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Determination of Heavy Metal Concentration and Proximate Composition In Tetrapleura Tetraptera (Uyayak) From Ikot Ekpene Local Government Area Of Akwa Ibom State

Etim I. G. and Jonah, A. E.
Department of Science Technology, Akwa Ibom State Polytechnic Ikot Osurua, Ikot Ekpene.

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ABSTRACT
The determination of heavy metal concentrations and Proximate composition in Tetrapleura tetraptera, was investigated using standard analytical methods. The result of the analysis revealed cadmium (Cd) (0.0205±0.001mg/kg),chromium (Cr)(0.1055±0.002mg/kg),lead (Pb) (0.0035±0.001mg/kg), copper (Cu) (0.512±0.001mg/kg) and zinc (Zn) (0.2885±0.002mg/kg). The proximate analysis revealed that, the moisture content (40.675±0.625%), Ash (2.58± 0.11%), crude fat (28.015±0.079%), crude fibre (5.570±0.430%), crude protein (19.795±0.315%) and carbohydrate (43.54±0.11%). The result showed that the concentration of cadmium (Cd), lead (Pb) and zinc (Zn) were within FAO/WHO permissible standard when compared, while chromium (Cr) and copper (Cu) concentrations were found to be above the FAO/WHO permissible limit. The concentration of crude fat and crude fiber were within the Recommended Dietary Allowance (RDI) permissible limit exceptions of ash content, crude protein and carbohydrate. This result obtained indicate that Tetrapleutera tetraptera are of good dietary benefit to man and animal but Standard measures should be adopted in order to reduce the concentrations of Chromium (Cr) and copper (Cu).
Keywords: Proximate composition, heavy metal, Tetrapleura tetraptera and RDI.

I. INTRODUCTION

Heavy metals are importance environment pollutants and their toxicity is a serious problem of great concern for environment. The term "heavy metals" refers to any metallic element

that has a relatively high density and is toxic or poisonouseven at low concentration (Lenntech, 2004). Iron, zinc and copper are essential metals whereas cadmium, lead and mercury have bio-important Heavy metals contamination in plants, animals and human are due to environment pollution through air emissions from automobile exhaust, pesticides leaching into water bodies, waste from mining and other industries (Divrikliet al., 2006).

Proximate composition reveals the major component or constituents of a food material and confirms whether the food is normal (non – hazardous) in composition or has been adulterated or contaminated by various environmental contaminants like mycotoxins, pesticides, intentional and unintentional additives and preservatives. The natural constituents of food determined by proximate analysis are moisture content, crude protein, crude fiber, crude fat, and total carbohydrate.

Tetrapleura tetraptera is one of the such valuable medicinal plant, locally known as Uyayak in Ibibio. This is a flowering plant that belongs to the Mimosaceae or pea family native to Western Africa.The plant is indigenous to tropical areas especially India and West Africa. In Nigeria, it is found in the Savannah and Coastal areas, it is cultivated in Ceylon, Southsea Islands and also within Nepal Bengal Chittagong and Deccan.

According to (Aladesanmi, 2007), The fruit of this plant possesses a fragment characteristic pungent, aromatic odour and flavour,

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which is responsible for its insect repressive characteristic.

Tetrapleuratetraptera level plays an important role in human nutrition as the fruit cultivated and often used to prepare soup or porridge for nursing mothers from the beginning of child birth to post-partum contractions (Nwaichi, 2013) and as lactation aid in nursing mothers(Enwere, 1998).

In West Africa, the plant Tetrapleura tetraptera is used as a spice, a medicine and as a dietary supplement rich in vitamins. It is rich in protein, lipid, potassium, iron, magnesium, phosphorous, and vitamin C. The medicinal uses of Tetrapleura tetraptera are as follows;

i. Tetrapleura tetraptera or uyayak is very beneficial in controlling type 2 diabetes mellitus. It was also found that Tetrapleura tetraptera fruit extract is beneficial in lowering blood glucose levels in both tasting and non-fasting condition.
ii. The fruit of the plant has a strong smell due to which it its often. Used as the mosquito repellent and is effective. This property is attributed to the presence of essential oils present in the plant.
iii. Tetrapleura tetraptera pod is used as an additive in the soup which is served to postpartum mother to avoid contraction. The pods are rich in potassium; iron and calcium which are the three most important ingredients required for the production of milk in new mothers and helps to restore the lost blood.

Locally, the fruit are used in flavoring. An infusion of the whole fruit is usually taken by convalescents to bathe in order to get relief from feverish conditions, for use as an enema, for constipation and as an enematic. The plant has many traditional medicinal uses mainly in the management of convulsion, leprosy, inflammatory and rheumatic pains. Infusion of the whole fruit is taken as a recuperative tonic (Okwu, 2003).

Tetrapleura tetraptera fruit is widely used in Western Nigeria amongst men as a birth control medicine. It has been reported that feeding of extracts to animals produced some toxic effects and pathological lesions in some organs.It is therefore, used as a popular seasoning spice in Southern and Eastern Nigeria. Its fruit is used for the management of convulsions, leprosy, inflammation, rheumatism, flatulence, jaundice. Plant is used to treat diabetes and its complications such as Oxidative Stress and Hyperlipidemia. The leaves, roots, and bark can also be added for optimal performance (Okwu, 2003).

II. MATERIALS AND METHOD

Sample Collection and Preparation

The dry fruit of Tetrapleura tetraptera (Uyayak) was collected from the market AbiakpoNtak Inyang in Ikot Ekpene Local Government Area, packaged in a polythene bag and transported to the Chemistry Laboratory in Akwa Ibom State Polytechnic, thereafter, the Uyayak was wash and were sliced into small pieces and was dried in the oven. The samples were placed in the mortar and crushed with pestle and stored in airtight plastic.

Digestion of the Sample (AOAC, 2000)

1g of the sample was measured with the use of weighing balance into a digestion flask. Thereafter, 10ml of hydrochloric acid and 20ml of nitric acid was introduced into the hydrate mixtures. The digestion flask containing the two mixtures was heated gently, gentle at a temperature of 130^0C until colour was colourless.

The contents in the flask was allowed to cool and 30ml of distilled was added to the different content and shacked well. Then digest was filtered with Whatman filter paper and the was made up to 100ml each with distilled water. The digest with sample bottles for reading of the absorbance concentration of the different solution using atomic absorption spectrophotometer (AAS).

Determination of moisture content (AOAC, 2000)

Weighing crucible was washed and dried in an oven at 80^0c for some minutes, cooled and weighed Two grammes(2g) of the sample was weighed crucible bottle, now weight of the crucible bottle and its content (sample) taken (b). The weighing crucible and its content was then dried in an oven at the temperature of 105^0c for 24 hours. At the end of the time, it was removed from the oven, allowed to cool in a dedicator to room temperature, weighed with a minimum exposure to atmosphere. This was repeated till a constant weight was obtained (c).

Calculation. Moisture (% wet weight) = $\frac{b-c}{b-a} \times \frac{100}{1}$

where: a= weight of weighing crucible only

b= weight of weighing + sample before oven drying

c= weight of weighing +sample after oven drying

Preparation of sample for subsequent analysis.**(**AOAC, 2000)

After taking part of the fresh sample for moisture content determination the remaining sample was dried to a constant weight before

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subsequent analysis. The low temperature (50-60^{0}C) is employed to reduce any possible effect of high temperature on the protein (and probably other nutrient) in the food sample. However, the oven dried material is ground in a mortar into a powered form, often necessary to pass through a serve of a particular mesh size tight container specifically having a plastic cover.

Determination of Ash content and organic matter (AOAC, 2000)

Ashing procedure: Crucible with lid was ignited in a muffle furnace at the temperature of 105^{0}C for an hour. It was transferred to a desiccator to cool and weighed.A few grams (1-5g) finely ground dry sample was put in to the pre-weighed crucible. The weight of the crucible and its content on a Bunsen flame in a fume cupboard, to drive off most of the smoke (until smoking ceases), then transfer it to a muffle furnace heated at (500-600^{0}C) to burn off all the organic matter, left at this temperature for 2hour. Take out the crucible, when cool, cover and was placed in a desiccators and weight (c).

Calculation:

$$\text{ASH\%} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times \frac{100}{1} = \frac{C - a}{b - a} \times \frac{100}{1}$$

where: a = weight of empty crucible
b= weight of crucible + sample before ashing
c= weight of crucible + ash
The portion of the sample which burnt off is organic matters.
Organic matter (%) = 100 - (%) Ash.

Estimation of Crude fiber (AOAC, 2000)

Defat about 2g of material with petroleum either for 2hour, boiledfor 30minutes with 200ml of a saluting contaminating 1.25% of H_2SO_4 per 100m solution. Filter the solution through linen or cotton cloth on a fluted funnel. Wash with boiling water until the washing are no longer acidic. Transferred the residue to a beaker and boiled for another 30minutes with 200ml of a solution containing 1.25g of NaOH per 100ml. filter the final residue and wash the residue with boiling water several times until it is base (NaOH) free. The residue was finally washed twice with ethanol, and qualitatively transferred into a pre weight crucible, oven dried at 105^{0}C (1o).
Indicating in a furnace at 550^{0}C, allow to stand at this temperature for 2 hours cool in a desiccators and weight (1o) (loss in weight).

Calculation: $\frac{1a-1o}{1a} \times 100$
Weight of original sample taken.
Where 1o= weight of empty crucible.
1a= weight of empty crucible and its content after incineration (Ash fibre).

Determination of crude fat(AOAC, 2000)

Weigh 2.0g of the sample into extractor thimble which has already been washed dried in an oven, plug lightly with cotton wool.
150ml of petroleum ether (Boiling point 60-80^{0}c) was poured into a 25ml capacity round bottle flask. The Soxhlet extraction was fitted into the round button flask which was seated on a heating mantle. assemble the Soxhlet apparatus and allow to reflux for about 4 hours.
Pour the extract into a dried pre-weighed beaker (W1) and rinse the thimble with a a little quantity of the ether back to the beaker. Heat the beaker on a steam bath or oven to cool the beaker in a desiccator and weighed (W2)

Calculation: Crude fat = $\frac{\text{Weight gain in flask}}{\text{Weight of sample}} \times 100$

Crude fat = $\frac{W2-w1}{\text{Weight of sample}} \times 100$

Where: W2 = weight of beaker + fat
W1 = weight of empty beaker only.

Determination of Carbohydrate (AOAC, 2000)

This was determined as the different obtained after subtracting total organic nitrogen (protein), lipid, and fibre from the total dry matter.
This was determined as the different obtained after subtracting total organic nitrogen (protein), lipid, ash and fiber from the total dry matter.

Determination of Crude Protein(AOAC, 2000)

kjeldahl method: 1g of the sample was accurately weighed into a standard 250ml kjeldahl flask containing 1.5g $CuSO_4$ and 1.5g of Na_2SO_4.
The kjeldahl flask (digestion) was placed on a heating mantle and was heated gently to prevent frothing for some hours until a clear bluish solution was obtained.
The digested solution was allowed to cool and this was quantitatively transferred to 100ml standard flask and up to the mark with distilled water.

20ml portion of the digest was pipette into a semi – micro kjednal distillation apparatus into a treated equal volume of 40% NaOH solution. The ammonia evolved was steam distilled into a 100ml conical flask containing 10ml solution of saturated boric acid to which 2 drops Tashirus indicator (double indicator) has been added. The lip of the condenser was immersed into the boric acid double indicator solution and then the distillation continued until about 2/3 of the original volume obtained.
The tip of the condenser was rinsed with a few millimeters of distilled water in the distillate which was then titrated with 0.1MHCl until a purple pink end point was observed. The blank determination was also carried out in the similar manner as described above except for the omission of the

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sample. The crude protein was obtained by multiplying the % nitrogen.

Estimation of caloric value (energy)

The caloric values of the sample were obtained by multiplying the value of the crude protein, lipid and carbohydrate by 4,9,4 kcal respectively and taking the sum of the product.

III. RESULTS AND DISCUSSION

Results

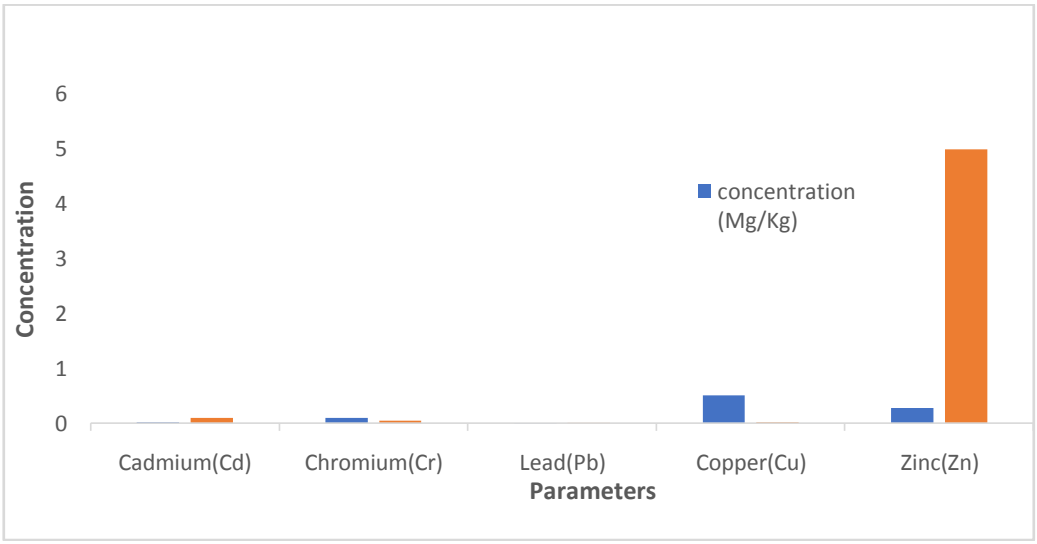

Figure 1: Heavy metal concentration in Tetrapleura tetraptera

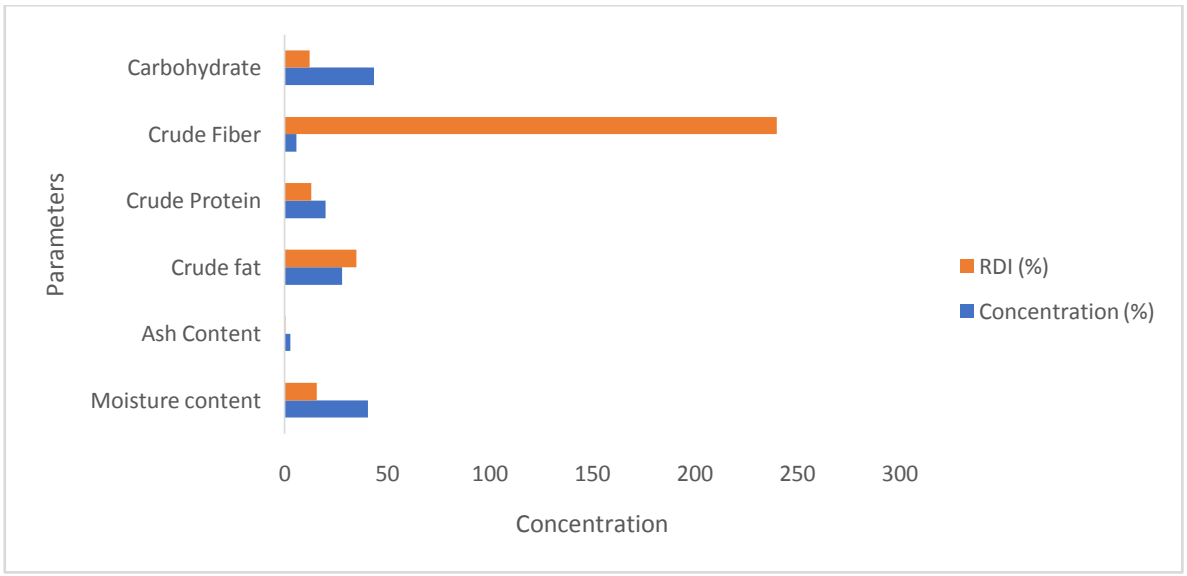

Figure 2:Proximate Composition of Tetrapleura tetraptera

IV. DISCUSSION

Heavy metal accumulation in plant tissues is affected by the characteristics of soil and atmosphere and the uptake ability of plants (Sofowora, 1993). Heavy metal ions present in plants are absorbed by roots, taken to aerial plant parts and are bioaccumulated. Heavy metals contamination affects the nutritive values of agricultural products and eliminates the benefits required from consuming them.

The concentration of cadmium (Cd) was 0.0205±0.001mg/kg. Cadmium is extremely toxic even at low levels. Cadmium causes kidney and liver problem including heart, brain and eyes problem on longer time of its accumulation (WHO, 1995). From the result obtained, cadmium (Cd)

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concentration was well within the 0.10mg/kg FAO/WHO permissible limit.

The concentration of chromium (Cr) was 0.1055±0.002mg/kg. The concentration of Cr was above the 0.05 FAO/WHO permissible limit. The high concentration of chromium may be attributed to the environment where the plant was harvested. Exposure to chromium compounds result in formation of ulcers, DNA damage, inhibition of erythrocyte glutathione reductase and respiratory cancer (WHO, 1995).

The concentration of lead (Pb) from the analysis was 0.035±0.001mg/kg. Lead (Pb) is a toxic element that can be toxic to plants although plants usually show ability to accumulate large amounts of lead without visible changes in their appearances or yield (Muhammed et al., 2008). Lead is a metal that is harmful to human when ingested through water and food. Lead toxicity causes encephalopathy (brain dysfunction), nausea, vomiting etc. Human body needs a very small quantity of lead for body functioning. The concentration of lead from this analysis was lower than the 0.01mg/kg set standard by FAO/WHO (2001), and thus considered safe.

The concentration of Copper (Cu) from the analysis recorded 0.512±0.001mg/kg. Copper is an essential element in the human body and exist as an integral part of Copper proteins cerulosis, which is concern with the release of iron from the cells into the plasma and is involved in energy metabolism (Bahl and Bahl, 2006). The concentration of Copper from this analysis was above the 0.02mg/kg set standard by FAO/WHO (2001), making the plant unsafe in regards to its high copper content.

The concentration of Zinc (Zn) from the analysis recorded 0.2885±0.002mg/kg. Zinc is one of the important metals needed in the body for normal growth and development of the sexual organs. Zn is an intercellular cation present in body tissues and next to iron-zinc is important for enzymatic function. It takes part in the synthesis of DNA, protein and insulin. It stimulates the activity of vitamin formation of red and white corpuscle (Claude and Paule, 1980).

High levels of Zn can lead to urinary tract infection, kidney stones and even kidney failures. Also, large quantities of Zn may cause anemia, nervous system disorders and damage to the pancreas and low levels of good cholesterol. The concentration of zinc from this analysis was well within the 5.0mg/kg permissible standard set by FAO/WHO (2001)

The proximate analysis of Tetraplura tetraptera revealed the presents of moisture content, Ash, Crude fat, crude protein, crude protein and carbohydrates. The moisture content was (40.675± 0.625 %), higher than the RDI permissible limit of 15.7 %. Also, higher than the values (7.37%- 10.64% reported by Uyohet al., (2013b), this could be attributed to the fact that the fruit were freshly harvested as reported by the dealer. The amount of moisture contained in a food is indicative of water activity of the food. Therefore, it is used to determine food's susceptivity and stability to spoilage micro-organism. The moisture content of the fruit is low when compared to other fruit and this proves its seining resistances in active to anti- microbial degradation thereby improving its shelf-life. However, moisture plays a helpful role in food digestion, weight loss, better productivity at work and relief of fatigue.

The ash content was (2.580±0.110%) higher than RDI permissible limit of 0.36% and also the value was almost inline when compared to the value 2.86% reported by (Uyohet al., 2013b). the differences in these values may be attributed partially to the choice of analytical method and the variation in soil micronutrient at the site of collection. High ash content in foods denotes high minerals content. Minerals function in water balance, bone health and body metabolism.

The Crude fiber was observed to contain (5.570 ± 0.430 %) while the Recommended Dietary Allowance (RDI) is 240%. The concentration of crude fibre is lower than RDI permissible limit but can be use as crude fiber supplement. The crude fiber value in the analysis was higher than 2.79-4.81% reported by Uyohet al.,(2013b). Fibre is one of the essential body nutrients. Its presence in food help to lower risk of constipation, high blood pressure, diabetes, cardiovascular disease, cancer and obesity. It is work while to mention that, high value reported in this study is not advantage to the usage of this plant since the fruit are utilized as spice and flavouring agent and not consumed directly.

The crude fat content was (28.015 ±0.079%) while the RDI is 35 %. The crude fat in the analysis was in line with RDI permissible limit. This value was higher than 11.79- 21.71 % reported by Uyohet al.,(2013b). Fats give palatability of foods, serve as storage and transport forms of metabolic fuel, serve as thermal/ electrical insulator for subcutaneous tissue, emulsifier for drug preparation and forms structural components of biomembrances. Essential fat-soluble vitamins are processed (transported) by dietary lipids and consumption of much fat are known to cause

cardiovascular diseases such as alcerolerosis, caner and aging.

The crude protein content was (19.795 ± 0.315%) while the RDI is 13 %. The crude protein in the analysia is higher than the RDI limit.The value was also higher than 5.45- 7.8% as reported by Uyohet al., (2013b). Hence, any plant food that cannot supply up to 12% of its caloric value from protein is generally considered as a good source of protein. Protein is very essential for healthy growth in children, repairs and maintenance in adult production of immunoglobulins for body defense, production of enzymes and increase muscular mass.

The carbohydrate contain was 43.540 ± 0.110) while the RDI for carbohydrate is 22 %, higher than the RDI permissible limit. Carbohydrates provides energy to body cells especially the brain cells that solely depend on glucose (component of carbohydrate) for its function. It is also essential for stability of plasma level and prevent easy degradation of body protein to obtain energy. Hence, it is the primary source of energy for organism. The high content of carbohydrate revealed by this study is in agreement with earlier research by Uyohet al., 2013b), who reported that carbohydrate had the highest composition in 20 accession of Tetrapleura tetraptera obtained from different locations in cross river state, Nigeria.

V. CONCLUSION AND RECOMMENDATION

Conclusion

The proximate analysis of T. tetraptera reveal that the spice is a rich source of food material and could be useful in the maintenance of proper body weights and treatment of disease.

The present study revealed the presence of cadmium, chromium, lead, copper and zinc in the fruit of Tetrapleura tetraptera**.** Cadmium, lead and zinc were well within the FAO/WHO stipulated standard, while chromium and copper were found to be above the FAO/WHO set standard. Heavy metals present in plants, which exceed the acceptable limits can bioaccumulate in human bodies overtime as they are consumed, resulting in poisoning with serious damaging effect. Thus, the presence of higher concentrations of chromium and copper above the FAO/WHO permissible limits make the Tetrapleura tetraptera fruit contaminated.

RECOMMENDATIONS

Based on the findings from this research work, the researcher hereby recommends that;

i. There should be continuous monitoring of the heavy metal content of Tetrapleura tetraptera fruit.
ii. Authorities should establish a more standardized and universally accepted value for safe levels of heavy metals in medicinal plants.
iii. Anthropogenic activities that introduce heavy metals into the soil should be reduced.
iv. Steps should be taken to prevent collection and marketing of medicinal plants that are contaminated with heavy metals

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