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Screening of Some Indigenous Wild Fruits for Anti-Nutritional Factors

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Authors' contributions

This work was carried out in collaboration between all authors. This work was designed and supervised by author BAA, sampling and analysis was done by authors RLT and PO. While author BWT complied and interpreted the results. All the authors read and approved the final copy of this work as presented.

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ABSTRACT

Aim: The levels of some anti-nutritional factors of some common indigenous wild fruits were assessed in order to determine their safety, since these fruits are widely eaten by the indigenes.
Study Design: Five samples each of *Chrysophyllum albidum* (White Star Apple or Local Cherry), *Persea americana* (avocado pear) *Dinnettia tripetala* (pepper fruit), *Diallium guineense* (velvet tamarind), *Annona muricata* (Soursop) and *Citrullus lanatus* (water melon) sold in Railway market in Makurdi metropolis were collected and analysed for some anti-nutritional factors (hydrogen cyanide, phytate, tannins, alkaloids and oxalate).
Place and Duration of Study: The study was conducted at the Department of Chemistry, Benue State University Makurdi between April and May, 2014.
Methodology: The analysis was conducted using standard methods.
Results: The results of hydrogen cyanide ranged between 0.01 mg/100g, to 0.31 mg/100g Tannins level in the fruits range between 0.03 mg/100g to 65.97 mg/100g. Alkaloids contents of the fruits were general very low (0.01 mg/100g to 0.11 mg/100g). The level of phytate observed in the fruits was found to be in the range 0.04 mg/100g to 0.43 g/100g. Oxalate was found to range between

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0.01 mg/100g to 0.53 mg/100g.
Conclusion: The result indicates that all the fruits had varying contents of the anti-nutritional factors which should be removed during processing hence continuous consumption may lead to a cumulative effect which may be hazardous to heath.

Keywords: Anti-nutritional; screening; fruits; wild; analysis.

1. INTRODUCTION

Apart from the use of wild plants for medicinal purposes, firewood, craft and building materials by the early man, the fruits produced by these wild plants served as sources of food before the study of the nutritional values of some of these fruits began. Motlhanka et al. [1], reported that some wild fruits were used as offerings during wedding ceremonies by the ancient people of Botswana. Some were preserved for consumption during times of food scarcity, whereas others served as ingredients for local traditional breweries. Wild fruits have been found to be valuable sources of lipids, carbohydrates, proteins, vitamins and minerals. Other nutrients provided by fruits includes; fibre and folate. Studies have shown that eating of fruits as a source of food may reduce the risk of heart disease, including heart attack and stroke. Fruits protect the body against certain types of cancers; reduce the chances of obesity and type 2 diabetes. It lowers blood pressure, reduces the risk of kidney stones and help to decrease bone loss [2].

Despite the nutritional values and health benefits of fruits, there is dearth knowledge about the anti- nutritional factors contents of these wild fruits. Information about the anti-nutritional contents of these fruits will aid their maximum utilization as food products. Umaru et al [3], observed that the nutritional content of wild fruits is usually very high when compared with cultivated fruits, nevertheless, they are also known to contain anti-nutritional factors that can interfere with the metabolic activities of the body which in most cases predispose negatively on growth and bioavailability of nutrients. Anti-nutritional factors (ANF) can be define as 'compounds which reduce the nutrient utilization and/or food intake of plants or plant products used as human foods or animal feeds' [4]. Some of the common examples are; saponins, tannins, flavonoids, alkaloids, trypsin (protease) inhibitors, oxalates, phytates, haemagluttinins (lectins), cyanogenic glycosides, cardiac glycosides, coumarins, gossypol, etc. Anti-nutritional factors are the determining factors for the exploitation of plants as food materials for human and animal consumption. They are also known as secondary metabolites and are generated naturally in feed stuffs through normal metabolism of the plant species. Some of these factors are known to impair some metabolic activities in human and animals bodies, a situation that may result to adverse biological effects or even death.

While measures are been taken to boost food production by conventional agriculture, and through the exploitation of unconventional plants resources in solving the problem of food shortage and malnutrition, concerted efforts must be made in identifying the types of anti-nutritional factors present in these plants. Information about the type of anti-nutritional factors present in a particular food material will assist in the processing of such food material with the view of eliminating them.

The present study therefore consider the screening of some wild *Chrysophyllum albidum* (White Star Apple or Local Cherry), *Persea americana* (avocado pear) *Dinnettia tripetala* (pepper fruit), *Diallium guineense* (velvet tamarind), *Annona muricata* (Soursop), and *Citrullus lanatus* (water melon) sold in Railway market in Makurdi metropolis for some anti-nutritional factors like hydrogen cyanide, phytate, tannins, alkaloids and oxalate.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The samples were collected from the Railway market in Makurdi, Benue State and were identified by a Botanist in the Department of Biological Science, Benue State University Makurdi. The samples were washed and the pulps of the fruits removed, air dried at room temperature to constant weight and further dried at 45ºC in an air-circulating oven; ground in porcelain mortar with pestle to fine particles and were stored in screw capped plastic containers. Chemical analyses were carried out on the ground samples.

2.2 Determination of Moisture Content

5g of ground sample was weighed into a cleaned crucible of known weight. A lid was placed on the dish and transferred into an air circulated oven set at 105ºC for four hours until a constant weight was obtained. The sample was removed and placed in a dessicator to cool and weighed. Replicate determinants were made and the percentage moisture was calculated [5].

2.3 Determination of Ash Content

The crucible was preheated in a muffle furnace at 550ºC for one hour, cooled in a dessicator and weighed. 2 g of the oven dried ground sample was transferred into the crucible and weighed. The crucible and its content were placed in the muffle furnace and the temperature was allowed to rise to 550ºC. After maintaining the temperature at 550ºC for four hours, the crucible with its residue was allowed to cool to 200ºC before transferring it into the dessicator to cool to room temperature and then weighed. Replicate determinants were made and the percentage ash was calculated [6].

2.4 Determination of Crude Fibre

Exactly 2.0 g of ground sample was placed in a round bottom flask, 100 cm^3 of 0.25 $moldm^{-3}$ H_2SO_4 was added and the mixture boiled under reflux for 30 minutes. The hot solution was filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. Thereafter, it was transferred into a flask containing 100 cm^3 of hot (0.312 $moldm^{-3}$) NaOH solution. The insoluble residue was washed with hot water until it was base free. The residue was dried to constant weight at 100ºC and cooled in a dessicator and weighed. The weighed sample was incinerated in a muffle furnace at 525ºC for two hours, cooled in a dessicator and re-weighed. The percentage fibre was then calculated. Three replicate determinations were made in each case and the average determined [7].

2.5 Determination of Cyanogenic Glycosides (HCN)

The AOAC 2012 [5] modified method for the analysis of cyanogenic glycosides was adopted**.** 10.0 g ground sample was weighted into an 800 ml Kjeldahl flask onto which 200 ml of distilled water was added and allowed to stand for four hours (for autolysis to occur). The mixture was steam distilled until about 150 -170 ml of distillate was collected into a 250ml conical flash containing 20 ml of 2.5 % NaOH and diluted to 250 ml. To 100 ml of the distillate, 2ml of 6 $moldm^{-3}$ NH_4OH and 2 ml of 5 % KI was added, the mixture was titrated with 0.02 M silver nitrate ($AgNO_3$) using a micro-burette to a faint but permanent turbidity was obtained (1ml of 0.02 $moldm^{-3}$ $AgNO_3$ ≡ 1.08 mg HCN).

2.6 Determination of Phytate

The method reported by Hassan et al [8] and Aina et al [9] was adopted for phytate quantification. 4 g of powder sample were soaked in 100 cm^3 of 2% HCl V/V for three hours and filtered. To 25 cm^3 of the filtrate in a conical flask 5 cm^3 of 0.3 % ammonium thiocyanate solution and 53.5 cm^3 of distilled water were added, mixed together and titrated against standard $FeCl_3$ solution containing 0.00195 g Iron/cm^3 until a brownish yellow colour persisted for five minutes. Blank was titrated in a similar manner (1cm^3 Fe = 1.19 mg) phytin phosphorus was determine and the phytate content was calculated by multiplying by a factor of 3.55 [8,9].

2.7 Determination of Tannins Content

The tannin contents were determined using Folin Denis reagent [10]. In that method, a standard calibration curve was prepared and the Absorbance (A) against concentration of tannins at specific wave length was estimated as follows: Suitable aliquots of the tannin-containing extract (initially: 0.05, 0.2 and 0.5 mL) were pipetted in test tubes, the volume was made up to 1.00 mL with distilled water, then 2.5 mL of sodium carbonate reagent were added. Then the tubes were shaken and the absorbance was recorded at 725 nm after 40 min. The amount of total phenols was calculated as tannic acid equivalent from the standard curve.

2.8 Determination of Alkaloids

The gravimetric method was adopted for alkaloid analysis [11].

Exactly 5 g of each sample was weighed using a weighing balance and dispersed into 50 ml of 10 % acetic acid solution in ethanol. The mixture was well shaken and then allowed to stand for about 4 hours before it was filtered. The filtrate was then evaporated to one quarter of its original volume on a hot plate. Concentrated ammonium

hydroxide was added drop wise in order to precipitate the alkaloids. A pre-weighed filter paper was used to filter off the precipitate and it was then washed with 1 % ammonium hydroxide solution. The filter paper containing the precipitate was dried on an oven at 60ºC for 30 minutes, transferred into a dessicator to cool and then reweighed until a constant weight was and recorded. The weight of the alkaloid was determined by weight difference of the filter paper and expressed in mg/100g. Replicates determinates were done and the average value determined.

2.9 Determination of Oxalate

This was determined using Dye method. 2.0 g of the sample was extracted with dilute HCl, 5 ml of concentrated ammonia and precipitated with $CaCl_2$ as calcium oxalate. The precipitate was washed with 20 ml of 25% H_2SO_4 and dissolved in hot water before titrating with 0.05 M $KMnO_4$ to determine the concentration of oxalate [12].

2.10 Statistical Analysis

The one-way analysis of variance (ANOVA) was used to compare the means of the variables. Through the general linear model program SAS and the Fisher multiple comparison procedures, the least significance different (LSD) at $p<0.05$ was determined [13].

3. RESULTS AND DISCUSSION

In (Table 1), watermelon was found to have the highest moisture content (92.01 %), followed by avocado pear (78.44 %). The moisture content of pepper fruit, soursop, cherry fruit and velvet tamarind was fund to be 71.10 %, 70.73 %, 70.04 % and 12.44 % respectively. There was significant difference in the moisture contents of cherry fruit, avocado pear velvet fruit and watermelon, whereas no significant difference exist between pepper fruit and soursop fruit at $P<0.05$. Moisture content is the measure of the water content of a food material and it is essential in processing and storage of the food products. A lower percentage of the moisture content signifies longer shelf life. While higher values indicate that the material cannot be stored for a long time. Naturally dried fruits have certain advantages over those preserved by other methods because they are lighter in weight than their corresponding fresh produce and at the same time, they do not require refrigerated storage.

The crude fibre content of, cherry fruit, avocado pear, pepper fruits velvet tamarind, Soursop and watermelon (Table 1) was found to be 4.04%, 3.16%. 3.12%, 6.27%, 5.21% and 2.52% respectively. The highest value (6.27%) was observed in Soursop while the lowest value (2.52%) was recorded for watermelon. There was significant difference in crude fibre content of the fruits at $P<0.05$. The lower crude fibre content of the fruits is an indication that it contained relatively low level of organic indigestible components. Apart from reducing the risk of chronic disease such as diabetes, obesity, cardiovascular disease and diverticulitis, dietary fibre lowers the concentration of low density lipoprotein cholesterol in the blood, by binding with bile acids. It is also a known fact that fibre helps eliminate waste from the gastrointestinal tract because of its ability to bind water and thus soften the stool.

Ash content of the fruits (Table 1) was found to range between 0.52 % to 4.24 %, There was a significant difference in the ash contents of the fruits. The ash content of a food material indicate the total amount of minerals content of the food material.

The results of hydrogen cyanide (Table 1) indicate its content in cherry fruit, avocado pears, pepper fruits velvet tamarind soursop and watermelon to be 0.01 mg/100g, 0.02 mg/100g, 0.01 mg/100g, 0.31 mg/100g, 0.01 mg/100g and 0.02 mg/100g respectively There was no significant difference in the hydrogen cyanide contents of cherry and pepper fruits, whereas significant difference existed in the hydrogen cyanide contents of avocado pear, velvet tamarind, soursop and watermelon at $p<0.05$. Hydrogen cyanide of soursop was higher (Fig. 1.) than the other fruits. The result showed that all the fruits had hydrogen cyanide content below the 1.0 mg/100g permissible limits adopted by WHO for hydrogen cyanide content in food [14].

The levels of tannins (Table 1) observed in cherry fruit, avocado pears, pepper fruits velvet tamarind, soursop and watermelon was found to be 13.11 mg/100g, 0.11 mg/100g, 39.10 mg/100g, 65.97 mg/100g, 13.13 mg/100g and 0.03 mg/100g respectively. Statistical analysis of variance indicated no significant difference in the tannin contents of cherry fruit and velvet tamarind, whereas significant difference existed between avocado pears, pepper fruits soursop and watermelon at $P<0.05$. Soursop fruit was found to contain the highest value of tannin

(Fig. 2.), although the result compared favourable with the value obtained by Onyechi et al. [15] for the same plant. According to Shivprasad et al. [16], tannins are anti-nutrients factor that inhibit the activities of digestive enzymes and the implication of which is some dietary diseases.

Alkaloids contents of the fruits were generally very low (Table1). The highest value 0.1075 mg/100g observed in velvet tamarind (Fig. 1), while the lowest value of 0.0097 mg/100g was found in avocado pear. Alkaloids are a group of chemical compounds that contain basically nitrogen atoms which may be neutral or acidic in nature. The presence of alkaloid in plant prevents insects and chordate animals from eating them. Alkaloids are known to regulate plant growths. The result obtained compared favourably with those obtained by Afiukwa et al. [17].

The level of phytate observed from the fruits ranges between from 0.43g/100g to 0.04 mg/100g (Table 1). Avocado pear was observed to have the highest content of phytate (Fig. 1). There was no significant difference at $p<0.05$ in the phytate content of pepper fruit and velvet tamarind. Phosphorus in plant is store in form of phytic acid. When phytic acid is bound to a mineral in the plant tissue it is known as phytate. Higher phytate content in the body is known to bind minerals and make them unavailable to digestive enzymes. It is known to reduce the digestibility of starches, proteins and fats. Despite some of the drawback, high phytate content in the body had been known to enhance the activity of natural killer cells and inhibit tumor growth [18].

In (Table 1), the highest level of oxalate (0.53 mg/100g) was observed in Cherry fruit (Fig. 1.) followed by soursop (0.02 mg/100g). Velvet tamarind had the lowest level of oxalate (0.01 mg/100g). Though the oxalate levels in most of the fruits analysed were below toxic level, with low composition in unit of 0.01 mg/100 g can exert a minimal effect on the physiological and biochemical activities in human metabolic system as they would bind to calcium even in small composition and render calcium unavailable. This situation may lead to deficiency of calcium that could affect bone development and proper metabolic functioning, particularly if the ingested value exceeds the recommended value [19].

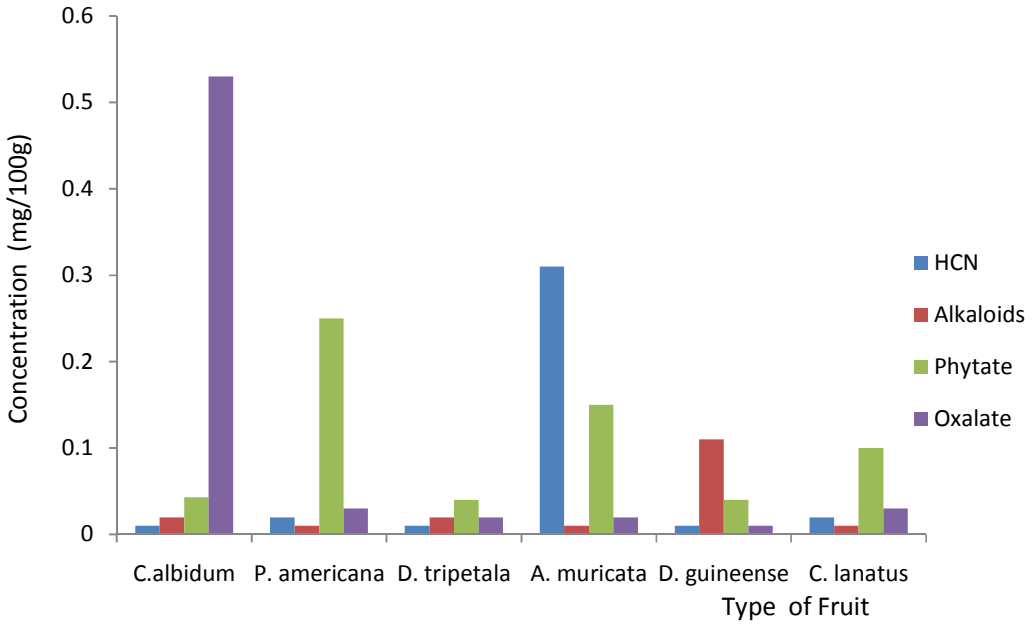

Fig. 1. Variation in hydrogen cyanide, phytate, alkaloids and oxalate contents of the fruits

Table 1. Moisture, crude fibre, ash contents and the anti-nutrition factors of the sampled fruits

	C. albidum	*P. americana*	*D. tripetala*	*A. muricata*	*D. guineense*	*C. lanatus*
Moisture (%)	70.04±0.02^{b}	78.44±0.04^{b}	71.10 ±0.10^{c}	70.73±0.03^{c}	12.44±0.04^{d}	92.01±0.00^{a}
Crude fibre (%)	4.04±0.02^{b}	3.16±0.02^{d}	3.12 ±0.02^{d}	6.27±0.01^{a}	3.21±0.01^{c}	2.52±0.02^{e}
Ash (%)	3.01±0.01^{c}	2.62±0.02^{e}	3.81 ± 0.01^{b}	4.21±0.02^{a}	2.82±0.02^{d}	0.51±0.01^{f}
HCN (mg/100g)	0.01±0.00^{d}	0.02±0.01^{b}	0.01±0.005^{d}	0.31±0.01^{a}	0.01±0.00^{e}	0.02±0.00^{c}
Tannins (mg/100g)	13.11±0.01^{c}	0.11±0.10^{d}	39.10 ±0.005^{b}	65.97±0.02^{a}	13.13±0.02^{c}	0.03±0.01^{e}
Alkaloids (mg/100g)	0.02±0.00^{d}	0.01±0.00^{f}	0.016±0.00^{e}	0.01±0.00^{e}	0.11±0.01^{a}	0.01±0.00^{d}
Phytate (mg/100g)	0.43±0.00^{a}	0.25±0.00^{b}	0.041± 0.00^{e}	0.15±0.01^{c}	0.04±0.00^{e}	0.10±0.01^{d}
Oxalate (mg/100g)	0.53±0.00^{a}	0.02±0.01^{c}	0.017 ±0.01^{c}	0.02±0.00b	0.01±0.00^{d}	0.03±0.00^{b}

(Values are mean ± standard deviation) Means in the same row with different superscripts are significantly different at ($p < 0.05$)

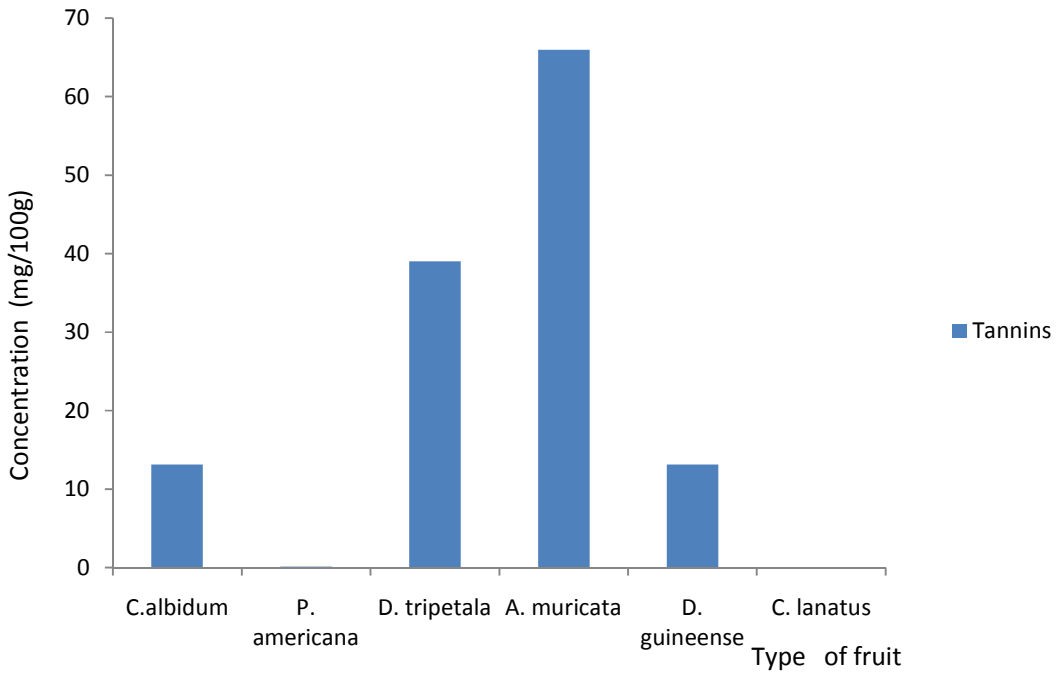

Fig. 2. Variation in tannins content of the fruits

4. CONCLUSION

The results of the study revealed that that all the fruits had varying contents of the anti-nutritional factors, though the levels were very low. However the level of tannins in the fruits was relatively high, continuous consumption may lead to a cumulative effect which may be hazardous to heath. It is therefore recommended that the raw consumption of these fruits should be discouraged; removal of some of the anti-nutritional factors through processing method like boiling should be encouraged hence continuous consumption may lead to a cumulative effect which may be hazardous to heath.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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