# PHYTOCHEMICAL COMPOSITION AND NUTRITIVE VALUE OF UNRIPE AND RIPE Dennettia tripetala (DT) FRUITS

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ABSTRACT

#### 14

Aims: Phytochemical composition and nutritive value of unripe and ripe pepper fruits (*Dennettia tripetala*-DT) were investigated.

**Place and Duration of Study:** Biochemistry Department, University of Port Harcourt, Rivers State, between the period of April and July, 2018

**Methodology:** Phytochemical, proximate, minerals and vitamin composition were carried out using standard methods.

**Results:** 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 ripe DT fruit. The energy value (kcal/100g 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 unripe fruit than the unripe fruit than the ripe fruit. The concentration of Na, Se, Pb, Ca, Fe and Cu did not differ significantly (p<0.05) between the two fruits. DT fruits possessed a significantly (p<0.05) higher concentrations of vitamin A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>5</sub>, B<sub>7</sub>, B<sub>9</sub>, B<sub>12</sub> and C than the ripe fruit; while the unripe fruits showed a significantly higher concentrations of Vitamin B<sub>6</sub> and K than the ripe fruit.

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16 *Keywords:* Phytochemical and proximate composition, mineral, vitamins, ripening *Dennetia tripetala* 17

# 19 1. INTRODUCTION

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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 neutraceuticals and pharmaceutical agents for the prevention and treatment of diseases. Phytochemicals are the non-nutritive principles or the bioactive components of

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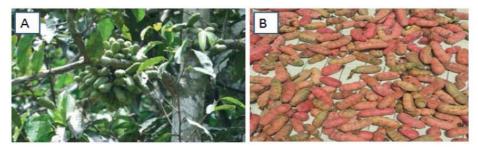
plants which are responsible for their biological activity. Vitamins and minerals are regarded as important micronutrients, which are required in the body in minute amount for performance of metabolic functions. Although minerals yield no energy, they are necessary for the maintenance of certain physicochemical 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]

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32 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. 33 34 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 35 36 may be due to failure of protective mechanisms and dietary deficiencies such as deficiency of antioxidants and nutrients. 37 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, 38 rendering them unavailable to perpetuate neuronal damage. Vitamins such as  $B_6$  and  $B_9$  are involved in neurotransmitter 39 40 synthesis while minerals such as magnesium and iron are important in cognition, oxidation-reduction reaction and ionic regulation respectively [6]. 41

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*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.



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

51 It is documented in literature that DT fruit contains important nutrients such as vitamins, minerals, carbohydrate and 52 fibre[12,11]; as well as also phytochemicals such as flavonoids, tannins and cyanogenic glycosides[13,14]. These 53 phytochemicals give the fruits their biological usefulness as anticancer, anti-diabetic, and anti-glaucoma effects.

54 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.

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## 59 2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

### 60

### 61 **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 lbom State, Nigeria.

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#### 66 2.2 Experimental design

67 *Denettia tripetala* fruits (both unripe and ripe) were ground and dried under room temperature and approximately 500g 68 was weighed using electronic weighing balance and used for phytochemical, proximate, vitamins and mineral analysis 69 using standard analytical methods.

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# 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% 72 73 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 74 75 venting to release excess pressure. The organic layer was allowed to separate from the water phase for a minimum of 10 76 min, and the methylene chloride extract was collected in 250ml flask. A second and third extraction was prepared in the 77 same manner. Finally, the combined extract was poured through a drying column packed with cotton wool and 0.1M 78 anhydrous sodium sulphate and silica; it was concentrated by boiling with nitrogen gas to 1.0ml. The remaining extract 79 was mixed with 1.0ml of the solvent and injected into a Flame Ionization Detector GC for analysis.

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## 81 **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<sup>-1</sup>, the carrier gas used was Helium 5.0 psi with a flow rate of 40 ml min<sup>-1</sup>. The oven operated from a temperature of 200 °C until it heated to 330 °C at a rate of 3 °C min<sup>-1</sup>. 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/100g.

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#### 87 2.4 Proximate analysis

#### 88 **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( $29 \pm 1^{\circ}$  C )and weighed. The procedure was done in triplicates and the percentage ash content of the sample was calculated thus;

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96 % Ash =<u>Weight of ash</u> x 100 =  $\frac{W_{32}}{W_1}$  x 100

97 Weight of sample W<sub>2</sub>-W<sub>1</sub>

98 Where

99  $W_1$  = weight of empty crucible;  $W_2$  = weight of crucible + sample before ashing;  $W_3$  = weight of crucible + sample after 100 ashing

#### 101

### 102 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;

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108 % Crude fat= <u>Weight of sample (extracted fat)</u> x 100 =  $\frac{W_2 - W_1}{W_1}$  x 100 109 Weight of sample W

110 Where

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

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### 113 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.

119

120 % Crude fibre =  $W_2 - W_1 \times 100$ 

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122 Where

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

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#### 125 2.4.4 Determination of crude protein content [15]

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126 A 1g weight of the sample was transferred into Kihedahl 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. 127 The flask was gently heated for frothing to occur and subside, and then heat was increased to 250°C. The complete 128 sample digestion was done in 5 hours. The digest was cooled to room temperature and diluted to 100ml with distilled 129 130 water. A 20ml aliguot 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 131 through a receiver adapter and 10ml of 2% boric acid and two drops of double indicator were pipetted into the distillation 132 flask. Then, 30ml of 40% sodium hydroxide was injected into the distillation flask through a cork with the aid of a syringe. 133 The flask was heated for 10min to digest the content. The distillate was collected in the boric acid and then titrated with 134 0.1M HCL. The volume of HCl added was recorded as the titre value. The % Crude protein was calculated thus: 135

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- 137 % Nitrogen = <u>Titre value x 1.4 x 100 x 10</u>
- 138 1000 x wt of sample x aliquot of digest
- 139 % Crude protein = % Nitrogen x 6.25

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#### 141 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.

144 Total Carbohydrate(%) = 100 – (% moisture + % crude fat + % ash+ % crude protein + % crude fibre).

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#### 146 **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.

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### 151 **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 HNO<sub>3</sub>, ratio of 3:1 ) at 130° C using electric hotplate for 30 min, filtered and the filtrate was made up to 100ml[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.

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#### 159 **2.6 Determination of water-soluble vitamins**

#### 160 **2.6.1 Vitamin B<sub>1</sub> (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<sub>4</sub>OH 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.

165 Calculation

166 Concentration of vit.  $B_1$  in sample (mg/g) = <u>Absorbance of sample x Concentration of sample</u> 167 Absorbance of standard

#### 168 2.6.2 Vitamin B<sub>2</sub> (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.

- 172 Calculation
- 173 Concentration of vit. B<sub>2</sub> in sample (mg/g) = <u>Absorbance of sample x Concentration of sample</u>
   174 Absorbance of standard

# 175 **2.6.3 Vitamin B<sub>3</sub> (nicotinamide**)

Two millilitres of the standard, sample and blank solution were taken in marked test tubes. In each test tube, 5 ml sulphanilic 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.

- 179 Calculation
- 180 Concentration of vit. B<sub>3</sub> in sample (mg/g) = <u>Absorbance of sample x Concentration of sample</u>

182 2.6.4 Vitamin B<sub>5</sub> (pantothenic acid) Five milliliters of sample solution was taken into 50 ml volumetric flask. In each volumetric flask, 2 ml of 1M hydrochloric 183 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% 184 hydroxylamine reagent (in 0.1M sodium hydroxide), 5 ml of 1 M sodium hydroxide and kept for five min. The pH was 185 adjusted to 2.7 ± 0.1 with hydrochloric acid and the volume was made up with water. Then, 5 ml of the standard and 186 hydrolysed sample solution was taken in marked test tubes. In each test tube, 1 ml of 1% ferric chloride solution was 187 added and mixed well and absorbance measured at 500 nm against blank. 188 189 Calculation Concentration of vit  $B_5$  in sample (mg/g) = Absorbance of sample x Concentration of sample 190 Absorbance of standard 191 2.6.5 Vitamin B<sub>6</sub> (pyridoxine hydrochloride) 192 Two millilitres of the standard and sample solution were taken in marked test tubes. In each test tube, 1ml of ammonium 193 buffer, 1 ml of 20% sodium acetate solution, 1 ml of 5% boric acid solution and 1ml 1M dye (2, 6-di-chloroguinine 194 chorimide) solution were added and mixed well. Absorbance was read in a spectrophotometer at 650nm against the 195 blank. 196 197 Calculation Concentration of vit.  $B_6$  in sample (mg/g)=Absorbance of sample x Concentration of sample 198 199 Absorbance of standard 2.6.6 Vitamin B<sub>7</sub> (biotin) 200 500 microgram of sample was weighed into a 100 ml volumetric flask and 10 ml of dimethyl sulfoxide was added to 201 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 202 with dilute water. It was filtered and absorbance read at 294 nm against blank. 203 204 Calculation 205 Concentration of vit.  $B_7$  in sample (mg/g) = Absorbance of sample x Concentration of sample Absorbance of standard 206 2.6.7 Vitamin B<sub>9</sub> (Folic acid) 207 Two millilitres of the standard and sample solution were taken in marked test tubes. In each test tube, 2 ml of 0.02% 208 potassium permanganate solution, 2 ml of 2% sodium nitrate solution, 2 ml of 4M hydrochloric acid solution, 1ml of 5% 209 210 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 535 nm against the blank. 211 212 Calculation Concentration of vit.  $B_9$  in sample (mg/g) = Absorbance of sample x Concentration of sample 213 214 Absorbance of standard 215 2.6.8 Vitamin B<sub>12</sub> (cyanocobalamin) 216 One microgram of sample was weighed into 25 ml volumetric flask and 10 ml of water was added to dissolve. Then, 1.25 217 g of diabasic sodium phosphate, 1.1 m of anhydrous citric acid and 1.0gm of sodium metabisulphate was added. The 218 219 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. 220

Absorbance of standard

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222	Calculation		
223	Concentration of vit. B <sub>12</sub> in sample (mg/g)=Absorbance of sample x Concentration of sample		
224	Absorbance of standard		
225	2.6.9 Vitamin C (ascorbic acid)		
226	Two millilitres each of the standard, sample and blank solution were taken in 25 ml volumetric flask. In each flask, 2 ml		
227	10% v/v sulphuric acid and 5ml 10% v/v ammonium molybdate were added and mixed well, then kept for 50 min at room		
228	temperature. It was diluted to 25 ml with distilled water and absorbance read at 450 nm against the blank.		
229	Calculation		
230	Concentration of sample (mg/g) = <u>Absorbance of sample x Concentration of sample</u>		
231	Absorbance of standard		
232	2.7 Determination of fat soluble vitamins		
233	Fat and water soluble vitamins were determined as described by [19].		
234			
235	2.7.1 Vitamin A (retinol)		
236	A 500IU sample was weighed into round bottom flask. Then, 2 ml 50% w/v potassium hydroxide solution, 10 ml glycerol		
237	and 50 ml methanol were added and mixed well, then refluxed for 45 min on boiling water bath and cooled. The flask was		
238	washed with distilled water and taken into the separator then extracted with 4x25 ml diethyl ether and washed with water.		
239	The water layer was discarded then the ether layer was taken into dry 100 ml volumetric flask by passing it through		
240	anhydrous sodium and made up to 100 ml with diethyl ether, mixed well. Absorbance was read at 325 nm against the		
241	blank.		
242			
243	Amount of vitamin (IU) = Sample Absorbance x factor (1830) x (1000/100) x (Sample Dilution/Sample		
244	Weight) x average weight		
245			
246	2.7.2 Vitamin $D_3$ (cholecalciferol)		
247	Standard preparation: A 25mg vitamin D3 working standard was weighed and taken into 25ml volumetric flask with		
248	solution mixture (chloroform and methanol in ratio 1:9), dissolved and diluted with solution mixture and made up to the		
249	mark well mixed.		
250	Sample Preparation: A 40, 00000 IU of sample was taken into 25 ml volumetric flask with solution mixture (chloroform and		
251	methanol in ratio 1:9) dissolved and dilute with solution mixture and made up to the mark well mixed. Absorbance was		
252	recorded at 264nm against blank.		
253	Calculation		
254	Amount of vitamin (IU) = (sample absorbance/ standard absorbance) x (standard weight/standard dilution) x (sample		
255	dilution/sample weight) x standard potency x average weight		
256			
257	2.7.3 Vitamin E acetate (tocopherol)		
258	Five milliliters of the standard, sample and blank solution were taken into 25 ml volumetric flask. In each flask, 2 ml 0.1%		
259	2, 2 bilyridil solution (in methanol) and 1 ml 0.1% ferric chloride solution (in water) were added and mixed well. It was		
260	diluted in 25 ml of methanol and absorbance recorded at 525 nm against blank.		
261	Calculation		

262	Amount of vitamin (IU) = (sample absorbance/ standard absorbance) x (standard weight/standard dilution) x (sample
263	dilution/sample weight) x standard potency x average weight
264	
265	2.7.4 Vitamin K (menadione)
266	Five milliliters of the standard, sample and blank solution were taken into test tube. In each test tube, 2 ml 2% solution of
267	2, 4-dinitrophenyl hydrazine (in hydrochloric acid and alcohol in ratio of 1:5 v/v) was added and mixed well. Then it was
268	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.

## 270 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

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## 274 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 ± SEM at confident level of determination (P=0.05).

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## 279 3. RESULTS AND DISCUSSION

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

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

Phytochemicals	Unripe DT fruit (mg/100g)	Ripe DT
		fruit(mg/100g)
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 ± 0.17 <sup>b</sup>	$5.12 \pm 0.27^{a}$
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 ± SEM of triplicate determinations (n=3).
 Values in the same row with the same superscript letters are not significantly different at 5% level.

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287 288 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 ± 0.05 <sup>b</sup>	11.81 ± 0.02 <sup>a</sup>	

Lipid	$1.85 \pm 0.00^{\circ}$	$2.35 \pm 0.00^{a}$
Crude fibre	20.11 ± 0.04 <sup>a</sup>	19.11 ± 0.03 <sup>b</sup>
Crude protein	$9.55 \pm 0.04^{a}$	$9.62 \pm 0.03^{a}$
Total carbohydrate	$73.08 \pm 0.06^{a}$	67.94 ± 0.06 <sup>b</sup>
Moisture	$8.56 \pm 0.00^{a}$	$8.28 \pm 0.00^{b}$
Energy content(kcal/100g) sample	347.2	331.4

289 Values are mean ± SEM of triplicate determinations (n=3).

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

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

Mineral	Unripe(mg/g)	Ripe(mg/g)	
Na	$2.12 \pm 0.00^{a}$	$2.01 \pm 0.00^{a}$	
К	12.22 ± 0.00 <sup>b</sup>	$12.53 \pm 0.14^{a}$	
Zn	$8.42 \pm 0.00^{a}$	$8.02 \pm 0.00^{b}$	
Са	$5.16 \pm 0.00^{a}$	$5.18 \pm 0.00^{a}$	
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	

<sup>294</sup> 295

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

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Parameters	Unripe DT fruit (mg/g)	Ripe DT fruit(mg/g)	
Vitamin B <sub>1</sub>	0.33 ± 0.01 <sup>b</sup>	$0.48 \pm 0.03^{a}$	
Vitamin B <sub>2</sub>	$0.14 \pm 0.00^{b}$	$0.28 \pm 0.01^{a}$	
Vitamin B <sub>3</sub>	$0.54 \pm 0.03^{b}$	$0.99 \pm 0.05^{a}$	
Vitamin B <sub>5</sub>	1.34 ± 0.07 <sup>b</sup>	$1.86 \pm 0.10^{a}$	
Vitamin B <sub>6</sub>	$1.85 \pm 0.10^{a}$	$1.63 \pm 0.09^{b}$	
Vitamin B7	$0.23 \pm 0.01^{b}$	$0.27 \pm 0.01^{a}$	
Vitamin B <sub>9</sub>	$0.14 \pm 0.01^{b}$	$1.21 \pm 0.06^{a}$	
Vitamin B <sub>12</sub>	$0.29 \pm 0.02^{b}$	$0.47 \pm 0.03^{a}$	
Vitamin C	66.99 ± 3.26 <sup>b</sup>	90.01 $\pm$ 4.74 <sup>a</sup> h same superscript letters are not significantly different at	

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

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Vitamin D

Parameters	Unripe DT fruit (IU)	Ripe DT fruit(IU)
Vitamin A	48.65 ± 2.56 <sup>b</sup>	129.37 ± 6.81 <sup>a</sup>

 $0.95 \pm 0.05^{a}$ 

 $0.97 \pm 0.05^{a}$ 

Vitamin E	1.11 ± 0.06 <sup>a</sup>	$1.17 \pm 0.06^{a}$	
Vitamin K	$1.57 \pm 0.08^{a}$	$1.2 \pm 0.06^{b}$	
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.			

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# 305 4. 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.

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# 315316 COMPETING INTERESTS

317 318 Declaration of competing interest should be placed here. All authors must disclose any financial and personal 319 relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of 320 potential conflicts of interest include employment, consultancies, honoraria, paid expert testimony, patent 321 applications/registrations, and grants or other funding. If no such declaration has been made by the authors, SDI reserves 322 to assume and write this sentence: "Authors have declared that no competing interests exist.".

# 324 AUTHORS' CONTRIBUTIONS

Authors may use the following wordings for this section: " 'Author A' designed the study, performed the statistical analysis,
 wrote the protocol, and wrote the first draft of the manuscript. 'Author B' and 'Author C' managed the analyses of the
 study. 'Author C' managed the literature searches..... All authors read and approved the final manuscript."

# 331 ETHICAL APPROVAL

332333 Authors have declared that no competing interests exist.

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