been found in breast milk. Chronic exposure to arsenic has been shown to affect child development, lowering their IQ scores (OSHA, 2014). Arsenic covalently bonds with most metals and non-metals (Adriano, 2001).

Cadmium may interfere with the metallothionein's ability to regulate zinc and copper concentrations in the body. Metallothionein is a protein that binds to excess essential metals to render them unavailable. When cadmium induces metallothionein activity, it binds to copper and zinc, disrupting the homeostasis

levels (Kennish, 1992). Cadmium is used in industrial manufacture and is a by-product of the metallurgy of zinc (Wei and Yang, 2010).

The presence of abundant chromium anions in the water is generally a result of industrial waste. The chronic adverse health effects are respiratory and dermatologic (Viessman and Hammer, 1985).

Plants since the earliest beginning have served human as a source of food and medicaments. When consumed, plants serves as food that provides essential nutrients for energy, growth, health and vitality, though might be a source of ailments especially when contaminated. Plants can also serve as treatment options or as lead for compounds for management, amelioration and treatment of human diseases, mainly due to their secondary metabolites. Studies in the use of plant extracts for control of diseases have shown the importance of natural chemicals as possible sources of non-phytotoxic and easily biodegradable alternative fungicides and antibiotics (Okigbo and Nmeke, 2005). Virtually all native plant species are used for the treatment of one ailment or another.

An antimicrobial is any substance of natural, synthetic or semisynthetic origin that kills or inhibits the growth of microorganisms, but cause little or no damage to the host. Antimicrobial resistance is one of the most serious public health threats that results mostly from the selective pressure exerted by antibiotic use and abuse (Pitout and Laupland, 2008). Therefore, it is necessary to search and develop new alternative compounds to ameliorate the problem of microbial resistance.

Free radical production occurs continuously in all cells as part of normal cellular function. However, excess free radical production originating from endogenous or exogenous sources might play a role in many diseases. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition (Halliwell and Gutteridge, 1995). The physiological role of antioxidants is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. In recent years, a substantial body of evidence has shown the contributions of plant and plant based products as natural sources of free radical scavengers.

Chrysophyllum albidum fruit, known locally as Agbalumo or Udara in Nigeria, has fleshy pulp which is widely consumed when in season by both adult and children, with either a very sweet taste (when fully ripe), or sour (when unripe). The plant, a forest tree, common throughout the tropical African, belongs to the family Sapotaceae.

METHODOLOGY

Sample collection and identification

Chrysophyllum albidum fruits and leaves were gotten from Mile-12 market, Lagos, Nigeria and identified at the Pharmacognosy laboratory, Faculty of Pharmacy, University of Lagos. Identification was also done at Botany department, University of Lagos where the identity of the plant was also confirmed and herbarium sample deposited with voucher number, BDP 434.

Extraction procedure

The fruits were chopped into bits and soaked in 1.0L of methanol for 72 hours in a closed vessel. The mixture was filtered using a fluted filter paper (to prevent sticking to the funnel) and a funnel into a clean glass vessel. After filtration, the extract was concentrated using a rotary evaporator at 35°C. The concentrated extract was then freeze dried. A gummy-like exudate was obtained after the drying process.

Phytochemical screening:

A portion of the gummy-like extract gotten was dissolved in methanol to form thick slurry. Standard phytochemical screening methods were followed (Sofowora, 1993; Trease and Evans, 2002) and the following phytoconstituents were tested for: alkaloids, tannins, flavonoids, saponins, steroidal and triterpenoid nucleus, and anthraquinones.

Heavy Metals Analysis

Sample digestion

Six different acids were used in the digestion of the extracts before quantifying the heavy metals (HClO₄, HNO₃ + HCl, HNO₃ + H₂O₂, HCl, HNO₃, and H₂O₂).

To 2.0g of the extract, 25ml of H₂O₂ was added. The resulting mixture was concentrated on a hot plate in a fume cupboard until brown fumes disappeared leaving white fumes. The sample was then removed from the hot plate, allowed to cool and made up to 50ml with distilled water. This was filtered to remove particles which can block the nebulizer in the Atomic Absorption Spectrophotometer "AAS" and then transferred into a reagent bottle. The procedure was repeated for the different acids used and each sample was sprayed into the Atomic Absorption Spectrophotometer.

Preparation of Standard Solutions

Standard preparation was done in order to ensure accurate analysis. A standard is required to calibrate the instrument (AAS) to ensure that Beer-Lambert's law is obeyed. The stock standard of the different metals (Cadmium, Chromium, Iron, Zinc, Lead and Copper) to be analysed were prepared and subsequent serial dilutions were done to obtain the different concentrations 0.1, 1.0, 2.0, 4.0 and 6.0 ppm required for the calibration curves.

Analysing *C. albidum* methanolic extract

In the analysis of heavy metals in the *C. albidum* fruit extract, a suitable lamp was fixed into the equipment at the desired wavelength relative to the metal to be analysed. The samples were aspirated into a nebulising chamber, where the samples mixes with the oxidant (compressed air and fuel) to form an aerosol, about 90% of the aerosol goes into the flame. The flame burned the sample and atomized the sample from the ground to excited state at which the absorbed light was measured. The monochromator selects the wavelength of the atom coming in based on the source of light. The procedure was followed for all metals and for the different digestion acids using their respective lamps and wavelengths as shown in table 1 (Adepoju-Bello et al. 2009).

ANTIMICROBIAL ANALYSIS

Sample preparation

Six working concentrations of the extract were prepared (400, 300, 150, 75, 50 and 25mg/ml) using 2% methanol as the diluent.

Preparation of Antimicrobial Reference Solution

Ciprofloxacin IV Infusion BP (200mg/100ml) was used as reference standard. A stock concentration of 2000µg/ml was prepared from which the working concentrations of 20, 10, 5 and 2.5µg/ml were prepared using sterile water as the diluent.

Assay organisms

Liquid culture of the test organisms (*Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus albus*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Klebsiella pneumonia*, *Proteus vulgaris*) was prepared from pure subculture organisms provided in the laboratory. Colonies of the pure assayed organism were picked using sterile loop. The colonies picked were then rubbed in the wall of a bottle containing normal saline. More colonies were picked when necessary and a turbid suspension was formed for calibration using 0.5 McFarland as the standard. The calibrated organism, (2.0ml) was then transferred into a petri dish.

Agar preparation and Inoculation

Mueller Hinton agar was used for the antimicrobial analysis. After preparation of the agar, 25.00mls was transferred to the petri dish already containing the assay organism and then mixed properly to ensure a thorough mix of the organism and the agar. After solidification of the agar, wells of 5mm were bored with the help of a sterile borer. Five holes were bored in each petri dish. The different working concentration of the extract (0.15ml) was then introduced into the wells with the help of a sterile

1ml pipette. The control of the experiment was done by the addition of 0.15ml of 2% methanol into the fifth hole after four working concentrations of the extract had been added to the other four holes. The agar plates were then incubated at 37°C for 24hrs and observed for zone of inhibition. This was done in triplicate and the average zone of inhibition was recorded.

Determination of antioxidant activity

The radical scavenging activity of the fruit extract of *Chrysophyllum albidum* against 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical was determined by UV/Visible spectrophotometry at 517nm. The working concentrations of the extract prepared were

100, 150, 200, 250 and 300µg/ml in methanol. Vitamin C served as reference standard at concentrations of 20, 40, 60, 80 and 100µg/ml in methanol. Each working concentration of the extract and standard (2ml) was treated with 0.5ml of 1mM DPPH in methanol. A blank solution was prepared containing 2ml of methanol and 0.5ml of 1mM DPPH. The determination was done in triplicates and the average absorbance calculated.

The radical scavenging activity was calculated using the formula;

$$\% \text{ inhibition} = \{(A_b - A_a) / A_a\} * 100$$

Where A_b is the absorption of the blank sample and A_a is the absorption of the extract.

Table 1: Parameters used for analysis of metals

Metal ion	Cadmium	Lead	Iron	Copper	Chromium	Zinc
Wavelength	228.80nm	283.31nm	248.33nm	324.7nm	357.9nm	213.9nm
Flame used	Oxy-acetylene	Oxy-acetylene	Oxy-acetylene	Oxy-acetylene	Oxy-acetylene	Oxy-acetylene
Lamp used	Hollow cathode	Hollow cathode	Hollow cathode	Hollow cathode	Hollow cathode	Hollow cathode
Max current(mA)	10	15	20	15	20	35

RESULTS

Phytochemical screening

From the result of the phytochemical analysis gotten, it was observed that *Chrysophyllum albidum* methanol extract contains saponin, tannin with steroidal and terpenoid nucleus while alkaloid and free anthraquinone were absent.

Heavy Metals Analysis

To determine the concentration of the metal ions present in the plant extract, a calibration curve of absorbance against concentration using standard stock solution for each metal was done (See figures 1-6).

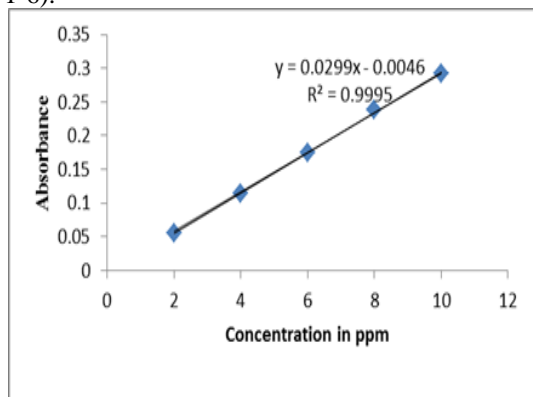


Figure 1: Calibration curve for Fe²⁺

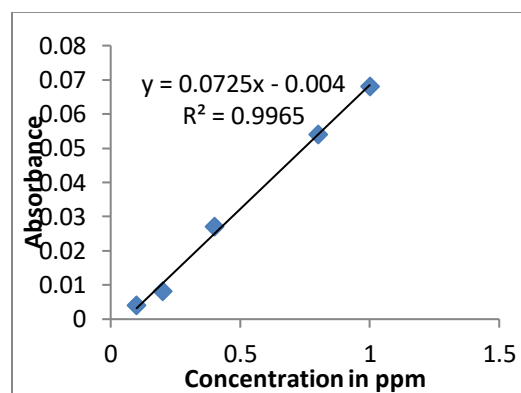


Figure 2: Calibration curve for Cu²⁺

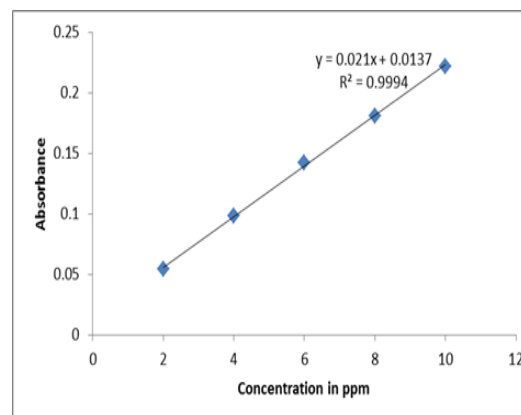


Figure 3: Calibration curve for Cr²⁺

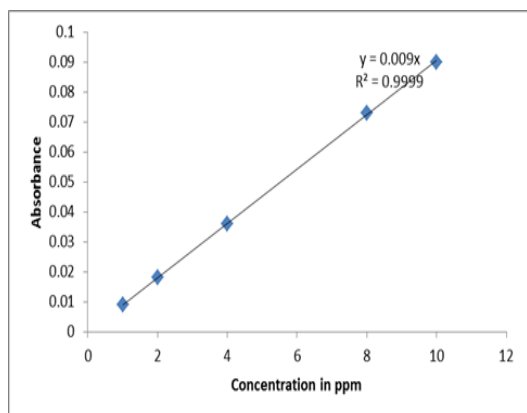


Figure 4: Calibration curve for Pb²⁺

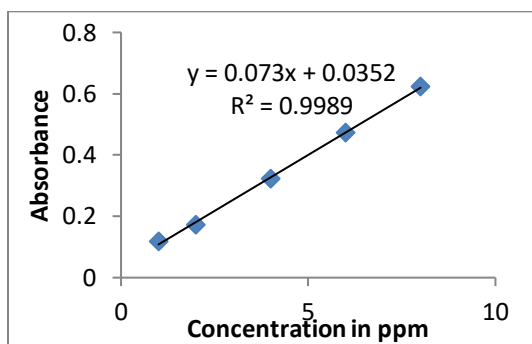


Figure 5: Calibration curve for Cd²⁺

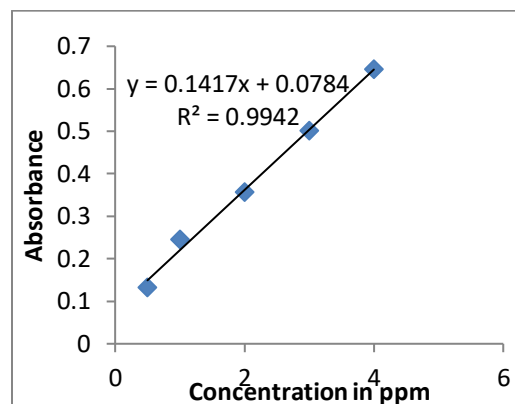


Figure 6: Calibration curve for Zn²⁺

Antimicrobial analysis

Tables 4 and 5 respectively, show the average zone of inhibition of *Chrysophyllum albidum* fruit extract as well as that of the reference standard (Ciprofloxacin) at different concentrations.

Antioxidant assay

Table 5 and 6 show the percentage inhibition of DPPH in *C. albidum* fruit extract and Vitamin C Standard respectively, while Figure 7 is a bar chart showing inhibition of DPPH by *C. albidum* extract and Vitamin C standard.

Table 2: Concentration of metal ions present in the *Chrysophyllum albidum* extract for the different digestion methods used and the mean concentrations

Digestion acid used	concentration of Fe in mg/L	concentration of Pb in mg/L	concentration of Cd in mg/L	concentration of Cr in mg/L	concentration of Cu in mg/L	concentration of Zn in mg/L
HClO ₄	15.72	0.70	ND	1.92	0.50	5.85
HNO ₃ +HCl	57.12	0.57	ND	17.05	0.49	6.08
HNO ₃ +H ₂ O ₂	72.50	0.80	ND	14.50	0.30	-0.61
HCl	121.02	1.33	ND	28.82	0.60	6.63
HNO ₃	1.66	0.66	ND	-0.24	0.31	0.21
H ₂ O ₂	6.13	0.84	ND	0.05	0.47	15.16
Mean	45.69	0.82	ND	10.35	0.45	5.55

Table 3: Average zone of inhibition of *Chrysophyllum albidum* fruit extract at different concentration

Organism/Concentration of extract	25mg/ml (mm)	50mg/ml (mm)	75mg/ml (mm)	150mg/ml (mm)	300mg/ml (mm)	400mg/ml (mm)	Control
<i>Bacillus subtilis</i>	NI	NI	13	14	19	20	NI
<i>Escherichia coli</i>	NI	NI	18	20	23	25	NI
<i>Staphylococcus aureus</i>	NI	NI	15	19	23	25	NI
<i>Staphylococcus albus</i>	NI	NI	15	18	22	25	NI
<i>Pseudomonas aeruginosa</i>	NI	NI	12	15	20	24	NI
<i>Shigella dysenteriae</i>	NI	NI	10	18	23	25	NI
<i>Klebsiella pneumonia</i>	NI	NI	12	14	15	20	NI
<i>Proteus vulgaris</i>	NI	NI	13	15	23	25	NI

Table 4: Average zones of Inhibition obtained for Ciprofloxacin standard**Table 5: Percentage inhibition of DPPH in *C. albidum* fruit extract**

Concentration of extract ($\mu\text{g/ml}$)	Percentage inhibition of DPPH
100	8.1

Organism	Concentration of Ciprofloxacin			
	2.5 $\mu\text{g/ml(mm)}$	5 $\mu\text{g/ml(mm)}$	10 $\mu\text{g/ml(mm)}$	20 $\mu\text{g/ml(mm)}$
<i>Bacillus subtilis</i>	20	21	23	25
<i>Escherichia coli</i>	18	20	22	24
<i>Staphylococcus aureus</i>	22	22	25	27
<i>Staphylococcus albus</i>	19	22	23	24
<i>Pseudomonas aeruginosa</i>	21	22	24	25
<i>Shigella dysenteriae</i>	19	20	22	23
<i>Klebsiella pneumonia</i>	19	22	23	24
<i>Proteus vulgaris</i>	22	23	24	25
150	16.6	60	66.9	
200	25.6	80	75.6	
250	33.2	100	83.7	
300	45.3			

Table 6: Percentage inhibition of DPPH in Vitamin C Standard

Concentration of vitamin C ($\mu\text{g/ml}$)	Percentage inhibition of DPPH
20	54.9
40	59.2

DISCUSSION

Chrysophyllum albidum fruit when in season is highly consumed by both young and old. Hence, there is the need to determine how safe it is, especially when consumed in large amounts. The results for the phytochemical screening revealed the presence of tannins, saponins, steroid and flavonoids while alkaloid and free anthraquinone are absent. Phytochemical screening of medicinal plants is very essential as it helps in understanding the use of these plants and the reason for the various activities they possess e.g. the presence of flavonoid in plants and plant extracts is linked to their antioxidant activities (Kamba and Hassan, 2011).

Saponins are thought to bind with cholesterol and pathogens in the body. Thus preventing them from being absorbed in the body, carrying them through

the body's digestive system instead, where they can be properly eliminated. This elimination process is thought to reduce the cholesterol level (Kamba and Hassan, 2011).

Tannins are naturally occurring plant phenols. Their main characteristic is that they bind and precipitate proteins. They can have a large influence on the nutritive value of many foods for humans and livestock. Tannins contribute to many aspects of our daily lives. They are responsible for the astringent taste we experience when we partake of wine or unripe fruits, and for the enchanting colours seen in flowers and in autumn leaves (Frutoset al., 2004).

Flavonoids are compounds found in fruits, vegetables, and certain beverages that have diverse beneficial biochemical and antioxidant effects. Their

dietary intake is quite high compared to other dietary antioxidants like vitamins C and E. The antioxidant activity of flavonoids depends on their molecular structure, and structural characteristics of certain flavonoids found in hops and beer confer surprisingly possess potent antioxidant activity exceeding that of red wine, tea, or soy (Kamba and Hassan, 2011).

Heavy metals are naturally-occurring components of the earth's crust that are, as a rule, neither created nor destroyed, but are simply redistributed. Distribution of heavy metals is not uniform, such that some soils may contain higher amounts of any of these chemicals, either due to natural processes or to pollution factors wherein heavy metals have been disbursed into the environment through human activities, such as mining, power generation, manufacturing, and the former use of lead contaminated gasoline. Many heavy metals can be absorbed into plants as they grow. Some plants have been reported to accumulate specific metals, such as is the case with cadmium and arsenic in numerous seaweed species (American Herbal Products Association, 2009).

According to US Environmental Protection Agency (US EPA, 2009), the maximum concentration level (MCL) of iron in vegetables is 0.3mg/L, Lead is 0.015mg/L, Cadmium is 0.01mg/L, Chromium is 0.1mg/L, Copper is 1.3mg/L and Zinc is 7.0mg/L. From the result obtained (Table 3) 45.69mg/L of Iron, 0.82mg/L of Lead, 10.35mg/L of Chromium, 0.45mg/L of Copper and 5.55mg/L of Zinc were obtained, with no detection of Cadmium. Only Cadmium, Copper and Zinc fell within the USEPA limits while Iron, Lead and Chromium were above the stated limits.

The heavy metals which were over the USEPA limits for vegetables could be as a result of contamination from the soil used for planting, pollution from the environment, pesticide use, oil and refineries etc. Hence there is a need to control predisposing factors of vegetables and other foodstuff to heavy metals to ensure their safety to consumers.

Chrysophyllum albidum has been used for the treatment of various ailments and infections even when there was no scientific proof (Amusa et al., 2003). After incubation for 24 hours there was a clear zone of inhibition for all the different organisms at the various concentrations (Table 5). Six working

concentrations were used for the extracts which were 25, 50, 75, 150, 300 and 400mg/ml. After 24 hours it was observed that at 25 and 50mg/ml there was no zone of inhibition showing that *Chrysophyllum albidum* fruit extract had no antibacterial activity at those concentrations. Antibacterial activity was observed from 75mg/ml concentration up to 400mg/ml, and the zone of inhibition increased with increasing concentration. Zone of inhibition was observed for all the organisms used showing that the fruit of *C. Albidum* has a good antibacterial activity at high concentrations and supports its use in the treatment of infections (Table 4).

The antioxidant activity of the fruit extract as determined by the DPPH scavenging activity using Vitamin C as standard reference revealed a low degree of DPPH inhibition. This result indicates *C. albidum* fruit extract has little antioxidant activity; which is attributable to the presence of flavonoid in the extract as revealed by the phytochemical analysis.

CONCLUSION

This study showed that *Chrysophyllum albidum* commonly called udara or agbalumo is a fair source of anti-oxidant and also possesses some levels of antibacterial effects. The *Chrysophyllum albidum* fruit extract showed higher concentrations for iron, lead and chromium based on Maximum Concentration Level according to USEPA for heavy metals in vegetables. *Chrysophyllum albidum* is good for consumption though there is need for environmental control to reduce this relatively high amount of heavy metals in the extract which could limit its potential therapeutic use.

RECOMMENDATION

There is a need to control the exposure of plants to heavy metals which pose health risk to the consumers. Policies can also be made to guide against indiscriminate disposal of home and industrial waste into the environment where they can come in contact with plants and plant products.

Furthermore, there is a need to inform the public and also the farmers about the danger of being exposed to toxic metals which can be ingested from the various fruits they consume. Proper washing and storage of fruits should be encouraged.

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Conflict of Interest: None declared

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