



### VARIATIONS IN VITAMIN COMPOSITION OF FOUR ACCESSIONS OF *Dialium guineense* (L.) FRUIT PULP

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#### ABSTRACT

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*Dialium guineense* is an important medicinal plant used in fighting diverse diseases because of the presence of vitamin c it contains. This study is aimed at varying the vitamin composition of *Dialium guineense* fruit pulp obtained from four accessions from different geopolitical locations (South-West, South-South, South-East, North-Central) in Nigeria. Different vitamins were considered which includes; Vitamin A, Vitamin B<sub>12</sub>, Vitamin C and Vitamin E. The vitamin composition of *D. guineense* fruit pulp were done according to the standard biochemical method. The result obtained for all the four accessions shows varying quantities in the vitamins investigated. Vitamin C has the highest amount in varying quantities with the trend: North-Central (Abuja) > South-South (Edo) > South-West (Oyo) > South-East (Abia), while Vitamin A: North-Central (Abuja) > South-West (Oyo) > South-South (Edo) > South-East (Abia), Vitamin B<sub>12</sub>: South-West (Oyo) > North-Central (Abuja) > South-South (Edo) > South-East (Abia), and Vitamin E: South-South (Edo) > South-West (Oyo) > North-Central (Abuja) > South-East (Abia). These accessions of *D. guineense* were high in Vitamin C, followed by Vitamin A, then Vitamin E, and Vitamin B<sub>12</sub>. The presence of all these vitamins are good indices that *D. guineense* is a very rich nutritional and medicinal plant with high potentials of vitamin content considering different accessions.

**Contribution/Originality:** The paper's primary contribution is finding how provenance/accession could alter the genetic make-up and vitamin composition of *Dialium guineense*. Since vitamin C is the main components of *Dialium* fruits, the experiment is set up to investigate how provenance could affect the different vitamin composition of *Dialium* fruit.

#### 1. INTRODUCTION

Consuming fruits and vegetables may usually aid in the prevention of chronic and degenerative diseases [1]. Vitamins, as part of the nutritional requirement of the human body, are gotten from fruits and plants in a substantial amount. Vitamins are a wide group of organic compounds that are needed only in minor quantities. However, they are essential constituents of food required for the normal growth, self-maintenance, and functioning of human and animal bodies [2]. Humans are not able to synthesize most vitamins and they consequently have to be obtained exogenously [3]. Vitamins are usually divided into two general classes: the fat-soluble vitamins, such

as A, D, E and K1, and the water-soluble vitamins, B1, B2, B6, nicotinamide, pantothenic acid, biotin, folic acid, B12 and C [4]. Usually, a balanced diet can adequately supply the vitamin requirement of the body. A deficiency can result in hypovitaminosis and, if more severe, in avitaminosis [5]. A vital dietary supplementary will also enhance the availability of these vitamins to the body, through plants, leaves, fruits, fruit pulps, and drugs e.t.c. Wild or commercialized fruits in tropical countries offer potential novel sources of macro and micro-nutrients as well as promoting health in rural areas. Among these wild fruits, *Dialium guineense* is found in many parts of sub-saharan Africa and remains a well-known nutritional and health-promoting fruit and food to the local population. *Dialium guineense* is a tree of an average height of 30 m with densely leafy crown, smooth greyish bark. Leaves are hairy and the flowers are usually whitish while the fruits are less circular and flattened. The pulp of the fruit is edible and sweet, with fairly low levels of ascorbic acid and tannin. It is a fairly good source of protein and minerals [6]. *Dialium guineense* also known as Velvet tamarind can be found in West African countries such as Ghana where it is known as Yoyi, Sierra Leone, Senegal, and Nigeria where it is known as Awin in Yoruba, Icheku in Igbo and Tsamiyar kurm in Hausa [7, 8]. The fruits are widely sold in local markets and are consumed fresh by people of all ages as a snack. Some elderly people consume a non-alcoholic drink made from the fruit. Also, the bark, roots and leaves of *Dialium guineense* have medicinal properties and are used for the treatment of a variety of health problems. The fruits of the plant are chewed by most women in Southeastern Nigeria to improve lactation and check genital infection [9]. The leaves and stem bark are also used as folklore remedies for the treatment of infections such as diarrhoea, severe cough, bronchitis, wound, stomachaches, malaria, fever, jaundice, ulcer and haemorrhoids [10]. The flour of *Dialium guineense* pulp can be incorporated in infant foods to enhance appetite and provide a remedy for the problem of scurvy and micronutrient deficiencies. Extracts from *Dialium guineense* plants have been shown to possess both anti-mutagenic and molluscicidal activities [11]. The fruits are used in medicinal remedies, as a source of vitamin C, as flavour in snacks and non-alcoholic beverages [12, 13]. Fruit pulp supplies high amount of micronutrients like sodium, magnesium and potassium. Bark and leaves are used against several diseases such as malaria [14]. The pulp is a source of Vitamin C which is needed by both adult and children. The leaves can be squeezed and apply on wounds, this is practised by wolofs of Senegal Devendra [15]. Okegbile, et al. [16] found a high content of vitamin C and other micronutrients in wild fruits when compared with nutrition supplied by other fruits such as oranges, Avogadro pear, pineapple, pawpaw and commercially produced fruits. Researchers have not focused on comparing vitamin c and other vitamin content of different accessions. Thus, the experiment is set up to investigate and evaluate how provenance could affect the genetic make-up and vitamin composition of *Dialium guineense*.

## 2. MATERIALS AND METHOD

### 2.1. Collection Area

Fruits of four accessions of *Dialium guineense* were collected from local markets in four geo-political zones; South-West (Oyo State) collected precisely from Oje market, South-South (Edo State) collected from Auchu market, South-East (Abia State) collected from Ahia Ukwu market and North-Central (FCT, Abuja) collected precisely from Gwagwalada market across Nigeria. All the accessions collected were identified and authenticated at the Taxonomy unit of Forestry Research Institute of Nigeria. Ibadan. Oyo State, Nigeria.

### 2.2. Seed Extraction

The *D. guineense* fruits collected from each accession were extracted in the nursery by manually de-pulping the seeds from the fruits. The pulp of the fruits was kept under a very cool condition and placed inside a closed container ready for analysis.

### 2.3. Vitamin Analysis

The vitamin composition present in the pulp of *D. guineense* obtained from four geo-political zones were determined using the standard method described by Achikanu, et al. [17]; AOAC [18]. The vitamins investigated include A, C, E, and B<sub>12</sub> for all the four accessions.

### 2.4. Determination of Vitamin A (Retinol)

A quantity, one gram, of the sample was weighed and macerated with 20mls of n-hexane in a test tube for 10 minutes. Then 3mls of the upper hexane extract was transferred into a dry test tube in duplicates and evaporated to dryness. Following this, 0.2ml of acetic anhydride chloroform reagent was added and 2ml of 50% trichloroacetic acid (TCA) in chloroform was also added. The absorbance was taken at 15 seconds and 30 seconds intervals at 620nm.

### 2.5. Determination of B<sub>12</sub> (Cobalamin)

9-10 g of the sample was ground and homogenized to a fine powder with the use of mortar and pestle, combined with the use of liquid nitrogen until the powder of the sample to be homogenized (the best possible). Afterwards, an aliquot was accurately weighed and transferred into a flask. 60 ml of 50 mM acetate buffer (pH 4.0), 1 ml of KCN (1% w/v), 1 g pepsin, 300 µl α-amylase were added, and the suspension was incubated at 37°C for 3 h under agitation. The enzymatic reaction was stopped by incubating the sample in boiling water at 100°C for 30-35 min. After cooling to room temperature, the solution was centrifuged at 4,000 rpm for 25 min at 4°C. The supernatant was collected and filtered through filter paper. The solution was quantitatively transferred to a volumetric flask. Plant samples with high moisture content were cut into small particles in paper containers. The containers were placed in an incubator and dried at 90°C for 24 h. The dry weights of the plant samples were derived from these procedures. All data also from fresh plant samples were corrected to dry weight. Heating (100°C, 30 min) was done, Cooking was also done using the ice bath, 30min, centrifugation 5000rpm 3°C 25mins. Filtration of supernatant was done and the PH was adjusted to 7. Water dilution was done up to 100ml. The sample was then purified and concentrated, after which the B<sub>12</sub> was analyzed [19].

### 2.6. Determination of Vitamin C (Ascorbic acid)

About 0.5g of the sample was weighed, macerated with 10mls of 0.4% oxalic acid in a test tube for 10 minutes, centrifuged for 5 minutes and the solution filtered. 1ml of the filtrate was transferred into a dry test tube in duplicates, 9mls of 2,6- di-chlorophenol indophenol was added and absorbance was taken at 15 seconds and 30 seconds interval at 520nm.

### 2.7. Determination of Vitamin E (Tocopherol)

One gram (1g) of the original sample was weighed, macerated with 20mls of n-hexane in a test tube for 10 minutes and centrifuged for 10 minutes. The solution was filtered, 3mls of the filtrate was transferred into a dry test tube in duplicates and evaporated to dryness in a boiling water bath. Following this, 2mls of 0.5N alcoholic potassium hydroxide was added and boiled for 30 minutes in a water bath. Then 3mls of n-hexane was added and was shaken vigorously. The n-hexane was transferred into another set of test tubes and evaporated to dryness. A volume, 2mls, of ethanol was added to the residue. Another volume, 1ml of 0.2% ferric chloride in ethanol was added. Then 1ml of 0.5% α<sup>1</sup> - α<sup>1</sup> di-pyridyl in ethanol was added followed by the addition of 1ml of ethanol to make it up to 5mls. The solution was mixed and absorbance was taken at 520nm against the blank.

### 2.8. Data Analysis

Data obtained were expressed in the graphical Figures where each bar represents the mean and the error bar, the standard deviation of triplicate measurement, analysis of variance and Duncan test was done to determine significant differences in the means of the measured vitamins from the four sources,  $p < 0.05$  was considered significant; thus, bars with same alphabets are not significantly different. Statistical analysis was done using IBM SPSS version 20.

### 3. RESULTS

The results of the vitamin's concentration in the accessions collected from the four locations are presented in Figure 1-4. Vitamin A content ranged from 1.09-6.32 mg/100g, vitamin C content ranged from 13.00-48.63 mg/100g, vitamin E content ranged from 0.61-3.26 mg/100g while vitamin B<sub>12</sub> content ranged from 1.03-2.59mg/100g. The result also shows that the highest content of vitamin A, C and B<sub>12</sub> was found in accession from Abuja, while for Vitamin E, the highest concentration was found in accession from Edo, however, the lowest levels of all the vitamins was found in accession from Abia. There are significant differences ( $p < 0.05$ ) in the means of all the vitamins concentrations in the four accessions

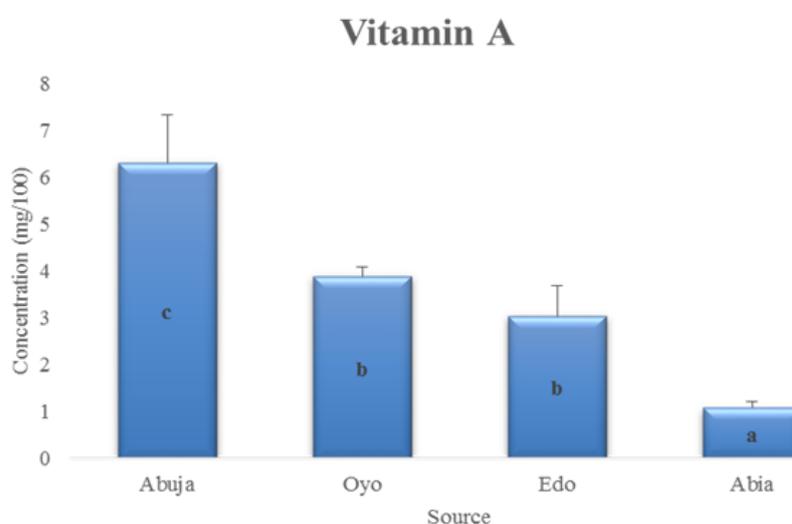


Figure-1. Vitamin A Concentration in Four Accessions of *Dialium guineense* Fruit Pulp.

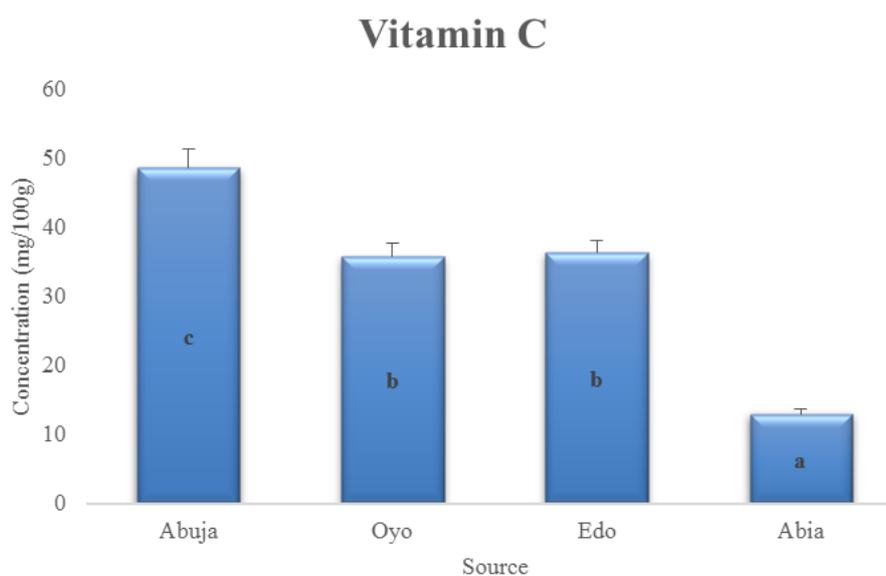


Figure-2. Vitamin C Concentration in Four Accessions of *Dialium guineense* Fruit Pulp.

## Vitamin E

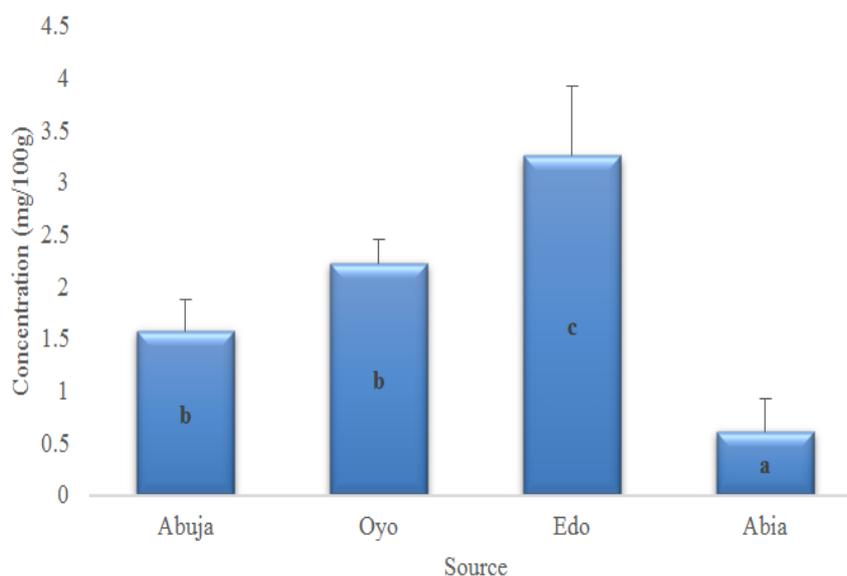


Figure-3. Vitamin E Concentration in Four Accessions of *Dialium guineense* Fruit Pulp.

## Vitamin B12

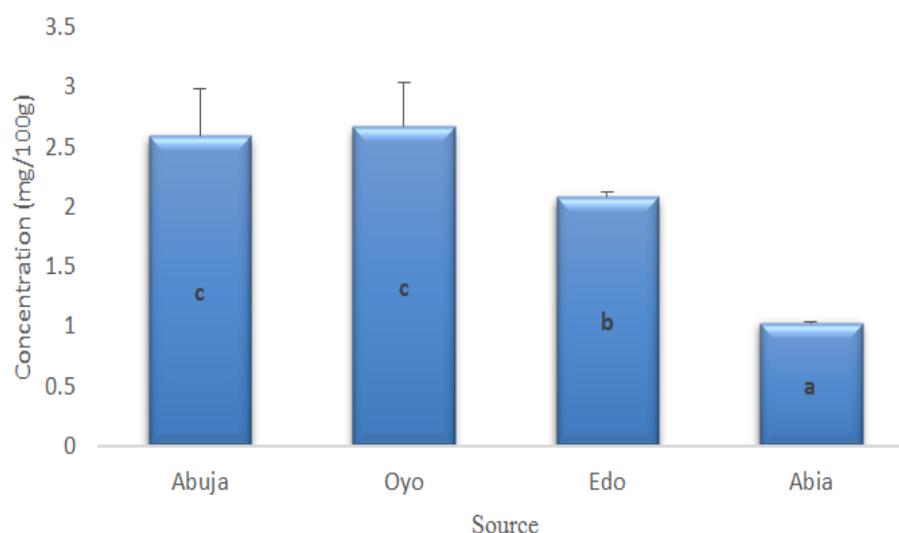


Figure-4. Vitamin B<sub>12</sub> Concentration in Four Accessions of *Dialium guineense* Fruit Pulp.

## 4. DISCUSSION

Vitamins are usually required in very small quantities. They are usually grouped into two as fat-soluble vitamins (A, D, E and K) and Water-soluble vitamins (B and C). The fat-soluble vitamins can be found in the liver and the adipose tissues, while the water-soluble are usually eliminated out of the body. Vitamin C possesses an antioxidant character and have the potential for maintaining the connective tissues, facilitates the absorption of dietary iron from the intestine and wound healing [20]. Vitamin A and C content are adequate to supplement other dietary sources. Vitamin A helps in the treatment of eye problem while lack of vitamin C causes scurvy and gingivitis. Vitamin E and C are important antioxidants which protect the outer membranes from oxidative stress/damage [21]. The recommended dietary allowance of vitamin C for adults is 45 mg/day [22]. Vitamin C is one of the major components of the fruit pulp of *D. guineensis*. In this study, the fruit pulp across the accession in all the locations contain ascorbic acid (Vitamin C), tocopherol (vitamin E), retinol (vitamin A) and Vitamin B<sub>12</sub> which recommends them as good dietary supplements, antioxidants, and also essential for clear vision.

Vitamins are also important nutrients, vitamin A is important for normal vision, gene expression, growth and immune function by its maintenance of epithelial cell functions [23]. For all the accessions investigated for Vitamin A, North-Central (Abuja) gave the highest concentration (6.32mg/100g), this level is significantly higher than those of the other accessions ( $p < 0.05$ ), this is followed by accessions from South-West (Oyo) (3.89mg/100g) and South-South (Edo) (3.05mg/100g) whose levels are comparable, then, accessions from South-East (Abia) which had the least value (1.09mg/100g). The vitamin A concentration range reported in this study is lower when compared with the findings of Achikanu, et al. [17] where the result for vitamin A in all the plant samples investigated gave very high values except for *Ficus capensis* which gave (25.22mg/100g). However, the value reported by Ocheja, et al. [24] for cashew nutshell for vitamin A was (8.12mg/100g) which was higher than the value reported for all the accessions across the four geo-political zones. In the research finding of Ogbonna, et al. [25] he reported lower figures for all the musa spp investigated compared to all the accessions except for Abia accession that gave 1.09mg/100g. *Terminalia catappa* flour gave a very high value of 6.45 mg/100g in the research work of Anuforo, et al. [26] and the value was higher than all the reported result for all the accessions across the four geo-political zones. The recommended dietary allowance and the daily dose of vitamin A for adults is 0.8-10 mg/day [4]. Vitamin B<sub>12</sub> plays an important role in promoting carbohydrate, protein and normal fat metabolism, it is essential in the formation of red blood cells, the normal functioning of the nervous system and on the translocation of the methyl group in DNA synthesis. The result of the Vitamin B<sub>12</sub> obtained for all the accessions across the four geo-political zones shows that South West (Oyo) has 2.67mg/100g which is the highest in all the accessions collected, this is followed by North-Central (Abuja) which has 2.59mg/100g, then South-South (Edo) which gave 2.08mg/100g, and finally South-East (Abia) which gave 1.03mg/100g. The vitamin B<sub>12</sub> result for all the accessions ranges from 1.03-2.67mg/100g. The result obtained in the findings of Achi, et al. [27] for *Ficus capensis* leaves gave 0.37mg/100g which is very low compared to all the values gotten for the accessions investigated in this studies across the country. Ocheja, et al. [24] reports that no vitamin B12 exist in his research findings for cashew nutshell which is contrary to the result obtained in this research study. *D. guineensis* leaves contains ascorbic acid (Vitamin C), tocopherol (vitamin E), retinol (vitamin A) and Vitamin B<sub>12</sub> which recommends them as good dietary supplements, antioxidants, and also essential for clear vision. The highest value gotten for vitamin C was recorded for North-Central accession (Abuja) which is (48.63mg/100g), followed by South-South (Edo) which as (36.34 mg/100g), South-West (Oyo) which gave (35.81mg/100g), and South-East (Abia) whose result was (13.00mg/100g). The results have gotten for all the vitamin accessed for all the fruit pulp varied significantly ( $p < 0.05$ ) among the four locations. The vitamin c content (33.33mg/100g) gotten for *Dialium guineense* fruit pulp in the finding of Ogungbenle [28] was lower than the value recorded for Abuja, Oyo and Edo but on the contrary higher than Abia. The vitamin C value obtained in this study for Abuja is on the high side compared to that reported by Ocheja, et al. [24] who in his findings reports a value of 46.41mg/100g for Cashew nutshell but comparably higher than that reported for other accessions. Also, all the values reported by Ogbonna, et al. [25] for the different stages of development of Musa spp for their vitamin C are all lower than the values reported in these studies. Offor and Igwe [29] also reported a very high vitamin c content in its findings for *Solanum aethiopicum* and *Solanum macrocarpon* which corroborates our findings too for all the accessions. There is an astronomical difference between all the result obtained in this experiment for vitamin c compared to that which was reported by Achi, et al. [27] for all the vitamins analyzed for *Ficus capensis* leaves. The vitamin c content reported for *Terminalia catappa* endocarp flour is 14.54mg/100g and it is far lower than the values reported for *D. guineense* accessions in this study, except for Abia accession that is 13.00mg/100g. Fully matured and not darkened fruits gave a very low vitamin c result in the findings of Duru, et al. [30] compared to the result gotten in this study for all the accession across the geo-political zones. Vitamin c content of *Adansonia digitata* fruit pulp in the findings of Chabite, et al. [31] from two districts of Mozambique which are Nacarôa (N) and Erâti (E) gave 62.38mg/100g and 52.83mg/100g which is higher than all the value gotten for *D. guineense* fruit pulp in all the four accession across the country. Also, the

result obtained for all the accessions studied is astronomically higher than the value gotten in the findings of Asoiro, et al. [32] who reported 0.3g/kg of *D. guineense* fruit pulp. Also, the value (4.5mg/100g) gotten for vitamin c in *D. guineense* fruit pulp growing in the Benin Republic by Gnansounou, et al. [33] was very low compared to that reported for all accessions in this study. Fruit pulp result obtained in the findings of Jacob, et al. [34] gave 39.93mg/100g for *Dialium guineense*, which is comparably lower than the value gotten for Abuja accession but higher than all the other accessions. The recommended dietary allowance of vitamin C for adults is 45 mg/day [22]. Vitamin E is a powerful antioxidant which helps to protect cells from damage by free radicals and it is vital to the formation and normal function of red blood cell and muscles [23]. The result gotten for all the different accession obtained across the four geopolitical zones for vitamin E shows that South-South (Edo) has the highest value (3.26mg/100g) reported followed by South-West (Oyo) which gave (2.23mg/100g), then North-Central (Abuja) which shows (1.58mg/100g) and finally South-East (Abia) whose report gave (1.03mg/100g). The vitamin E result for all the accessions ranges from 1.03-3.26mg/100g. All the values reported for vitamin E in the findings of Duru, et al. [30] for all the fruits at different stages of maturation are very low compared to the values reported in this studies for all the accessions across the country. Also, all the green leafy vegetables in the finding of Achikanu, et al. [17] gave higher values compared to all our accessions collected. Ogbonna, et al. [25] reported in his findings for cashew nutshell a lower value of 0.46mg/100g which is lesser than all the values reported in our findings. The recommended dietary allowance and the daily dose of vitamin E for adults is 8-10 mg/day [4].

## 5. CONCLUSION

The presence of all these vitamins is good indices that *Dialium guineense* is a very effective nutritional plant and a good dietary supplement for human being. Moreover, the high content of vitamin C indicated in all the accessions, and the varying quantities of other vitamins across the geopolitical zones depict that variation in environment and location could affect the genetic make-up, medicinal value and nutritional composition of *D. guineense*. The following trend was observed for vitamin composition in the four accessions obtained across different geo-political zones: North-Central (Abuja) gave the highest value for Vitamin C and Vitamin A, while South-West (Oyo) gave the highest amount for Vitamin B<sub>12</sub> and South-South (Edo) gave the highest value for Vitamin E.

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