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Ascorbate Content and Antinutritional profile of some Wild Fruits sold in Mubi Market, Nigeria.

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ABSTRACT

Wild fruits constitute significant sources of essential nutrients to human health, but some contain antinutritional factors capable of diminishing nutrient bioavailability when present in high concentration. This study evaluates proximate content with emphasis on ascorbate and antinutrient in four wild fruits species sold in Mubi market. Fruits sample were collected and blended into fine powder. Ascorbate, macronutrient and antinutrient were determined using standard analytical techniques. Ascorbate content in *Phoenix dactylifera*, *Cyperus esculentus, Ziziphus mauritiana and Dialium indium* was 1.65mg/100g, 4.58mg/100g, 2.34mg/100g and 8.98mg/100g respectively. *Dialium indum* showed highest level of trypsin inhibitors (0.0280mg/100g), tannin (1.56 mg/100g) and cyanogenic glycosides (1.67 mg/100g), while the least trypsin inhibitors (0.0043 mg/100g), tannin (0.47 mg/100g) and cyanogenic glycosides (0.38 mg/100g) content was observed in *P. dactylifera*. Oxalate and phytate content were highest in *Z. mauritiana* (0.95 mg/100g) and *C. esculentus* (0.66 mg/100g) respectively, while *P. dactylifera* contained least (0.14mg/100g, 0.18 mg/100g). Carbohydrate content was highest in *Z. mauritiana* (67.96%) followed by *D. indum* (59.55%) and *P. dactylifera* (59.21%). Crude fibre and ash content was higher in *P. dactylifera* (14.54%) and *D. indum* (11.50%) respectively, while moisture content ranged between 9.98% - 11.46% with *Z. mauritiana* having the lowest and *D. indum* the highest moisture content. Lipid content was highest in *C. esculentus* (26.38%), followed by *D. indum* (5.73%), while values in *P. dactylifera* (2.44%) and *Z. mauritiana* (2.18%) were comparable. Crude protein was highest in *C. esculentus* (8.35%) followed by *P. dactylifera* (7.32%) and least in *D. indum* (5.25%). The wild fruits generally present good sources of carbohydrate despite serving as poor sources of vitamin C and protein. While levels of antinutrients in the fruits were comparatively lower than standard permissible limits, *Z. mauritiana* and *D. indum* may not be safe for consumption due to high content of hydrogen cyanide.

KEYWORDS: Ascorbate, antinutrient, proximate content, wild fruits.

Introduction

Wild fruits are plant resource collected outside agricultural area for the purpose of human consumption (Zade *et al*., 2016). In Nigeria, wild fruits are commonly consumed mostly by rural as well as urban dwellers, especially during the dry season when most cultivated fruits are out of season or beyond reach due to its expensive nature (Barminas *et al.*, 1998). Generally, fruits are significant sources of minerals, fibre and vitamins, which provide essential nutrients to the human health. They are one of the oldest forms of food known to man, they retain easy digestibility and exercise a cleansing effect on blood and digestive tract. Wild fruits popularly used by local communities contain appreciable amount of nutrients and energy; thus they are useful food supplement (Hegazy *et al*., 2013; Anhwange *et al*., 2015). Although wild fruits represent a minor contribution to family meals especially in urban centers, they are potentially important nutrient and cultural resources for local people around the world. They often contain higher amount of nutrients and bioactive compounds than many cultivated species, especially those that have been under cultivation for many generations (Hegazy *et al*., 2013). In view of the nutritive qualities associated with wild fruits, several works have recognized its potentials to meet household food and income security especially in rural areas (Kebu and Fessil, 2006). A few of the wild fruits sold in mubi market include *Cyperus esculentus, Dialium indum, Phoenix dactylifera* and *Ziziphus mauritiana. Cyperus esculentus*; commonly called tiger nut in English and aya in Hausa belongs to the family cyperaceaea. It is a grass-like tuber and has starch and oil as its major macronutrient. It also contain essential amino acid ie glycine which is lacking in many cereals (Olaide *et al*., 2010; Si-qun *et al*., 2016). *Dialium indum* commonly known as tamarind plum in English and Tsamiyar kurmi in Hausa belongs to the family Fabaceae. Its fruits are usually circular and black in colour with a stalk of about 6mm long and contain carbohydrate, protein, fat amidst other nutrients (Osaolube and James, 2014). *Phoenix dactylifera* is commonly called date palm in English and dabino in Hausa. It belongs to Aracaceae botanical family, which contains about 200 genera with over 3000 species. In view of its high nutritive and therapeutic value, it has been sometimes referred to as the tree of life (Sheba *et al.,* 2015). Its leaves, bark, pits, fruits and pollen have antioxidant, anticancer, hepatoprotective, neuroprotective, nephroprotection, gastrointestinal protective, antidiabetic, antihyperlipidemic, sexual improvement and antimicrobial potential attributed to phenols, flavonoids, carotenoid, vitamins, minerals, amino acids, fatty acid and organic acid (El-Far *et al.,* 2016). *Ziziphus mauritiana* belongs to the Rhamnaceae, it is a spiny evergreen shrub. Its fresh fruits contain protein, fat and carbohydrate, while the dried fruits are used as anodyne, anticancer, pectoral, refrigerant, sedative, styptic and tonic. They are known to purify blood and their roots are used in treatment of dyspepsia and fever (Palejkar *et al*., 2012)

Ascorbate is one of the most important nutritional constituent in many fruits and has momentous biological activities in human body (Naidu, 2003). More than 90% of vitamin C in human diets is supplied by fruits and vegetables, and it is usually adopted as a quality index of nutrients in food processing and storage, thus if a significant amount of vitamin C content is retained after processing, other nutrients are also likely to be preserved because ascorbic acid is extremely unstable to heat, oxygen, light, pH, moisture contents and heavy metallic ions (Cu^{2+}, Ag^{+}, Fe^{3+}), (Marques, *et al.,* 2007; Chang, *et al.,* 2006). Notwithstanding the importance of wild fruits to human health, some are known to contain antinutritional factors such as phytate, oxalate, and cyanogenic glycosides that diminish nutrient bioavailability especially when they are present in high concentration (Hassan *et al*., 2014). This study was undertaken to evaluate the proximate content with emphasis on ascorbic acid and antinutrient in four wild fruits sold in Mubi market.

Materials and Methods

Collection and processing of fruits samples

Healthy fruits samples of *Cyperus esculentus*, *Dialium indum*, *Phoenix dactylifera* and *Ziziphus mauritiana* were collected from vendors in Mubi main market in May 2016. The Samples were washed to remove dirt and dried at room temperature. Samples were pounded with mortar and pestle and then blended into fine powder with an electric blender (Binatone model BLG-400), and sieved with 1 mm size sieve (Umaru *et al*., 2007).

Determination of Ascorbate

100g of each powdered fruit sample was dissolved in 50ml of distilled water. The mixture was stirred thoroughly and then strained through cheesecloth. The pulp was rinsed with 10ml distilled water and all the filtrate was collected in a volumetric flask and the extracted solution made up to 100 ml with distilled water. Ascorbic acid was determined by titrimetric method (Onwuka, 2005).

Macronutrient analysis

Moisture, ash, crude fibers, crude protein, lipid and carbohydrate content were determined in accordance with standard methods of AOAC (2000) and as described by Hegazy *et al*. (2013). Briefly, moisture content was determined by drying the fruits at 105°C in an oven (Genlab-210 UK) until constant weight was obtained. Ash content was determined by incineration in muffle furnace (Vecstar Furnaces-USA) at temperature 450±10°C. Fiber estimate was obtained from the loss in weight on ignition of dried residue following digestion of fat free samples with 1.25% each of sulfuric acid and sodium hydroxide solutions. Crude protein content (N × 5.25) was estimated by the macro Kjeldahl (Markham 230 Foss USA) nitrogen assay. The lipid content was determined by petroleum ether (60-80^{o}C) in a soxhelt apparatus (Markman 230 Foss USA). Total carbohydrate was determined by; 100 – (% moisture + % ash + % total lipid + % protein + % crude fibre).

Determination of Antinutrients

Oxalate content of the fruits samples was determined as described by Anhwange *et al.* (2015). Briefly, 2.0g of the sample was extracted with diluted HCl and 5ml 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 0.05M $KMnO_4$ to determine the concentration of oxalate.

Phytate content was determined by soaking 4g of sample in 100 ml of 2% HCl for three hours and filtered. To 25ml of the filtrate in a conical flask, 5ml of 0.3 % ammonium thiocyanate solution and 53.5ml of distilled water was added, mixed together and titrated against standard $FeCl_3$ solution containing 0.00195g Iron/cm^3 until a brownish yellow colour persisted for five minutes. Blank was titrated in a similar manner (1ml Fe = 1.19 mg), phytin phosphorus was determine and phytate content calculated by multiplying with the factor of 3.55 (Anhwange *et al.,* 2015; Hassan *et al*., 2011).

Cyanogenic glycoside was determined by dispensing 10g of sample into 800ml Kjeldahl flask (Markham 230 Foss USA), and 200ml 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 flask containing 20 ml of 2.5 % NaOH, and diluted to 250 ml. To 100 ml of the distillate, 2ml of 6 mol/dm^3 NH_4OH and 2 ml of 5 % KI was added, the mixture was titrated with 0.02M silver nitrate ($AgNO_3$) using a micro-burette to a faint but permanent turbidity (1ml of 0.02 mol/dm^3 $AgNO_3 \equiv 1.08$ mg HCN) (Anhwange *et al.,* 2015).

Tannin content in the fruits was determined using Folin Denis reagent. 1ml of ethanolic extract of fruit sample was treated with 5 ml Folin Dennis reagent in a basic medium (2.5 ml of sodium carbonate) and allowed to stand for about 40 minutes. The absorbance was recorded at 725nm with a spectrophotometer (Jenway 6405-UK). The amount of total phenols was calculated as tannic acid equivalent from the standard curve (Anhwange *et al.,* 2015).

Trypsin inhibitor was determined by dissolving 1g of fruit sample in 50 ml of 0.5 M NaCl solution. The mixture was stirred for 30 minutes at room temperature and centrifuged at 1500 rpm for 5 min. The supernatant was filtered and filtrate used for further assay. Two ml of standard trypsin solution was added to 10ml of the filtrate. Absorbance of the mixture was taken at 410 nm (Jenway 6405-UK) using 10ml of the same filtrate as blank (Prokopet and Unlenbruck, 2002).

Results

Results obtained from the evaluation of ascorbate content in 100g each of *P. dactylifera, C. eculentus, Z. mauritiana and D. indum* was 1.65mg, 4.58mg, 2.34mg and 8.98mg respectively. Antinutrient analysis of 100g each, for all the fruits revealed tannin as comparatively higher (Table 1). *Dialium indum* showed the highest level of trypsin inhibitors (0.0280mg/100g), tannin (1.56mg/100g) and cyanogenic glycosides (1.67mg/100g), while *P. dactylifera* revealed the least of trypsin inhibitors (0.0043mg/100g), tannin (0.47mg/100g) and cyanogenic glycosides (0.38mg/100g). Oxalate and phytate content were highest in *Z. mauritiana* (0.95mg/100g) and *C. esculentus* (0.66mg/100g) respectively, while *P. dactylifera* contained least (0.14mg/100g, 0.18mg/100g) (Table 1).

Table 1: Ascorbic acid content and antinutritional factors of sampled fruits (mg/100g)

Parameters	*Phoenix dactylifera*	*Cyperus esculentus*	*Ziziphus mauritiana*	*Dialium indum*
Ascorbate	1.65	4.58	2.34	8.98
Trypsin inhibitor	0.0043	0.0064	0.0160	0.0280
Tanins	0.47	1.43	3.62	1.56
Cyanogenic glycocides	0.38	0.49	1.08	1.67
Oxalates	0.14	0.84	0.95	0.65
Phytates	0.18	0.66	0.23	0.39

Results of proximate analyses revealed that fruits of *Z. mauritiana* (67.96%) had the highest carbohydrate content followed by *D. indum* (59.55%) and *P. dactylifera* (59.21%) (Table 2). Crude fibre and ash content were significantly higher in *P. dactylifera* (14.54%) and *D. indum* (11.50%) respectively, while moisture content ranged between 9.98% - 11.46% with *Z. mauritiana* having the lowest moisture and *D. indum*, highest (Table 2). The highest content of lipid was observed in *C. esculentus* (26.38%), followed by *D. indum* (5.73%), while values in *P. dactylifera* (2.44%) and *Z. mauritiana* (2.18%) were comparable. Crude protein was highest in *C. esculentus* (8.35%) followed by *P. dactylifera* (7.32%) and least in *D. indum* (5.25%) (Table 2)

Table 2: Macronutrients in sampled fruits (%)

Parameter	*Phoenix dactylifera*	*Cyperus esculentus*	*Ziziphus mauritiana*	*Dialium indum*
Crude protein	7.32	8.35	6.77	5.25
Lipids	2.44	26.38	2.18	5.73
Crude fibre	14.54	5.13	8.35	6.53
Ash	6.35	1.45	4.68	11.50
Moisture	10.15	10.35	9.98	11.46
Carbohydrate	59.21	48.40	67.96	59.55

Discussion

Although wild fruits contribute significantly to healthy dietary requirements of humans most especially within rural areas, due to cheap and rich source of vitamins, mineral and antioxidant like vitamin C, the presence of antinutrient such as phytate, cyanogenic glycosides, oxalate, tannin and trypsin inhibitor, beyond permissible concentrations could affect bioavailability of some of these nutrients thereby leading to malnutrition. This study reports presence of appreciable quantity of ascorbates in each 100g sample of wild fruits analysed as well as antinutritional factor that are below permissible limits except for cyanogenic glycosides in *D. indum* and *Z. mauritiana* where the content is beyond the WHO permissible limits per 100g of the sample (Anhwange *et al*., 2015).

Ascorbate is a dietary antioxidant required as a co-factor for many enzymes. The importance of antioxidants in health and disease has long been recognized in medicine and biological sciences (Oliveira *et al*., 2009). While this study reports a range of 1.65g/100g - 8.98mg/100g ascorbic acid content in all the wild fruits, values as high as 30 mg/100g have been reported for guava, 112 mg/100g for pawpaw (*Caricapapaya*), 43mg/100g for orange and 179.8 mg/100g for cashew nuts (Oyenuga, 1968). Similar studies have also reported high vitamin C content in *Chrysophyllum albidum, Spondias mombin, Irvingia gabonensis, Cola millenii, Adansonia digitata, Sclerocarya birrea* and *Treculia Africana* (Eromosele *et al*.,1991; Olayiwola *et al*., 2013; Edet *et al*. 1984). Albeit the low ascorbate content observed in the wild fruits, other nutrient could still be present in substantial amount owing to the fact that ascorbic acid is extremely unstable to heat, oxygen, light, pH, moisture contents and heavy metallic ions (Cu^{2+},

Ag^{+}, Fe^{3+}) and its presence indicate that other nutrients are also likely to be preserved (Marques, *et. al.,* 2007; Chang, *et. al.,* 2006). In view of the low vitamin C content observed in this study, consumption of these fruits should be supplemented with other sources of vitamin C.

Trypsin inhibitors are proteins that are found in some raw foods and have long been known to cause diminished growth in rats, chickens and other experimental animals The presence of trypsin inhibitors in foods can lead to the formation of irreversible trypsin and trypsin inhibitor complexes, that can cause a drop in intestine trypsin and chemotrypsin known to play vital role in protein digestibility in animal, thus leading to retarded growth (Bolhuis, 1954; Ogbe and Obeka, 2013). The trypsin inhibitor complex in this study was found to be within the range of 0.0043mg/g - 0.0280mg/g. This value is very low compared to 1.857 – 3.476 µ/mg reported by a study on three mushroom varieties (Oly-Alawuba and Obiakor-Okeke, 2014), indicating that the wild fruits might not pose trypsin associated health risk, considering the lethal dose of 2.50g/kg reported by Rathod and Valvi (2011).

The highest tannin content was observed in *Z. mauritiana* (3.63mg/100g) which is comparably higher than an earlier reported level of 2.42% for *Z. mauritiana*, however, tannin content of other wild fruits in this study (Table1) are comparatively lower (Umaru *et al*., 2007). A similar studies reported high of level tannin within a range of 13.11mg/100g – 65.97mg/100g in *C. albidum, D. tripetala*, *D. guineense* and *A. muricata*, as well as low level for *P. Americana* (0.11mg/100g) *and C. lanatus* (0.03mg/100g) (Anhwange *et al*., 2015). Although tannin contents for fruits analysed in this study are low to be of any nutritional importance, except in *Z. mauritiana* (Table 1), tannin in fruits imposes astringent taste that affects palatability, reduce food intake and consequently body growth. It also binds to both exogenous and endogenous proteins including enzymes of the digestive tract, thereby affecting the utilization of protein (Umaru *et al*., 2007). Pharmacologically, polyphenol to which tannin belongs possess antioxidant activity, thus preventing oxidative stress that causes coronary heart disease, some types of cancer and inflammation (Tapiero *et al*., 2002; Bello *et al*., 2008). This implies that fruit like *Z. mauritiana* is likely to have antioxidant activity.

Cyanogenic glycosides are present in a number of food plants and seeds, and Hydrogen cyanide is usually released when fresh plant materials are macerated (chewed). Although cyanide is a normal constituent of the blood, it is usually at low concentrations of less than 12µmol (Osagie *et al*., 1996). At high concentration, it is a potent inhibitor of respiratory chain, inhibiting the oxidative processes of cells causing them to die very quickly. It can precipitate dysfunction of central nervous system, respiratory failure and cardiac arrest. Because the body rapidly detoxifies cyanide, an adult human can withstand 50-60 ppm for an hour without serious consequences. However, exposure to concentrations of 200-500 ppm for 30 minutes is usually fatal (Lary and Toumi, 2004). Besides death, acute cyanide toxicity at small doses can cause headache, tightness in throat and chest, and muscle weakness. The cyanide content in *P. dactylifera, C. esculentus, Z. mauritiana* and *D. indum* was 0.38 mg/100g, 0.49 mg/100g, 1.08 mg/100g and 1.67 mg/100g respectively. While a similar study on wild fruits reported hydrogen cyanide level 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, results from this study showed that cyanide content for *Z. mauritiana* (1.08 mg/100g) and *D. indum* (1.67 mg/100g) are above the 1.0 mg/100g permissible limits adopted by WHO for hydrogen cyanide content in food (Emurotu *et al*., 2012). This implies that *Z. mauritiana* and *D. indum* may not be fit for human consumption especially in large amount.

Oxalate is an antinutrient that binds to calcium and other minerals, making them insoluble thus decreasing their bioavailability. Ingestion of foods containing high concentrations of oxalates may cause decreased bone growth, kidney stones, renal toxicity, vomiting, diarrhea, convulsions, coma and impaired blood clotting (Jones, 1995). The significant role oxalate plays in kidney stone development is exemplified by the fact that approximately 65% of kidney stones consist of calcium oxalate (Finkelstein *et al*., 2006). It has been estimated that ingestion of approximately 22 g of oxalic acid could be lethal to a 59 kg human (Lowry, nd). Oxalate content in wide fruits studied was found to be within 0.14 – 0.95mg/100g. These values are lower than acceptable limit given by WHO (105mg/100g) indicating that utilization of the fruits do not have any negative impact that is connected with abundance of oxalate (Oly-Alawuba and Obiakor-Okeke, 2014)

Phytate is an effective chelator of divalent cations such as zinc, copper, iron, magnesium and calcium and these complexes are insoluble in the intestinal tract, thus reducing mineral bioavailability (Jones, 1995). It inhibit digestive enzymes such as trypsin, pepsin, α-amylase and ß-glucosidase, therefore, ingestion of foods containing high amounts of phytate could literally cause mineral deficiencies or decreased protein and starch digestibility (Oly-Alawuba and Obiakor-Okeke, 2014). Since phytate-rich foods are digested at slower rate and produce lower blood glucose responses than foods that do not contain phytate, it has been hypothesized that phytate could have a therapeutic role in management of diabetes and it utility as an antioxidant have been highlighted (Deshpande, 2002, Kumar *et al*., 2010). However, because the beneficial effects of phytate are outweighed by its ability to cause essential mineral deficiencies, consumption of diet containing high amounts of phytate is not recommended. Phytate content for *P. dactylifera*, *C. esculentus*, *Z. mauritiana* and *D. indum* in this study was 0.18mg/100g, 0.16mg/100g, 0.23mg/100g, and 0.39mg/100g respectively. Although values obtained for *P. dactylifera* and *Z. mauritiana* in this study does not compare favourably to values for *P. dactylifera* (0.52mg/100g) *Z. mauritiana* (1.57mg/100g) obtained in previous study (Umaru *et al*., 2007), they are lower than the safe limit (22.1mg/100g) (WHO, 2013).

Crude protein serves as enzymatic catalyst, mediate cell responses, control growth and cell differentiation. Crude protein content observed for all the wild fruits (Table 2) was comparatively higher than values reported for *Arbutus pavarii* (2.23%), *Nitraria retusa* (1.51%), and *Ficus palmate* (2.17%) (Hezgazy *et al*., 2013). Although fruits are usually not considered as excellent protein sources, Plant foods that provide more than 12% of their calorific value from protein are a good source of protein (Aberoumand, 2011). In this context, the wild fruits studied are not good sources of protein.

Fibre helps in the maintenance of human health and has been known to reduce cholesterol level in the body, aids in diarrhoea treatment and detoxification of poisonous metals (Adepoju, 2009; Aberoumand, 2011). Fibre content for *P. dactylifera* (14.54%) was higher than values for *C. esculentus, Z. mauritiana* and *D. indum* (Table 2). These values were more than that reported for *S. mombin* (4.2g/100g), *D. guineense* (0.6/100g) but comparable to *M. whytii* (11.8g/100g) (Adepoju, 2009). *Cyperus esculentus* (1.45%) was lowest in ash content; an indication of low mineral value, especially the macro-minerals. *Phoenix dactylifera* (6.35%) and *Z. mauritiana* (4.68%) had moderately high value of ash while *D. indum* (11.58%) had the highest value which is also indicative of corresponding high mineral content. These values are similar to reported values of ash content for some wild fruits in Nigeria (Oluyemi *et al*., 2006; Bello *et al*., 2008). Moisture content for the species of fruits studied ranges between 11.46% - 9.98%. Low values of moisture content is usually indicative of dry matter content and possible long shelf life (Adepoju, 2009)

Carbohydrate is an essential source of energy for the body to perform its normal functions. A diet that does not contain carbohydrate can lead to muscle breakdown, ketosis and dehydration (Neeru *et al.*, 2015). The fruits examined in this study revealed high carbohydrate content (Tables 2); comparatively higher than some best sources of carbohydrate in some commercially available edible fruits (banana – 27.7%, apple – 13.4%, and fresh date – 33.8%) (Hegazy *et al*., 2013). This implies that the fruits could serve as good sources of carbohydrate.

Conclusion

The study revealed varying levels of antinutritional factors and proximate contents in wild fruits analyzed. Although levels of antinutrients in the fruits were relatively lower than standard permissible limits, *Z. mauritiana* and *D. indum* may not be safe for consumption due to high content of hydrogen cyanide. The fruits generally represent good sources of carbohydrate despite serving as poor sources of vitamin C and protein. Therefore its consumption should be supplemented with other known sources of vitamin C and protein.

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