Original Research Article

NUTRITIVE AND NON-NUTRITIVE PRINCIPLES OF RIPE AND UNRIPE *Dennettia tripetala* FRUITS

ABSTRACT

The present study was based on the assessment of nutrient and non-nutrient principles of ripe and unripe DT fruits. Phytochemical, proximate, vitamins and minerals composition were done 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 mineral 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, Ar, Ca, Fe and Cu did not differ significantly ($p<0.05$) between the two fruits. DT fruits possessed a significant ($p<0.05$) higher concentrations of vitamin A, B_1, B_2, B_3, B_5, B_7, B_9, B_{12} and C than the ripe fruit; while the unripe fruits showed a significant higher concentrations of Vitamin B6 and K than the ripe fruit. Therefore, ripe and unripe DT fruits are rich in macronutrients, micronutrients and phytochemicals and is of tremendous medicinal significance as an antioxidant, antimalarial, anticancer, immune booster and in the treatment of heart diseases vis-à-vis its phytochemicals.

KEYWORDS: vitamins; mineral; phytochemical; proximate; *Dennetia tripetala;* pepper fruit

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 its utilization in medicine as

neutraceuticals and pharmaceutical agents for the prevention and treatment of disease. The non-nutritive principles of plants are the bioactive components or phytochemicals which are responsible for thier 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, muscle contraction, 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 its 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 B_6 and B_9 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 belong to the family of annonaceae is one of the major fruit tree grown in Cameroun, Ivory Coast and Southern Nigeria[10]. DT 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.

It is documented in literature that DT fruit contains important nutrients such as vitamins, minerals, carbohydrate and fibre[12,11] also phytochemicals such as flavonoids, tannins and cyanogenic glycosides[13,14]. These phytochemicals gives the fruits its biological usefulness as anticancer, anti-diabetic, and anti-glaucoma effects. The present study is a part of an elaborate ongoing study, which tends to elucidate on the functional roles of nutritive and non-nutritive principles of ripe and unripe DT fruits.

MATERIALS AND METHODS

All materials and reagents used in this work were of analytical standards and grades.

2.1 Collection/preparation of plant Sample

The fruits of *Denettia tripetala* (both ripe and unripe) 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.

2.2 Experimental design

Denettia tripetala fruits (both ripe and unripe) were ground and dried under room temperature and approximately 500g 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 ripe and unripe DT samples were poured 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 wasconcentrated by boiling with nitrogen gas to 1.0ml. 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 ^{o}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 $^{\circ}$C until it heated to 330 $^{\circ}$C at a rate of 3 $^{\circ}$C min^{-1}. This temperature was maintained for 5 min and the detector operated at a temperature of 320 $^{\circ}$C. The concentration of the different phytochemicals were expressed in mg/100g.

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 and weighed. The procedure was done in triplicates and the percentage ash content of the sample was calculated thus;

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

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 poured into a thimble and was extracted with petroleum ether 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 2g weight of the defatted sample was put in a conical flask and 200ml 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 12.5cm filter paper. The sample was washed back into the original flask with 200ml of 1.25% NaOH, and boiled for 30min. 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 1g weight of the sample was transferred into Kjhedahl flask. A few chips of antibumping granules and 4g of digestion catalyst made up of 20ml 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, 30ml of 40% sodium hydroxide was injected into the distillation flask through a cork with the aid of a

syringe. The flask was heated for 10min 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;

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

% Crude protein = % Nitrogen x 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.

Total Carbohydrate = 100 – (% moisture + % crude fat + % ash+ % crude protein + % 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 of crude protein, crude fat and total carbohydrate by the Attwater factors of 4, 9, 4 respectively, summation of the products and expressing the result in Kcal per 100g.

2.5 Determination of mineral content of fruit

The fruit sampes were digested by weighing 2g of each of the samples into 250ml crucible; then adding aqua regia (HCL and HNO3, ratio of 3:1) at 130ºC using electric hotplate for 30 min, filtered and the filtrate was made up to 100ml. 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 minutes. The standard metal solutions were injected to calibrate the AAS using acetylene gas. An aliquot of the digest solutions were injected and the concentrations displayed by the AAS.

2.6 Determination of water-soluble vitamins

2.6.1 Vitamin B_1 (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 NH_4OH and 0.5 ml 0.1 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 B_1 in sample = $\frac{\text{Absorbance of sample x Concentration of sample}}{\text{Absorbance of standard}}$

2.6.2 Vitamin B_2 (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 B_2 in sample = $\frac{\text{Absorbance of sample x Concentration of sample}}{\text{Absorbance of standard}}$

2.6.3 Vitamin B_3 (nicotinamide)

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

Calculation

Concentration of vitamin B_3 in sample = $\frac{\text{Absorbance of sample x Concentration of sample}}{\text{Absorbance of standard}}$

2.6.4 Vitamin B_5 (pantothenic acid)

Hydrolysis of standard and sample

Five milliliters of sample solution was taken into 50ml volumetric flask. In each volumetric flask, 2ml of 1M hydrochloric acid was added and mixed well, then heated for 5 hr at 69^{o}C ± 1^{o}C and cool at room temperature. Then, 2ml 7.5% hydroxylamine reagent (in 0.1M sodium hydroxide), 5ml of 1M 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, 5ml of the standard and hydrolysed sample solution was taken in marked test tubes. In each test tube, 1ml of 1% ferric chloride solution was added and mixed well and absorbance measured at 500nm against blank.

Calculation

Concentration of vitamin B_5 in sample = $\frac{\text{Absorbance of sample x Concentration of sample}}{\text{Absorbance of standard}}$

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, 1ml of ammonium buffer, 1ml of 20% sodium acetate solution, 1ml of 5% boric acid solution and 1ml 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 B_6 in sample = $\frac{\text{Absorbance of sample x Concentration of sample}}{\text{Absorbance of standard}}$

2.6.6 Vitamin B7 (biotin)

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

Calculation

Concentration of vitamin B_7 in sample = $\frac{\text{Absorbance of sample x Concentration of sample}}{\text{Absorbance of standard}}$

2.6.7 Folic acid (vitamin B9)

Two millilitres of the standard and sample solution were taken in marked test tubes. In each test tube, 2ml of 0.02% potassium permanganate solution, 2ml of 2% sodium nitrate solution, 2ml of 4M hydrochloric acid solution, 1ml of 5% ammonium sulphate solution and 1ml 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 535nm against the blank.

Calculation

Concentration of vitamin B_9 in sample = $\frac{\text{Absorbance of sample x Concentration of sample}}{\text{Absorbance of standard}}$

2.6.8 Vitamin B_{12} (cyanocobalamin)

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

Calculation

Concentration of vitamin B_{12} in sample = $\frac{\text{Absorbanceof sample x Concentration of sample}}{\text{Absorbance of standard}}$

2.6.9 Vitamin C (ascorbic acid)

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

Calculation

Concentration of sample = $\frac{\text{Absorbance of sample x Concentration of sample}}{\text{Absorbance of standard}}$

2.7 Determination of fat soluble vitamins

2.7.1 Vitamin A (retinol)

A 500IU sample was weighed into round bottom flask. Then, 2 ml 50% w/v potassium hydroxide solution, 10ml glycerol and 50ml 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 4x25ml diethyl ether and washed with water. The water layer was discarded then the ether layer was taken into dry 100ml volumetric flask by passing it through anhydrous sodium and made up to 100ml with diethyl ether, mixed well. Absorbance was read at 325nm against the blank.

$$\text{Concentration of vitamin A in sample} = \frac{\text{Absorbance of sample} \times \text{Concentration of sample}}{\text{Absorbance of standard}}$$

2.7.2 Vitamin D3 (cholecalciferol)

Standard preparation*: A* 25mg vitamin D3 working standard was weighed and taken into 25ml 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 25ml 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

$$\text{Concentration of vitamin D3 in sample} = \frac{\text{Absorbance of sample} \times \text{Concentration of sample}}{\text{Absorbance of standard}}$$

2.7.3 Vitamin E acetate (tocopherol)

Standard preparation: A 25mg vitamin E acetate working standard was taken into 100ml volumetric flask and 50ml of methanol was added to dissolve. The volume was made up to the mark with methanol.

Sample preparation: A 25IU sample was weighed and taken in round bottom flask. Then, 2ml 50% w/v potassium hydroxide solution, 10ml glycerol and 25ml methanol were added and mixed well, then refluxed for 45 min on boiling water bath, The solution was left to cool, transferred into separating funnel and extracted with 50ml ether for 5 min. The water layer was discarded and ether layer was filtered through anhydrous sodium sulphate. Then, the ether layer was evaporated to dryness and dissolved with methanol in 100ml volumetric flask.

Procedure: 5ml of the standard, sample and blank solution were taken into 25ml volumetric flask. In each volumetric flask, 2ml 0.1% 2, 2 bilyridil solution (in methanol) and 1ml 0.1% ferric chloride solution (in water) were added and mixed well. It was diluted in 25ml of methanol and absorbance recorded at 525nm against blank.

Calculation

$$\text{Concentration of vitamin E in sample} = \frac{\text{Absorbance of sample} \times \text{Concentration of sample}}{\text{Absorbance of standard}}$$

2.7.4 Vitamin K (menadione)

***Standard preparation*:** A 25mg of menadione working standard was weighed and taken into 100ml volumetric flask and 50ml of chloroform was added to dissolve. The volume was made up to the mark with chloroform. The solution was filtered and further 1ml was taken into 50ml volumetric flask made up to the mark with chloroform.

***Sample preparation*:** 250 mcg of sample was weighed and taken into a separator. In a separator, 5ml of water was added, mixed well and extracted with 4x10ml chloroform. The water layer was discarded then chloroform was taken into dry 50ml volumetric flask by passing it through anhydrous sodium sulphate and made up to 50ml with chloroform.

Procedure: 5 millilitres of the standard, sample and blank solution were taken into test tube. In each test tube, 2ml 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. 15ml 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

$$\text{Concentration of vitamin K in sample} = \frac{\text{Absorbance of sample} \times \text{Concentration of sample}}{\text{Absorbance of standard}}$$

2.8 Data 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 ± SEM at confident level of determination (P=0.05).

RESULTS

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

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

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

Values are mean ± SEM of triplicate determinations (n=3).
Values in the same row with different superscripts are significantly different at P<0.05.

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 ± 0.05b	11.81 ± 0.02a
Lipid	1.85 ± 0.00b	2.35 ± 0.00a
Crude fibre	20.11 ± 0.04a	19.11 ± 0.03b
Crude protein	9.55 ± 0.04a	9.62 ± 0.03a
Carbohydrate	52. 97 ± 0.06a	48.83 ± 0.06b
Energy content(kcal/100g)	266.73	254.95

Values are mean ± SEM of triplicate determinations (n=3).
Values in the same row with different superscripts are significantly different at P<0.05.

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

Mineral	Ripe(/100g)	Unripe(/100g)
Na	2.01 ± 0.00a	2.12 ± 0.00a
K	12.53 ± 0.14a	12.22 ± 0.00b
Zn	8.02 ± 0.00b	8.42 ± 0.00a
Ca	5.18 ± 0.00a	5.16 ± 0.00a
Fe	3.45 ± 0.00a	3.39 ± 0.00a
Cu	0.01 ± 0.00a	0.02 ± 0.00a
Se	3.31 ± 0.00a	4.01 ± 0.00a
Pb	0.00 ± 0.00a	0.00 ± 0.00a
Ar	0.00 ± 0.00a	0.00 ± 0.00a

Values are mean ± standard deviation of triplicate determinations (n=3).
Values in the same row with different superscripts are significantly different at p<0.05.

Table 4: Vitamin composition of ethanol extract of ripe and unripe *Dennettia tripetala* fruits

Parameters	Ripe DT fruit(mg)	Unripe DT fruit (mg)
Vitamin B1	0.48 ± 0.03^{a}	0.33 ± 0.01^{b}
Vitamin B2	0.28 ± 0.01^{a}	0.14 ± 0.00^{b}
Vitamin B3	0.99 ± 0.05^{a}	0.54 ± 0.03^{b}
Vitamin B5	1.86 ± 0.10^{a}	1.34 ± 0.07^{b}
Vitamin B6	1.63 ± 0.09^{b}	1.85 ± 0.10^{a}
Vitamin B7	0.27 ± 0.01^{a}	0.23 ± 0.01^{b}
Vitamin B9	1.21 ± 0.06^{a}	0.14 ± 0.01^{b}
Vitamin B12	0.47 ± 0.03^{a}	0.29 ± 0.02^{b}
Vitamin C	90.01 ± 4.74^{a}	66.99 ± 3.26^{b}
Vitamin A	129.37 ± 6.81^{a}	48.65 ± 2.56^{b}
Vitamin D	0.95 ± 0.05^{a}	0.97 ± 0.05^{a}
Vitamin E	1.17 ± 0.06^{a}	1.11 ± 0.06^{a}
Vitamin K	1.2 ± 0.06^{b}	1.57 ± 0.08^{a}

Values are mean ± SEM of triplicate determinations (n=3).Values in the same row with different superscripts are significantly different at P<0.05.

RESULT/DISCUSSION

The non-nutritive bioactive compounds which are found in plants and are beneficial to humans are known as phytochemicals or secondary metabolites [18]. 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 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 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 [19]. The results imply that the unripe whole fruit of *Dennettia tripetala* is a potential source of dietary fibre (roughages) and diets mixed with fibre has been reported to protect against coronary heart disease [20]. This work of Olufunke and Adeola, [21] who reported that the crude fibre content of some unripe African fruits were higher than the ripe ones corroborates with the present study.

Carbohydrate content of unripe fruits was higher than unripe 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, [21] thus, indicating that the carbohydrate content reduces as maturity progresses. In a similar study, the crude carbohydrate content of ripe DT was reported by Ihemeje *et. al.* [10] as well as Borokini *et al.* [22] and the values were higher than what is 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 fruit contain mineral components such as zinc, calcium and potassium which were present in macro quantities; Fe, Se and Na were found in minor quantities; while, Cu, Pb and Ar were found in very minute quantities. 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 (Ca^{2+}) plays a role in formation and stability of cell walls and in 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 [23]. 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 little or none of these metals. These findings corroborates with 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 vitamins (water and fat-soluble) were estimated in ripe and unripe *Dennettia tripetala* fruits and are shown Table 4. 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 B_6. 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.* [24] 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 [25]. Studies have shown that, supplementation with vitamin B6, vitamin B_{12} or folate has positive effects on memory performance in women of various ages [26]. Vitamin D has a neuroprotective role in clearing amyloid plaques from the brain thereby preventing Alzheimer's disease [27]

CONCLUSION

The rich presence of nutritive and non nutritive principles in ripe and unripe DT fruits suggests that the consumption of DT fruits can boost metabolism, reproductive function, neuronal function and antioxidant function. Therefore, ripe and unripe fruits of DT may be taken as food to augment the mineral and vitamin needs of the body.

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