



Phytochemical Composition and Nutritive Value of Unripe and Ripe *Dennettia tripetala* (DT) Fruits

Ekam I. Akpakpan^{1*}, Eugene N. Onyeike¹ and Charity. U. Ogunka-Nnoka¹

¹*Department of Biochemistry, University of Port Harcourt, East West Road, P.M.B 5323, Port Harcourt, Rivers State, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. Author EIA designed, carried out the study, managed the literature searches and performed the statistical analysis. Author ENO wrote the protocol, edited and supervised the study. Author CUON managed the analyses, edited and supervised the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2019/v10i330036

Editor(s):

- (1) Dr. Rasha Mousa Ahmed Mousa, Associate Professor, Department of Biochemistry, University of Jeddah, Saudi Arabia.
- (2) Dr. R. C. Ranveer, Department of Meat, Poultry and Fish, PG Institute of Post – Harvest Management, Killa – Roha, BSKKV, Dapoli, India.
- (3) Dr. Amjad Iqbal, Assistant Professor, Department of Agriculture, Abdul Wali Khan University Mardan, Pakistan.

Reviewers:

- (1) Charles Emeka Umenwa, University of Ibadan, Nigeria.
- (2) Liu Changjin, Tianjin University of Science and Technology, China.
- (3) Akinyemi Bosede Kemi, Benue State University, Nigeria.

Complete Peer review History: <http://www.sdiarticle3.com/review-history/48885>

Original Research Article

Received 14 March 2019
Accepted 30 May 2019
Published 03 June 2019

ABSTRACT

Phytochemical composition and nutritive value of unripe and ripe pepper fruits (*Dennettia tripetala*-DT) were investigated. Phytochemical, proximate, minerals and vitamin composition were carried out using standard methods. The unripe fruit of DT had significant ($P<0.05$) higher concentrations of terpenes, phytosterol, steroid, oxalate, saponin, alkaloid and phytate when compared to the ripe fruit; while tannins, phenol, flavonoid, coumarin, cardiac and cyanogenic glycosides were higher in the ripe fruit compared to the unripe fruit. The ash and lipid content of DT fruits were significantly ($p< 0.05$) higher in the ripe DT fruit than unripe DT fruit; while the carbohydrate and the crude fibre content were significantly ($p<0.05$) higher in the unripe than the ripe DT fruit. The energy value (kcal/100 g sample) calculated from Atwater factors of 4, 9, 4 for carbohydrates, lipids and proteins was 347.2 for unripe and 331.4 for ripe DT fruits. Potassium composition of DT fruits were significantly ($p<0.05$) higher in the ripe fruit than the unripe fruit, while zinc was higher in the unripe fruit than the ripe fruit. The concentration of Na, Se, Pb, Ca, Fe and Cu did not differ significantly

*Corresponding author: Email: akpakpan85@yahoo.com;

($p < 0.05$) between the two fruits. DT fruits possessed a significantly ($p < 0.05$) higher concentrations of vitamin A, B1, B2, B3, B5, B7, B9, B12 and C than the ripe fruit; while the unripe fruits showed a significantly higher concentrations of Vitamin B6 and K than the ripe fruit. Therefore, unripe and ripe DT fruits may be consumed as potential sources of nutrients and phytochemicals which may be significant as dietary supplements.

Keywords: Phytochemical and proximate composition; mineral; vitamins; ripening *Dennettia tripetala*.

1. INTRODUCTION

Fruits, leaves, seeds and roots of plants contain diverse principles that are required for normal functioning of cells. Little wonder the upsurge of interest in their utilization in medicine as nutraceuticals and pharmaceutical agents for the prevention and treatment of diseases. Phytochemicals are the non-nutritive principles or the bioactive components of plants which are responsible for their biological activity. Vitamins and minerals are regarded as important micronutrients, which are required in the body in minute amount for performance of metabolic functions. Although minerals yield no energy, they are necessary for the maintenance of certain physicochemical processes, which are essential to life [1,2]. Such roles include: serving as cofactors in metabolic reactions, muscle contraction, nervous transmission, antioxidant functions etc. Fruits and vegetables are known as rich reservoir of micronutrients, as such, it is recommended that the consumption of fruits and vegetables, as well as grains, is strongly associated with reduced risk of cardiovascular disease, cancer, diabetes, Alzheimer disease, cataracts, and age-related functional decline [3,4,5].

Of particular interest are the neurons and other brain cells which require nutrients to build and maintain their structure and function in order to

prevent premature aging [6]. Negative consequences are observed when brain is deprived of nutrients. These include loss of cognition, dementia, cretinism etc.; For example, the deficiency of copper could be linked to Alzheimer's disease, severe cerebral dysfunction during pregnancy leading to cretinism [7,8,9]. Brain disorders in old age may be due to failure of protective mechanisms and dietary deficiencies such as deficiency of antioxidants and nutrients, which are effective against free radicals. Phytochemicals have been linked to reductions in the risk of chronic disease. For example, flavonoids and terpenes play functional roles as antioxidant thereby having the ability to stabilize free radicals, rendering them unavailable to perpetuate neuronal damage. Vitamins such as B₆ and B₉ are involved in neurotransmitter synthesis while minerals such as magnesium and iron are important in cognition, oxidation-reduction reaction and ionic regulation respectively [6].

Dennettia tripetala (pepper fruit) which belongs to the family of Annonaceae is one of the major fruit tree grown in Cameroun, Ivory Coast and Southern Nigeria [10]. *Dennettia tripetala* fruits are obtained within the period of March and May yearly. Like most fruits, DT fruits are green when unripe and red when ripe. The root, leaves, and fruits possess strong pungent and spicy taste [11], thus, their usage as spice in making dishes.



Fig. 1. (A) *Dennettia tripetala* tree with leaves and unripe fruits. (B) Ripe (red) and unripe (green) *Dennettia tripetala* fruit [2]

It is documented in literature that DT fruit contains important nutrients such as vitamins, minerals, carbohydrate and fibre [12,11]; as well as also phytochemicals such as flavonoids, tannins and cyanogenic glycosides [13,14]. These phytochemicals give the fruits their biological usefulness as anticancer, anti-diabetic, and anti-glaucoma effects.

The various principles (fat and water soluble vitamins, minerals, phytochemicals) of this important fruit has not been fully documented. The present study is a part of an elaborate ongoing study, which tends to expand as well as confirm existing literature on the functional roles of nutritive and phytochemical composition of both ripe and unripe DT fruits.

2. MATERIALS AND METHODS

2.1 Collection/preparation of Plant Sample

The fruits of *Denettia tripetala* (both unripe and ripe) were obtained from Marian Market, Calabar, Cross River State. The samples were authenticated by a Botanist in the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State, Nigeria.

2.2 Experimental Design

Denettia tripetala fruits (both unripe and ripe) were ground and dried under room temperature and approximately 500 g was weighed using electronic weighing balance and used for phytochemical, proximate, vitamins and mineral analysis using standard analytical methods.

2.3 Quantitative Phytochemical Analysis Using Gas Chromatography (GC-5890, Series 11)

Two grams each of unripe and ripe DT samples were weighed into a 1000 ml separatory funnel. A 50 ml volume of 50% methylene chloride was added to a sample bottle, sealed, and shaken for 30 s to rinse the inner surface. The resultant solvent was transferred to the separatory funnel and samples were extracted by shaking the funnel for 2 min with periodic venting to release excess pressure. The organic layer was allowed to separate from the water phase for a minimum of 10 min, and the methylene chloride extract was collected in 250ml flask. A second and third extraction was prepared in the same manner. Finally, the combined extract was poured through a drying column packed with cotton wool and 0.1M anhydrous sodium sulphate and silica; it

was concentrated by boiling with nitrogen gas to 1.0 ml. The remaining extract was mixed with 1.0ml of the solvent and injected into a Flame Ionization Detector GC for analysis.

2.3.1 Quantification using a flame ionization detector

The injector temperature was set to 280°C with splitless injection of 2µl of sample and a linear velocity of 30 cms⁻¹, the carrier gas used was Helium 5.0 psi with a flow rate of 40 ml min⁻¹. The oven operated from a temperature of 200°C until it heated to 330°C at a rate of 3°C min⁻¹. This temperature was maintained for 5 min and the detector operated at a temperature of 320°C. The concentration of the different phytochemicals were expressed in mg/100 g.

2.4 Proximate Analysis

2.4.1 Determination of ash content [15]

An empty crucible was ignited in a muffle furnace for 1 min and allowed to cool in a desiccator containing silica gel. A 5 g weight of the ripe and unripe DT fruit was put in the preheated dish and the weight of the porcelain dish and the samples were noted. The dish was heated with a Bunsen burner in a fume cupboard until smoking ceased and later transferred into a muffle furnace at 550°C for 18 hr to burn off all organic matter. After ashing, the crucible was removed from the furnace and placed in a desiccator to cool at room temperature (29 ± 1°C) and weighed. The procedure was done in triplicates and the percentage ash content of the sample was calculated thus;

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100 = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

where

W_1 = weight of empty crucible; W_2 = weight of crucible + sample before ashing

W_3 = weight of crucible + sample after ashing

2.4.2 Determination of crude fat content [15]

A 5g weight of the sample was put into a thimble and was extracted with petroleum ether (b.p. 40-60°C) until it refluxed using the Soxhlet extraction method. The fats were exhaustively extracted using petroleum ether (b.p 40-60°C) for 6hr. The sample in the thimble was removed and dried at 105°C - 110°C for 1hr, cooled in a desiccator and weighed. The procedure was done in triplicates and the % crude fat was calculated as follows;

$$\% \text{ Crude fat} = \frac{\text{Weight of sample (extracted fat)}}{\text{Weight of sample}} \times 100 = \frac{W_2 - W_1}{W} \times 100$$

Where

W_1 = weight of empty thimble; W_2 = weight of thimble + sample; W = weight of sample used.

2.4.3 Determination of crude fibre content [15]

A 2 g weight of the defatted sample was put in a conical flask and 200 ml of 1.25% boiling tetraoxosulphate (VI) acid was added within a minute. The content of the flask was filtered through a buchner funnel prepared with wet 12.5 cm filter paper. The sample was washed back into the original flask with 200 ml of 1.25% NaOH, and boiled for 30 min. All insoluble matter was transferred to the crucible and washed with boiling water until the sample was free from acid. The sample was again heated in a muffle furnace at 550°C for one hour. The crucible was then cooled in a desiccator and reweighed.

$$\% \text{ Crude fibre} = \frac{W_2 - W_1}{W} \times 100$$

Where

W = weight of sample; W_1 = weight of crucible+ sample; W_2 = weight of crucible+ filter paper after ashing.

2.4.4 Determination of crude protein content [15]

A 1 g weight of the sample was transferred into Kjhedahl flask. A few chips of antibumping granules and 4g of digestion catalyst made up of 20 ml of conc. Tetraoxosulphate (VI) acid were added with a retort stand on an electrothermal heater. The flask was gently heated for frothing to occur and subside, and then heat was increased to 250°C. The complete sample digestion was done in 5 hours. The digest was cooled to room temperature and diluted to 100ml with distilled water. A 20ml aliquot of the digest was transferred into a round-bottomed flask for distillation. This flask was connected to a Liebig condenser through a monoarm steel head (Adaptor). The Liebig condenser was connected to a receiving flask through a receiver adapter and 10ml of 2% boric acid and two drops of double indicator were pipetted into the distillation flask. Then, 30 ml of 40% sodium hydroxide was injected into the distillation flask through a cork with the aid of a syringe. The flask was heated for 10 min to digest the content. The distillate was collected in the boric acid and then titrated with 0.1M HCL. The volume of HCl added was

recorded as the titre value. The % Crude protein was calculated thus;

$$\% \text{ Nitrogen} = (\text{Titre value} \times 1.4 \times 100 \times 10/1000 \times \text{wt of sample} \times \text{aliquot of digest})$$

$$\% \text{ Crude protein} = \% \text{ Nitrogen} \times 6.25$$

2.4.5 Determination of total carbohydrate content [16]

The total carbohydrate content of ripe and unripe DT fruit was determined by the difference method according to the formula below.

$$\text{Total Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ crude fat} + \% \text{ ash} + \% \text{ crude protein} + \% \text{ crude fibre}).$$

2.4.6 Determination of the energy content of fruits [17]

The energy content of the fruits were calculated by multiplying the mean values for crude protein, crude fat and total carbohydrate by the Atwater factors of 4, 9 and 4 respectively, taking the sum of the products and expressing the result in Kcal per 100 g sample.

2.5 Determination of Mineral Content of the Fruit

The fruit samples were digested by weighing 2 g of each of the samples into 250 ml digestion flask; then adding 40 ml of aqua regia (HCL and HNO₃, ratio of 3:1) at 130°C using electric hotplate for 30 min, filtered and the filtrate was made up to 100 ml [18]. Standard solutions of the metal to be analysed were prepared. The Atomic Absorption Spectrophotometer (AAS) (Model: Varian Spectra 100, Australia) was set with power on for ten min. The standard metal solutions were injected to calibrate the AAS using acetylene gas. An aliquot of the digest solutions were injected and the concentrations were digitally displayed by the AAS.

2.6 Determination of Water-soluble Vitamins

2.6.1 Vitamin B₁ (thiamine hydrochloride)

Five millilitres each of the standard and that of sample was taken in marked test tubes. In each

test tube, 5 ml 0.1M NH₄OH and 0.5 ml 0.1M 4-Amino phenol solution was added and mixed well, then kept for 5min and 10ml of Chloroform was added and the chloroform layer separated. The absorbance of chloroform layer was measured in a spectrophotometer at 430nm against blank.

Calculation

Concentration of vitamin B₁ in sample(mg/g) = (Absorbance of sample x Concentration of sample/ Absorbance of standard)

2.6.2 Vitamin B₂ (riboflavin)

Five millilitres of the standard and sample solution were taken in marked test tubes. In each test tube, 2 ml of 1M hydrochloric acid, 2 ml glacial acetic acid, 2ml hydrogen peroxide, 2ml of 15% w/v potassium permanganate and 2ml phosphate buffer (pH 6.8) were added and mixed well and absorbance read at 444nm against blank.

Calculation

Concentration of vitamin B₂ in sample(mg/g) = (Absorbance of sample x Concentration of sample/ Absorbance of standard)

2.6.3 Vitamin B₃ (nicotinamide)

Two millilitres of the standard, sample and blank solution were taken in marked test tubes. In each test tube, 5 ml sulphanic buffer (pH 4.5), 5 ml distilled water and 2 ml 10% w/v cyanogen bromide solution were added and mixed well and absorbance was read at 450 nm against blank and recorded at interval of 2 min.

Calculation

Concentration of vitamin B₃ in sample(mg/g) = (Absorbance of sample x Concentration of sample/ Absorbance of standard)

2.6.4 Vitamin B₅ (pantothenic acid)

Five milliliters of sample solution was taken into 50 ml volumetric flask. In each volumetric flask, 2 ml of 1M hydrochloric acid was added and mixed well, then heated for 5 hr at 69°C ± 1°C and cooled at room temperature. Then, 2 ml 7.5% hydroxylamine reagent (in 0.1M sodium hydroxide), 5 ml of 1 M sodium hydroxide and kept for five min. The pH was adjusted to 2.7 ± 0.1 with hydrochloric acid and the volume was made up with water. Then, 5 ml of the standard

and hydrolysed sample solution was taken in marked test tubes. In each test tube, 1 ml of 1% ferric chloride solution was added and mixed well and absorbance measured at 500 nm against blank.

Calculation

Concentration of vitamin B₅ in sample(mg/g) = (Absorbance of sample x Concentration of sample/ Absorbance of standard)

2.6.5 Vitamin B₆ (pyridoxine hydrochloride)

Two millilitres of the standard and sample solution were taken in marked test tubes. In each test tube, 1 ml of ammonium buffer, 1 ml of 20% sodium acetate solution, 1 ml of 5% boric acid solution and 1 ml 1M dye (2, 6-di-chloroquinine chorimide) solution were added and mixed well. Absorbance was read in a spectrophotometer at 650nm against the blank.

Calculation

Concentration of vitamin B₆ in sample(mg/g) = (Absorbance of sample x Concentration of sample/ Absorbance of standard)

2.6.6 Vitamin B₇ (biotin)

500 microgram of sample was weighed into a 100 ml volumetric flask and 10 ml of dimethyl sulfoxide was added to dissolve. The flask was submerged in a water bath and heated at 60° to 70°C for 5 min. The volume was made up to mark with dilute water. It was filtered and absorbance read at 294 nm against blank.

Calculation

Concentration of vitamin B₇ in sample(mg/g) = (Absorbance of sample x Concentration of sample/ Absorbance of standard)

2.6.7 Vitamin B₉ (Folic acid)

Two millilitres of the standard and sample solution were taken in marked test tubes. In each test tube, 2 ml of 0.02% potassium permanganate solution, 2 ml of 2% sodium nitrate solution, 2 ml of 4M hydrochloric acid solution, 1 ml of 5% ammonium sulphate solution and 1 ml of dye solution (0.1% N, N diethyl aniline dye solution in iso-propyl alcohol) were added and mixed well, then kept for 15 min at room temperature. Absorbance was read at 535 nm against the blank.

Calculation

Concentration of vitamin B₉ in sample(mg/g) = (Absorbance of sample x Concentration of sample/ Absorbance of standard)

2.6.8 Vitamin B₁₂ (cyanocobalamin)

One microgram of sample was weighed into 25 ml volumetric flask and 10 ml of water was added to dissolve. Then, 1.25 g of dibasic sodium phosphate, 1.1 m of anhydrous citric acid and 1.0 gm of sodium metabisulphate was added. The volume was made up to the mark with water. The solution was autoclaved at 121 °C for 10 min. It was then filtered and absorbance read at 530nm against the blank.

Calculation

Concentration of vitamin B₁₂ in sample(mg/g) = (Absorbance of sample x Concentration of sample/ Absorbance of standard)

2.6.9 Vitamin C (ascorbic acid)

Two millilitres each of the standard, sample and blank solution were taken in 25 ml volumetric flask. In each flask, 2 ml 10% v/v sulphuric acid and 5 ml 10% v/v ammonium molybdate were added and mixed well, then kept for 50 min at room temperature. It was diluted to 25 ml with distilled water and absorbance read at 450 nm against the blank.

Calculation

Concentration of sample(mg/g) = Absorbance of sample x Concentration of sample/ Absorbance of standard

2.7 Determination of fat Soluble Vitamins

Fat and water soluble vitamins was done as described by [19].

2.7.1 Vitamin A (retinol)

A 500IU sample was weighed into round bottom flask. Then, 2 ml 50% w/v potassium hydroxide solution, 10 ml glycerol and 50 ml methanol were added and mixed well, then refluxed for 45 min on boiling water bath and cooled. The flask was washed with distilled water and taken into the separator then extracted with 4x25 ml diethyl ether and washed with water. The water layer was discarded then the ether layer was taken into dry 100 ml volumetric flask by passing it through anhydrous sodium and made up to 100

ml with diethyl ether, mixed well. Absorbance was read at 325 nm against the blank.

Amount of vitamin (IU) = Sample Absorbance x factor (1830) x (1000/100) x (Sample Dilution/Sample Weight) x average weight

2.7.2 Vitamin D3 (Cholecalciferol)

Standard preparation: A 25 mg vitamin D3 working standard was weighed and taken into 25 ml volumetric flask with solution mixture (chloroform and methanol in ratio 1:9), dissolved and diluted with solution mixture and made up to the mark well mixed.

Sample Preparation: A 40, 00000 IU of sample was taken into 25 ml volumetric flask with solution mixture (chloroform and methanol in ratio 1:9) dissolved and dilute with solution mixture and made up to the mark well mixed. Absorbance was recorded at 264nm against blank.

Calculation

Amount of vitamin (IU) = (sample absorbance/ standard absorbance) x (standard weight/ standard dilution) x (sample dilution/sample weight) x standard potency x average weight

2.7.3 Vitamin E acetate (tocopherol)

Five milliliters of the standard, sample and blank solution were taken into 25 ml volumetric flask. In each flask, 2 ml 0.1% 2, 2 bilyridil solution (in methanol) and 1 ml 0.1% ferric chloride solution (in water) were added and mixed well. It was diluted in 25 ml of methanol and absorbance recorded at 525 nm against blank.

Calculation

Amount of vitamin (IU) = (sample absorbance/ standard absorbance) x (standard weight/ standard dilution) x (sample dilution/sample weight) x standard potency x average weight

2.7.4 Vitamin K (menadione)

Five milliliters of the standard, sample and blank solution were taken into test tube. In each test tube, 2 ml 2% solution of 2, 4-dinitrophenyl hydrazine (in hydrochloric acid and alcohol in ratio of 1:5 v/v) was added and mixed well. Then it was heated on water bath until almost dry and cool at room temperature. 15 ml solution mixture (Ammonia and alcohol in ratio of 1:1) was added in each test tube. Absorbance was read at 635 nm against blank.

Calculation

Amount of vitamin (IU) = (sample absorbance/standard absorbance) x (standard weight/standard dilution) x (sample dilution/sample weight) x standard potency x average weight

2.8 Statistical Analysis

All data collected were subjected to descriptive and T-test analysis using Statistical Package for

Social Sciences (SPSS), Inc. 20.0 software. All data were represented in mean \pm SEM at confident level of determination (P=0.05).

3. RESULTS

Tables 1-4 shows the phytochemical, proximate mineral and vitamin composition of DT fruits.

Table 1. Phytochemical composition of ethanol extract of unripe and ripe *Dennettia tripetala* fruits

Phytochemicals	Unripe DT fruit (mg/100 g)	Ripe DT fruit(mg/100 g)
Terpenes	6.09 \pm 0.32 ^a	4.08 \pm 0.22 ^b
Phytosterol	1.96 \pm 0.10 ^a	1.45 \pm 0.08 ^b
Oxalate	0.29 \pm 0.01 ^a	0.15 \pm 0.01 ^b
Steroid	0.02 \pm 0.00 ^a	0.00 \pm 0.00 ^b
Tannin	0.30 \pm 0.02 ^b	0.49 \pm 0.3 ^a
Phenol	1.50 \pm 0.08 ^b	1.98 \pm 0.11 ^a
Saponin	0.47 \pm 0.02 ^a	0.14 \pm 0.01 ^b
Alkaloid	3.81 \pm 0.20 ^a	1.32 \pm 0.07 ^b
Coumarin	0.01 \pm 0.00 ^b	0.04 \pm 0.00 ^a
Flavonoid	3.13 \pm 0.17 ^b	5.12 \pm 0.27 ^a
Phytate	6.93 \pm 0.36 ^a	3.12 \pm 0.17 ^b
Cardiac glycoside	1.39 \pm 0.07 ^b	3.72 \pm 0.19 ^a
Cyanogenic glycoside	0.14 \pm 0.01 ^b	0.85 \pm 0.05 ^a

Values are mean \pm SEM of triplicate determinations (n=3).

Values in the same row with the same superscript letters are not significantly different at 5% level

Table 2. Proximate composition of ethanol extract of ripe and unripe *Dennettia tripetala* fruit

Parameters	Unripe whole fruit (%)	Ripe whole fruit (%)
Ash content	6.96 \pm 0.05 ^b	11.81 \pm 0.02 ^a
Lipid	1.85 \pm 0.00 ^b	2.35 \pm 0.00 ^a
Crude fibre	20.11 \pm 0.04 ^a	19.11 \pm 0.03 ^b
Crude protein	9.55 \pm 0.04 ^a	9.62 \pm 0.03 ^a
Total carbohydrate	73.08 \pm 0.06 ^a	67.94 \pm 0.06 ^b
Moisture	8.56 \pm 0.00 ^a	8.28 \pm 0.00 ^b
Energy content(kcal/100g) sample	347.2	331.4

Values are mean \pm SEM of triplicate determinations (n=3).

Values in the same row with same superscript letters are not significantly different at 5% level

Table 3. Mineral composition of ethanol extract of ripe and unripe *Dennettia tripetala* fruit

Mineral	Unripe(mg/g)	Ripe(mg/g)
Na	2.12 \pm 0.00 ^a	2.01 \pm 0.00 ^a
K	12.22 \pm 0.00 ^b	12.53 \pm 0.14 ^a
Zn	8.42 \pm 0.00 ^a	8.02 \pm 0.00 ^b
Ca	5.16 \pm 0.00 ^a	5.18 \pm 0.00 ^a
Fe	3.39 \pm 0.00 ^a	3.45 \pm 0.00 ^a
Cu	0.02 \pm 0.00 ^a	0.01 \pm 0.00 ^a
Se	4.01 \pm 0.00 ^a	3.31 \pm 0.00 ^a
Pb	ND	ND
Ar	ND	ND

Values are mean \pm standard deviation of triplicate determinations (n=3). ND-not detected

Values in the same row with same superscript letters are not significantly different at 5% level

Table 4. Water soluble-vitamin composition of ethanol extract of ripe and unripe *Dennettia tripetala* fruits

Parameters	Unripe DT fruit (mg/g)	Ripe DT fruit(mg/g)
Vitamin B ₁	0.33 ± 0.01 ^b	0.48 ± 0.03 ^a
Vitamin B ₂	0.14 ± 0.00 ^b	0.28 ± 0.01 ^a
Vitamin B ₃	0.54 ± 0.03 ^b	0.99 ± 0.05 ^a
Vitamin B ₅	1.34 ± 0.07 ^b	1.86 ± 0.10 ^a
Vitamin B ₆	1.85 ± 0.10 ^a	1.63 ± 0.09 ^b
Vitamin B ₇	0.23 ± 0.01 ^b	0.27 ± 0.01 ^a
Vitamin B ₉	0.14 ± 0.01 ^b	1.21 ± 0.06 ^a
Vitamin B ₁₂	0.29 ± 0.02 ^b	0.47 ± 0.03 ^a
Vitamin C	66.99 ± 3.26 ^b	90.01 ± 4.74 ^a

Values are mean ± SEM of triplicate determinations (n=3). Values in the same row with same superscript letters are not significantly different at 5% level

Table 5. Fat-soluble vitamin composition of ethanol extract of ripe and unripe *Dennettia tripetala* fruits

Parameters	Unripe DT fruit (IU)	Ripe DT fruit(IU)
Vitamin A	48.65 ± 2.56 ^b	129.37 ± 6.81 ^a
Vitamin D	0.97 ± 0.05 ^a	0.95 ± 0.05 ^a
Vitamin E	1.11 ± 0.06 ^a	1.17 ± 0.06 ^a
Vitamin K	1.57 ± 0.08 ^a	1.2 ± 0.06 ^b

Values are mean ± SEM of triplicate determinations (n=3). Values in the same row with same superscript letters are not significantly different at 5% level

4. DISCUSSION

The non-nutritive bioactive compounds which are found in plants and are beneficial to humans are known as phytochemicals or secondary metabolites [20]. Determination of the non-nutritive bioactive compounds of a plant gives information about the qualitative and quantitative composition of biologically active components of the plant material. The increased utilization of plants in medicine is attributed to the presence of one or more predominant secondary metabolites in the plant, which have medicinal potential against certain diseases. The non-nutritive compounds in this study include: terpenes, phytosterol, steroid, oxalate, saponin, alkaloid and phytate which were observed to be generally higher in the unripe than ripe fruits. The concentration of tannins, phenol, flavonoid, coumarin, cardiac and cyanogenic glycosides were higher in the ripe fruit than the unripe fruit (Table 1). The predominant phytochemical in the fruits were flavonoids and terpenes which are known to have antioxidant and anti-inflammatory potentials. Earlier findings had reported that alkaloids, tannins, saponins, flavonoids, terpenoids, steroids and cardiac glycosides are present in *Dennettia tripetala* fruits [10].

The proximate analysis of ripe and unripe DT fruits revealed that the ripe fruits had the greater concentration of lipid and ash than the unripe fruits; while the unripe fruits had the greater amount of carbohydrate and fibre than the ripe fruits (Table 2). There was no significant difference between the concentrations of proteins in both fruits. The energy value was higher in the unripe fruits than the ripe fruits. Fibre is known to possess anti-cancer and cholesterol reducing potentials [21]. The results show that the unripe and ripe whole fruit of *Dennettia tripetala* are potential sources of dietary fibre (roughages) and diets mixed with fibre have been reported to protect against coronary heart disease [22]. The present study corroborates the work of Olufunke and Adeola, [23] who reported that the crude fibre content of some unripe African fruits were higher than in the ripe fruits.

Carbohydrate content of unripe fruits was higher than the ripe fruits in this study. This finding supports the previous study, which reported that the carbohydrate content in unripe fruit is higher than that of ripe fruits, [23] thus, indicating that the carbohydrate content reduces as maturity progresses. In a similar study, the total carbohydrate content of ripe DT was reported by

Ihemeje et al. [10] and the values were higher than values obtained in the present study.

The ash content of a food material which represents the amount and various types of minerals present in a food material was seen to be higher in the ripe fruit than the unripe fruit. The result in Table 3 showed that the ripe and unripe DT fruits contain mineral components such as zinc, calcium and potassium which were present in macro quantities; Fe, Se and Na were found in micro quantities; while, Cu, was found in very minute quantity. However, the concentration of K was higher in ripe than unripe fruits; while Zn was higher in unripe than ripe fruits. Sodium and potassium helps in maintaining membrane potential responsible for muscle contraction and nerve impulses; calcium ion (Ca^{2+}) plays a role in the formation and stability of cell walls and in the maintenance of membrane structure and permeability; activation of enzymes, regulation of many responses of cells to stimuli. Zinc plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity [24]. Zinc acts as cofactors in many enzymatic reactions. These include DNA and RNA polymerase, alkaline phosphatase, carbonic anhydrase, carboxypeptidase and superoxide dismutase reactions. In addition, it plays a role in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. Lead and Arsenic are heavy metals, which play detrimental roles to human health. The fruits of DT are relatively safe for consumption because they contained none of these heavy metals. These findings corroborates the work of Ihemeji and colleagues [10], who reported that the fruits of *Dennettia tripetala* contain high amount of potassium and calcium while sodium, zinc, iron, copper, manganese, cobalt, nickel and cadmium were available in trace quantities.

The concentration of water and fat-soluble vitamins were determined in ripe and unripe *Dennettia tripetala* fruits and are shown in Table 4 and 5 respectively. Vitamins are mandatory for optimal functioning of the body. Vitamins are divided into water and fat-soluble vitamins; the B vitamins and vitamin C are only soluble in water while vitamins A, D, E and K are only soluble in lipids. Both ripe and unripe fruits showed considerable presence of both water and fat-soluble vitamins. Generally, all the vitamins evaluated in the present study were higher in the ripe fruits than in unripe fruits except for vitamins K and B₆. This finding implies that most vitamins

increase as ripening progresses. The predominant vitamins in both ripe and unripe fruits were Vitamin A and C. These vitamins are found mostly in fruits and vegetables and they play mainly antioxidant roles. According to Weber et al. [25] vitamin A helps in boosting the immune system by assisting in the recycling of tocopherol radical and dihydroascorbic acid generated by recycled reduced glutathione(GSH) and converting it to vitamin E (α -tocopherol) thus helping in prevention of lipid peroxidation. Vitamin C plays essential role in the production of neurotransmitters thus assisting in communication between neurons and prevention of neurodegenerative diseases [26]. Studies have shown that, supplementation with vitamin B₆, vitamin B₁₂ or folate has positive effects on memory performance in women of various ages [27]. Vitamin D has a neuroprotective role in clearing amyloid plaques from the brain thereby preventing Alzheimer's disease [28].

5. CONCLUSION

The rich presence of phytochemicals, minerals, fats and water soluble vitamins particular in the ripe DT fruits suggest that ripening improves the nutrient and the phytochemical composition of fruits. These findings also validates the traditional and medicinal uses of DT fruits. Therefore, ripe and unripe fruits of DT may be taken as food to augment the mineral and vitamins as well as antioxidant needs of the body.

ACKNOWLEDGEMENTS

We extend our sincere gratitude to Mr U. E. Evans, Dr E.O. Etim, U. U. Prof. Ekanemesang and project students: Mr. G. Isaiah, Mr. D. O. Simonson and Miss V. John for their support and technical assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Malhotra VK. Biochemistry for students. Tenth Edition. Jaypee Brothers Medical Publishers(P) Ltd, New Delhi; 1998.
2. Eruvbetine D. Canine nutrition and health. A paper presented at the seminar organized by Kensington Pharmaceuticals Nig. Ltd., Lagos; 2003.

3. Temple NJ. Antioxidants and disease: More questions than answers. *Nutritional Research*. 2000;20:449–59.
4. Willett WC. Diet and health: What should we eat? *Science*. 1994;254:532–37.
5. Willett WC. Diet, nutrition, and avoidable cancer. *Environmental Health Perspective*. 1995;103(8):165–70.
6. Bourre JM. Effects of nutrients (in food) on the structure and function of the nervous system: Update on dietary requirements for brain. Part 1: Micronutrients. *The Journal of Nutrition, Health & Aging*. 2006; 10:5.
7. Paleologos M, Cumming R, Lazarus R. Cohort study of vitamin C intake and cognitive impairment. *American Journal of Epidemiology*. 1998;148:45-50.
8. Larner AJ, Janssen JC, Cipolotti L, Rossor MN. Cognitive profile in dementia associated with vitamin B12 deficiency due to pernicious anaemia. *Journal of Neurology*. 1999;246:317-319.
9. Bryan J, Calvaresi E. Associations between dietary intake of folate and vitamins B-12 and B-6 and self-reported cognitive function and psychological well-being in Australian men and women in midlife. *Journal of Nutrition, Health and Aging*. 2004;8:226-232.
10. Ihemeje A, Nwachukwu CN, Ekwe CC. Production and quality evaluation of flavoured yoghurts using carrot, pineapple and spiced yoghurts using ginger and pepper fruit. *African Journal of Food Science*. 2015;9:163–169.
11. Achinewhu SC, Barber LI, Ijeoma IO. Physicochemical properties and garification (gari yield) of selected cassava cultivars in Rivers State, Nigeria. *Plant Foods for Human Nutrition*. 1998;52(2): 133.
12. Okwu DE, Morah FN, Anam EM. Isolation and characterization of phenanthrenic alkaloid uvariopsine from *Dennettia tripetala* fruits. *Journal of Medicine and Aromatic Plant Science*. 2005;27:496–498.
13. Nwaogu LA, Alisi CS, Ibegbullem CO, Igwe CU. Phytochemical and antimicrobial activity of ethanolic extract of *Landolphia owariensis* leaf. *African Journal of Biotechnology*. 2007;6(7).
14. Adedayo BC, Oboh G, Akindahunsi AA. Changes in the total phenol content and antioxidant properties of pepper fruit (*Dennettia tripetala*) with ripening. *African Journal of Food Science*. 2010;4(6):403-409.
15. Association of official analytical chemists. *Method of analysis*. Washington DC, U.S. 1990;15:1250-1255.
16. Onyeike EN, Acheru GN. Chemical compositions of selected Nigerian oil seeds and physicochemical properties of the oil extracts. *Food Chemistry*. 2002;77:431-437.
17. Onyeike EN, Ihuga AC, George C. Influence of heat processing on the nutrient composition of vegetable leaves consumed in Nigeria. *Plant Foods for Human Nutrition*. 2003;58:1-11.
18. McGrath SP, Cunliffe CH. A simplified method for the extraction of the metals Fe, Zn, Cu, Ni, Cd, Pb, Cr, Co and Mn from soils and sewage sludges. *Journal of the Science of Food and Agriculture*. 1985;36(9):794-8.
19. Ashok K, Kumar RG. To develop a simple (UV-VIS spectrometric) method for the estimation of multivitamin with special reference to capsules & tablets. *Int J Pharmagenes*. 2011;2(1):43-8.
20. Ebong PE, Atangwho IJ, Eyong EU, Egbung GE. The antidiabetic efficacy of combined extracts from two continental plants *Azadirachta indica* and *Vernonia amygdalina* (Del). *American Journal of Biochemistry and Biotechnology*. 2008;4(3):239-244.
21. Goozee KG, Shah TM, Sohrabi HR, Rainey-Smith SR, Brown B, Verdile G, Martins RN. Examining the potential clinical value of curcumin in the prevention and diagnosis of Alzheimer's disease. *British Journal of Nutrition*. 2016;115(3): 449-465.
22. Lupton JR, Turner ND. Dietary fiber and coronary disease: Does the evidence support an association? *Current Atherosclerosis Reports*. 2003;5(6):500-505.
23. Olufunke OO, Adeola EO. Comparative food contents of ripe and unripe fruits of African star apple, *Chrysophyllum albidum* G. Don (Syn. Gambeyaalbida). *Journal of Research in Forestry, Wildlife and Environment*. 2017;9(4):90-95.
24. Bhatnagar S, Taneja S. Zinc and cognitive development. *British Journal of Nutrition*. 2001;85(S2):S139-S145.
25. Weber P, Bendich A, Machlin LJ. Vitamin E and human health; rationale for

- determining recommended intake levels. Nutrition. 1997;13(5):450-460.
26. Harrison FE, May JM. Vitamin C function in the brain: Vital role of the ascorbate transporter SVCT2. Free Radical Biology and Medicine. 2009;46(6): 719-730.
27. Bryan J, Calvaresi E, Hughes D. Short-term folate, vitamin B-12 or vitamin B-6 supplementation slightly affects memory performance but not mood in women of various ages. Journal of Nutrition. 2002; 132:1345–1356.
28. Soni M, Kos K, Lang IA, Jones K, Melzer D, Llewellyn DJ. Vitamin D and cognitive function. Scandinavian Journal of Clinical and Laboratory Investigation. 2012; 72(243):79-82.

© 2019 Akpakpan et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle3.com/review-history/48885>