Phytochemicals and some Heavy Metal detection in Ripe Healthy Mesocarp *Raphia hookeri* fruits.

2 Okungbowa, F. I 1 Esiegbuya, O. D*. and 1 Okogbenin O.B,

1 Plant Pathology Division, Nigerian Institute for Oil Palm Research (NIFOR), Benin City.
2 Department of Plant Biology and Biotechnology, University of Benin, Benin City.

ABSTRACT

A study of the phytochemical and heavy metal composition of ripe *R. hookeri* fruits was carried out using the Buck 200 Atomic Absorption Spectrophotometer (AAS) method described by the Association of Official Analytical Chemists. The study revealed the presence of phytochemicals such as phenols, flavonoids, alkaloids, saponin, oxalate and quinines at 0.0302%, 23%, 4% 0.0185%, 0.0225% and 0.015% respectively. Tannin was not detected. The heavy metals were copper (1.93ppm), cadmium (1.43ppm) and chromium (1.93ppm). Lead was not detected. Oxalic acid and the heavy metal cadmium detected in the ripe healthy mesocarp of *R. hookeri* fruits are undesirable components in human food because of their potential risk in causing urinary disease and increasing the nutritional toxic level of food samples. It is therefore recommended that the consumption of *R. hookeri* fruit be discouraged due to its possible health risk. However, other economic benefits due to the presence of phytochemicals, such as its use in herbal medicine, tissue culture, as a purgative, anti-microbial, anti-tumor and anti-cardiovascular agents can be exploited.

KEYWORDS: *Raphia hookeri,* Phytochemicals and Heavy metals

INTRODUCTION

The economic importance of *R. hookeri* fruits as reported by Ndon , (2003) includes its oil which can be used for cooking and making of confectionery and the mature and ripe fruit used as food by coastal people of Akwa Ibom state, Nigeria. During the course of this study, some indigenes of the Niger delta area of Nigeria have indicated their preference for the consumption of the ripe mesocarp of R. hookeri fruits due to the bitter nature of unripe fruit. However, some do not consume them at all because of prolonged stomach upset/pain it causes after eating. Ndon (2003) also reported that the fruit contains plant growth regulators such as auxins, cytokinins, ethylene, gibberellins and other chemicals which can be used in tissue culture and to stupefy fish. Ethanoic extract of the epicarp, mesocarp and seed of R. hookeri fruits have been reported by Adaigbe et al. (2013) to posses some phytochemical agents which were toxic for the control of termite workers at 280C and 75% humidity.

Esiegbuya *et al.* (2013) previously reported on proximate value and mineral composition of healthy and black rot infected *R. hookeri* fruits. The study showed that ripe *R. hookeri* fruit contain high amount of mineral elements and nutritional compounds which can contribute to good health and fitness in humans. However, these elements are not readily available for economic benefits because of the postharvest effect of the black and dry rot disease on the ripe *R. hookeri* fruits caused by *Chalara paradoxa* and *Xylaria feejensis*.

According to Ndon (2003), the *R. hookeri* palm is the most popular among the twenty species of Raphia palm that have been identified. Its advantage over the other species includes its ability to mature between 3-6 years and also being able to yield 115 to 1145 litres of palm wine within its life time when compared to the other species. Based on the previous report on the health and economic benefits of *R. hookeri* fruits, there is therefore need to study the phytochemical and heavy metal composition of the ripe *R. hookeri* fruits which confers on it some of its economic benefits.

This study will help to give more insight into the understanding on the nutritional and phytochemical composition of the ripe *R. hookeri* fruits, thereby paving way for its full economic utilization

* Corresponding author: esiegbuya@gmail.com (07062470649)

MATERIALS AND METHODS

Source of Plant Materials:

Raphia fruits used in this investigation were obtained from the gene pools harvested from wild grooves plantation in Onuebum Bayelsa State of Nigeria. The species investigated was *R. hookeri* Man and Wendle. Four bunches containing about five hundred fruits were harvested; the healthy ones were separated from the infected ones. The average weight of the fruit was 10.75g. The fruit samples were washed with distilled water to remove sand particles before analysis.

Heavy metal detection

Heavy metals were analyzed using the BUCK 200 Atomic Absorption Spectrophotometer (AAS) according to the methods described by (A.O.A.C., 1990).The digest of the ash of the fruit sample was obtained by taking 2g of the sample which was weighed into a porcelain crucible. This was transferred into the muffle furnace set at 550ºC and left for about 4 hours. About this time it had turned to white ash. The crucible and its content were cooled to about 100ºC in air, then room temperature in a dessicator and weighed. The sample was washed into a 100 ml volumetric flask with deionised or distilled water and made up to mark. This diluent was aspirated into the Buck 200 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements was read at their respective wavelengths with their respective hollow cathode lamps, using appropriate fuel and oxidant combination.

Detection of phytochemicals

This was done using the official methods of analysis (A.O.A.C, 1990)

Alkaloids

A measured weight of the sample was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4 hours at 28^{o}C. It was then filtered via Whatman No 42 grade filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation in a water bath and treated with drop wise addition of conc. aqueous ammonium hydroxide (NH4OH) until the alkaloid was precipitated. The precipitated akaloids was received in a weighed filter paper, washed with 1% ammonia solution and dried in the oven at 800C. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

Flavonoids

0.5g was boiled in 50 ml of 2M HCl solution for 30 minutes under reflux. It was allowed to cool and then filtered through Whatman No 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate, first drop wise and later transferring the remaining volume to the mixture. The flavonoid precipitate was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

Tannins:

0.2 g of finely ground sample was transferred into a 50 ml beaker followed by 20 ml of 50% methanol. The beaker was covered with paraffin and placed in a water bath at 77-80^{o}C for 1 hour and stirred with a glass rod to prevent lumping. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper into a 100 ml volumetric flask. This was made up to mark with distilled water and thoroughly mixed. One millilitre of sample extract was pipetted into a 50 ml volumetric flask, 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na_2CO_3 were added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 20 min when a bluish-green colouration developed. Standard Tannic Acid solutions of range 0-10 ppm were treated similarly as 1 ml of sample above. The absorbances of the Tannic Acid Standard solutions as well as samples were read after colour development on a Spectronic 21D Spectrophotometer at a wavelength of 760 nm.

Percentage tannin was calculated using the formula:

Tannin (%) = Absorbance of sample x Average gradient x Dilution factor/ Weight of sample x 10,000

Saponin

One gram of finely ground sample was weighed into a 250 ml beaker and 100 ml Isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman No. 1 filter paper into a 100 ml beaker and 20 ml of 40% saturated solution of magnesium carbonate (Mg_2CO_3) added. The mixture obtained with saturated Mg_2CO_3 was again filtered through a Whatman No 1 filter paper to obtain a clear colourless solution. Then 1 ml of the colourless solution was pipetted into 50 ml volumetric flask and 2 ml of 5% iron (lll) chloride ($FeCl_3$) solution was added and made up to mark with distilled water. It was allowed to stand for 30 minutes for blood red colour to develop. This was followed by the preparation of 0-10 ppm standard saponin solutions from saponin stock solution. The standard solutions were treated similarly with 2 ml of 5% $FeCl_3$ solution as done for 1 ml sample.

The absorbances of the sample as well as standard saponin solutions were read after colour development on a Spectronic 2lD Spectrophotometer at a wavelength of 380 nm.
Percentage saponin was calculated using the formula:
Saponin (%) = Absorbance of sample x Average gradient x Dilution factor/ Weight of sample x 10,000

Anthraquinone content
Fifty milligrams of the fine powder sample was soaked in 50 ml of distilled water for 16 hours. This suspension was heated in water bath at 70 0C for one hour. After the suspension was cooled, 50ml of 50% methanol (MeoH) was added and then filtered. The clear solution was measured by spectrophotometer at a wavelength of 450nm and compared with a standard solution containing 1mg/100ml alizarin and 1mg/100ml purpurin with the absorption-maximum 450nm

Total polyphenols
Two-hundred microlitres of extracted sample, in triplicate, was added to 1 ml of 0.2 N Folin–Ciocalteau reagent and 0.8 ml of 7.5% sodium carbonate solution, mixed well and allowed to stand for 30 min at room temperature. Absorption at 765 nm was read using a Shimadzu 300 UV–Vis spectrophotometer (Shimadzu UV-1601).
Quantification was based on the standard curve generated with 100– 400 mg/l of gallic acid.

Determination of oxalic acid
One gram of the sample was weighed into 100 ml conical flask. 75 ml of 3 M H 2SO4 was added and the solution carefully stirred intermittently with a magnetic stirrer for about 1hr and then filtered using Whatman No.l filter paper. 25 ml of the sample filterate was collected and titrated against hot 0.1N KMno4 solution to the point when a faint pink color appeared and persisted for at least 30 seconds. The concentration of oxalate in each sample was determined as 1ml 0.l permanganate = 0.006303g oxalate

RESULTS
Phytochemicals detected in the ripe mesocarp of *R. hookeri* fruits as shown in Table 1 below are phenols, flavonoid, alkaloids saponins oxalate and quinines 0.0302%, 23%, 4%, 0.0185%, 0.0225% and 0.015% respectively. Tannin was absent. There is however the presence of oxalic compound. The heavy metal and mineral elements detected were copper, cadmium, and chromium at 1.93 ppm, 1.43 ppm and 1.93ppm respectively. Lead was absent..

Table1: Phytochemicals detected in ripe mesocarp of *R. hookeri* fruits

.

S/N	PARAMETER	AMOUNT
1	Phenols (%)	0.0302
2	Tannins	N.D
3	Flavonoid (%)	23
4	Alkaloids (%)	4
5	Saponin (%)	0.0185
6	Oxalate (%)	0.0225
7	Quinones (%)	0.015

ND: not detected

Table 2: Heavy metals detected in ripe mesocarp of *R. hookeri* fruits.

S/N	PARAMETER	AMOUNT
1	Copper (ppm)	1.93
2	Cadmium (ppm)	1.43
3	Lead (ppm)	ND
4	Chromium (ppm)	1.93

ND: not detected

DISCUSSION

Phytochemical compounds are desirable components in our food because of their antioxidant properties.

Phenolic compounds are essential for the growth and reproduction of plants. The amount of phenolic compound detected in this study was 0.0302%. This suggests the possible use of extracted phenolic compound from the ripe R. hookeri fruits as plant growth regulator according to Ndon (2003). According to Doherty et al., (2010), the presence of phytochemical compounds such as phenols in plant extracts indicates it as an antimicrobial agent. This is because phenols and phenolic compounds have been extensively used in disinfection and remain the standard with which other fungicides are compared.

Flavonoids are not only among the most common antioxidants in nature, but also those with the highest antioxidant potency in vitro (Miller et al., 1996; Rice-Evans, et al., 1996). Although their best-described property is to act as antioxidants (Nijveldt et al., 2001). Flavonoids can display a huge array of biochemical and pharmacological effects that affect the function of the immune system and inflammatory processes (Middleton and Kandaswami, 1992). The amount of flavonoids in the ripe R. hookeri was 23% suggesting its possible use an antioxidant, antiviral, anti-allergic and anti-inflammatory agent. There have been no recommended daily allowance for the consumption of phytochemicals, however, a recent, valuable contribution to the phytochemical debate has been made by Wahlqvist et al., (1998) who have proposed developing a food-based Index of Preferred Phytochemical Intake (IPPI). Under this proposal phytochemical rich foods which are known to be good sources of a particular class of beneficial phytochemicals are aggregated, thereby providing for optimum intake and synergy, but at the same time avoiding potential toxicity from excessive intakes Literature shows that some saponins are toxic to cold-blooded organisms and insects at particular concentrations. Most saponins, which readily dissolve in water, are poisonous to fish. The amount of saponin obtained from the ripe R. hookeri fruits in this study was 0.0185% supporting the fact that in ethnobotany, saponins are primarily known for their use by indigenous people in obtaining aquatic food sources Ndon (2003).

Quinones possess a number of biological properties, including some claims in herbal medicine. These applications include purgative, antimicrobacterial, anti-tumor and anti-cardiovascular disease. (Coe et al., 2005).

Generally, consumption of fruits and vegetables rich in phytochemical compounds according to Hung et al., (2004) have been reported to reduce the risk of chronic disease. Absence or deficiency of phytochemicals in processed foods may contribute to increased risk of preventable diseases (Rao and Rao 2007).

Oxalic acid is regarded as an undesirable component of our food not only because it raises the risk of urinary stones but also because it sequesters calcium, which is one of the essential ions, as insoluble calcium oxalate. The concentration of oxalate found in this study was 22.5mg/100g which is quite high. Therefore the consumption of this fruit should be discouraged since studies have shown that oxalic compounds can easily form bond with other mineral elements found in this fruit and cause kidney stone.

Heavy metal contamination of food is one of the most important aspects of food quality assurance. Heavy metals are non-biodegradable and are persistent environmental contaminants which may be deposited on the surfaces of plant and then absorbed into the plant tissues. The heavy metals detected in this study were copper, cadmium, and chromium. Prolonged exposure to heavy metals such as cadmium copper, lead, nickel and zinc have been reported by to cause deleterious health effects in humans. In this study lead and nickel were not detected. However, copper, cadmium and chromium were detected at 1.93ppm, 1.43ppm and 1.93ppm respectively.

Cadmium is a non-essential element in foods and natural waters and it accumulates principally in the kidneys and liver (Divrikli et al., 2006). The guideline value for cadmium in soil from plant uptake is 1 mg/ kg dry soil weight (DEFRA, 2002). In the present study the concentration of cadmium in fruits was 1.43ppm. Cadmium concentrations were within the advisory interval (0.5-5 mg/kg) (Danish, 2000).

Cadmium is taken up through the roots of plants to edible leaves, fruits and seeds. During the growth of grains such as wheat and rice, cadmium taken from the soil is concentrated in the core of the kernel. Cadmium also accumulates in animal milk and fatty tissues (Figueroa, 2008). Therefore, people are exposed to cadmium when consuming plant- and animal-based foods such as molluscs and crustaceans (Castro-González and Méndez-Armenta, 2008; WHO 2004; WHO 2006). Cadmium accumulates in the human body affecting negatively several organs: liver, kidney, lung, bones, placenta, brain and the central nervous system (Castro-González & Méndez-

Armenta, 2008). Other damages that have been observed include reproductive, and development toxicity, hepatic, haematological and immunological effects (Apostoli and Catalani, 2011).

The Joint FAO/WHO has recommended the provisional tolerable weekly intake (PTWI) as 0.007 mg/kg for cadmium (JECFA, 2004). The environmental protection agency (EPA) maximum contaminant level for cadmium in drinking water is 0.005 mg/L whereas the WHO adopted the provisional guideline of 0.003 mg/L (WHO, 2004).
Copper plays a critical role in a variety of biochemical processes. Copper is an essential micronutrient which functions as a biocatalyst, required for body pigmentation. In addition to iron, it maintains a healthy central nervous system, prevents anaemia and is interrelated with the function of Zn and Fe in the body (Akinyele and Osibanjo, 1982). However, most plants contain an amount of copper which is inadequate for normal growth which is usually ensured through artificial or organic fertilizers (Itanna, 2002). In this study, the concentration Cu was 1.93ug/g.
The Estimated Safe and Adequate Daily Dietary Intake of copper is between 1.5 - 3 mg/day (NRC 1989).

According to RTI (2000), chromium is an essential nutrient for man in amounts of 50 - 200 µg/day. Chromium is necessary for the metabolism of insulin. It is also essential for animals, whereas it is not known whether it is an essential nutrient for plants, but all plants contain the element.
The absence of lead in the fruit sample suggests that the fruit sample is non-toxic. The presences of these mineral elements in the ripe healthy mesocarp of R. hookeri fruits indicate its nutritional toxic property.

CONCLUSION

Raphia hookeri fruit contains high amounts of phytochemicals and heavy metals. The presence of compounds such as the oxalate, chromium and cadmium which have been reported to be toxic to man calls for caution in the consumption R. hookeri fruit. Other economic benefits of R. hookeri such as its use in herbal medicine, as purgative, antimicrobacterial, anti-tumor and anti-cardiovascular agents can be harnessed.

REFERENCES

1.Adaigbe, V. C., Ataga, C.D., Obibuzor, J.U., Ogbebor, C.O., Udoh, M.E., Okere, C. I and Okogbenin, O.B (2013). Efficacy of Raphia fruit extract in the management of termites.

2.Akinyele, I.O. and Osibanjo, O (1982). Levels of trace elements in hospital diet. Food Chemistry, 8: 247-251.

3.Apostoli, P and Catalani, S. (2011). Metal ions affecting reproduction and development. Metal Ions in Life Science, 8: 263-303.

4.Castro-González, M.I. and Méndez-Armenta, M. (2008). Heavy metals: Implications associated to fish consumption. Environmental Toxicology & Pharmacology, 26, 263-271.

5.Coe, F.L., Evan, A. and Worcester, E. (2005). Kidney stone disease. Journal of Clinical Investigation, 115 (10): 2598–608.

6.Danish Environmental Protection Agency. (2000). Guidance regarding Advice of inhabitants of slightly contaminated soil (in Danish) Danish EPA, Office of soil contamination: Copenhagen.75p.

7.DEFRA and Environmental Agency. (2002). Contaminated land exposure assessment model (CLEA): technical basis and algorithms. Reports CLR 10. Available from the R&D Dissemination centre, WRC plc. Swindon, Witts.

8.Elinder, CG. and Jarup, L. (1996).Cadmium exposure and health risks. Recent findings. Ambio. 25 5:370-373.

9.Esiegbuya, D.O., Okungbowa, F.I., Oruade-Dimaro, E.A and Airede, C.E (2013) Proximate and phytochemical analyses of Raphia hookeri fruits. Nig. J. Mycol. 5: 1-7

10.Hung, H.C., Joshipura, K.J., Jiang, R., Hu, D.Hunter, F.B. and Smith-Warner, S.A. (2004) Fruit and vegetable intake and risk of major chronic disease: Journal National Cancer Institute, 96(21):1577-1584.

11.Itanna, F. (2002). Metals in leafy vegetables grown in Addis Ababa and toxicology implications. Ethiopian. Journal of Health Development, 16: 295-302.

12.Middleton, E. and Kandaswani, C. (1992). Effect of flavonoids on immune and inflammatory functions. Biochemistry and Pharmacology, 43:1167-1171.

13.Miller, N.J., Castellucio C., Tijburg, L. and Rice-Evans, C. (1996): The antioxidant properties of the aflavins and their gallate esters – radical scavengers or metal chela¬tors? FEBS Letters, 392: 40–44.

14.Ndon B. A. (2003). The Raphia Palm Economic Palm Series. 1st ed. Concept publication series. Lagos, Nigeria. 156p

15.Nijveldt, R.J., Nood, E., Hoorn, D.E., Boolens, P.G., Norren, K. and Leeuwen, P.A. (2001) Flavonoids: A review of probable mechanisms of action and potential implications European Journal of Cancer Prevention, 32: 401-405

16.NRC National Research Council 1989 USA Recommended Dietary Allowances 10th ed. National Academy Press Washington DC.

17.Rao, A.V. and Rao, (2007) Carotenoids and human health Pharmacological Research, 55 (3):207–216

18.Reilly, C. (1991) Metal contamination of food Elsevier Science Publishers Lt 2nd Ed. London and New York

19.RTI. (2000). Toxicological profile for chromium. Syracuse Research Corporation for U.S. Department of Health and Human Services. Agency for Toxic Substances and Disease Registry, Atlanta.

20.Wahlqvist, M. L., Wattanpenpaiboon, N, Kouris-Blazos, A., Mohandoss, P. and Savige, G.S.(1998) Dietary reference values for phytochemicals? Procedure of Nutritional Society Australia 22: 34–40.

21.WHO. (2004). Evaluation of certain food additives contaminants. Sixty-first report of the joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 922.

22.WHO. (2006). Evaluation of certain food contaminants. Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 930.