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Article

Phytochemical constituents, vitamin composition and in vitro antioxidant potential of *Dialium guineense* fruit pulp

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Abstract: Medicinal herbs have been extensively utilized in the remediation of various health conditions. *Dialium guineense* fruit pulp, also well known as Velvet Tamarind is widely consumed in West Africa for its dietary and medicinal properties. The study aims to analyze the phytochemical constituents, vitamin content and the in vitro antioxidant effect of *Dialium guineense* fruit pulp (DGFP). The phytochemical constituents, vitamins (C, E, B1-12) composition, and in vitro antioxidant activity were examined utilizing standardized analytical methods. The qualitative and quantitative phytochemical screening of the fruit pulp of *Dialium guineense* was also carried out; the result indicated the presence of flavonoids, alkaloids, saponins, tannins, terpenoids, phenols, steroids, and cardiac glycosides in varying concentrations. The vitamin composition revealed that vitamin C was higher than other vitamins in the fruit pulp. The DPPH (2,2-diphenyl-1-picrylhydrazyl) and nitric oxide scavenging assay showed high radical scavenging activity while the FRAP (Ferric reducing antioxidant power) assay revealed significant reducing power. This indicates that *Dialium guineense* fruit pulp has potential health benefits.

Keywords: *Dialium guineense* fruit pulp; antioxidant activity; phytochemical; vitamin; phenolic acid

1. Introduction

Natural compounds have been deployed as medicaments for a long time, though some have been recently replaced by newer pharmaceutics. In modern times, natural agents such as botanical extracts have garnered the attention and fascination of the scientific community due to their rich source of chemical diversities with potential applications in managing chronic diseases [1]. They are an important source for the prevention and management of several ailments. The capability for most plants to have medicinal tendencies lies in their phytochemical constituents which enable them to show off certain chemical responses in the human body [2]. These compounds produced by these plants serve as a kind of protection against microbial attack, insects, and herbivores. In addition to these phytochemicals, plants are also a powerful source of significant vitamins, such as vitamins B, C and E, which both play an important role in various biological functions. These add to the therapeutic values of plants along with the other bioactive compounds. Plant parts like bark, leaves, stems, and even fruits and seeds synthesize these compounds naturally [3].

Dialium guineense also known as African black velvet tamarind, is a relatively sizable tree species prevalent across various regions of Africa, particularly West

Africa, the Central African Republic, and Chad. This tree falls under the family Fabaceae and the sub-family Dialioidaea; at many times, it grows up to an average height of 30 meters with a densely leaved crown and, in some cases, may look shrubby. The leaves are hairy, the flowers mostly white, the fruits flattened and more oval in shape. In Eastern Nigeria, the Igbo call it "icheku"; the Yorubas of Western Nigeria refer to it as "awin"; while among the Hausas of Northern Nigeria, it is known as "tsamiyar kurm." All parts of *D guineense*. The roots, bark, stems, leaves, fruits, and seeds of guineense are utilized medicinally [4]. It is one of those fruits that are annually consumed as a snack by all categories of individuals. The food industry has also employed it in the manufacture of drinks, sweets, and jams. [5].

The primary objective of this study is to assess the phytochemicals, vitamins present in the fruit pulp of *Dialium guineense*, and as well, as evaluate its antioxidant potential.

2. Materials and methods

2.1. Plant collection

The study utilized the fruit pulp of *Dialium guineense*. The fruit was obtained from the Forestry Department Garden at Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Botanical identification was performed by the Department of Plant Science and Biotechnology at the same university, and a voucher specimen was kept in the Herbarium for future reference.

2.2. Plant extract preparation

The fruits of *Dialium guineense* were harvested at full maturity and was collected early in the morning. The seeds were removed from fresh *Dialium guineense* fruit pulp, the fruit pulp was air-dried and then into a fine powder. Then, a known quantity (500 g) of the pulverised pulp of *Dialium guineense* was soaked in 1.5 litres of ethanol for 48 h The obtained mixture was filtered with Whatman No. 1 filter paper and concentrated by a rotary evaporator to give a solid residue.

2.3. Qualitative phytochemical analysis

To elucidate the bioactive profile of *Dialium guineense* fruit pulp, a phytochemical analysis was performed following established methodologies described by Evans, Harborne and Sofowora [6–8].

2.3.1. Flavonoid screening

A two-gram sample of the extract was subjected to reflux extraction in ethyl acetate for a duration of three minutes. The resulting filtrate was subsequently analyzed for flavonoid content. A qualitative test was conducted by adding a diluted ammonium solution to a four-milliliter aliquot of the filtrate. The formation of a yellow precipitate served as an indicator for the presence of flavonoids. Furthermore, an aluminum chloride test was performed using a 1% solution of aluminum chloride. A slight yellow coloration in the mixture confirmed the presence of flavonoids.

2.3.2. Alkaloids screening

The presence of alkaloids in the sample was verified by a series of chemical tests. A 0.2-gram aliquot was subjected to acid hydrolysis with 5 milliliters of 2% hydrochloric acid, followed by filtration. Ten milliliters of the filtrate were subsequently treated with Mayer's reagent, Wagner's reagent, and 1% picric acid, respectively. The formation of a creamy white precipitate with Mayer's reagent, a reddish-brown precipitate with Wagner's reagent, and a yellow precipitate with 1% picric acid unequivocally confirmed the presence of alkaloids in the original sample.

2.3.3. Tannins screening

Two grams of extract heated in 5 mL 45% ethanol for 5 min. After cooling, the mixture was filtered. Filtrate used for screenings, starting with Lead sub-acetate: 1 mL filtrate + 3 drops lead sub-acetate solution. Tannins detected by gelatinous precipitate. Bromine water test: 1 mL filtrate + 0.5 mL bromine water. Tannins are indicated by a light brown precipitate. Ferric chloride screening: Filtrate diluted with 1 mL distilled water + 2 drops ferric chloride solution. Tannins' presence is shown by color shift from greenish to black.

2.3.4. Cardiac glycosides screening

A weighed quantity of 2 grams of the extract was combined with 30 milliliters of distilled water and 15 milliliters of diluted sulfuric acid. The mixture was subsequently subjected to a 5-minute water bath treatment. Following filtration, the resulting filtrate was utilized for further analysis. A 5-milliliter aliquot of the filtrate was combined with 0.3 milliliters each of Fehling's solutions A and B, then rendered alkaline. Gentle heating of the mixture in a water bath for 2 min led to the formation of a brick-red precipitate, indicative of the presence of glycosides.

2.3.5. Tannins screening

To 2 grams of the extract, about 5 mL of 45% ethanol solution was added; this was then boiled in a water bath for 5 min. After cooling and filtering, the filtrates were used for the following tests: Bromine water test: 1 mL of filtrate was added to 0.5 mL of bromine water, a light brown precipitate showed the presence of tannins. In the lead subacetate test, filtrate was mixed with three drops of lead subacetate solution; the appearance of a creamy gelatinous precipitate showed positivity for tannins. Finally, in the ferric chloride screening, the filtrate was diluted with 1 mL of distilled water and 2 drops of ferric chloride solution. The color shifting from greenish to black after the addition of the ferric chloride solution confirmed the presence of tannins.

2.3.6. Saponin screening

A 0.1 g extract was boiled in 5 mL of water for 5 min and then filtered. The filtrate was used for tests: Emulsion screening 1 mL mixed with olive oil formed emulsion. Bubbling test 1 mL mixed with water, shaken, observed for froth.

2.3.7. Steroids screening

About 1 gram of the extract was refluxed with 9 mL ethanol, then filtered. Liquid reduced to 2.5 mL. 5 mL hot water added to filtrate, left for 1 h. Waxy

substance removed by filtration. After filtration, 2.5 mL was chloroform added. 0.05 mL chloroform extract was added to 1.0 mL concentrated sulfuric acid in the test tube. Reddish-brown coloration indicates the presence of steroids.

2.3.8. Terpenoids screening

Exactly 1 g of the plant extract was soaked in 10 mL of chloroform and the mixture was stirred occasionally. A volume of 0.5 mL of the chloroform extract was evaporated to dryness in a water bath. The grey-colored residue obtained indicated the presence of terpenoids.

2.3.9. Phenolic acids screening

Ferric chloride Test: The extract was mixed with 3–4 drops of ferric chloride. The development of a bluish-black hue shows that phenolic acids are present.

2.4. Quantitative phytochemicals analysis

2.4.1. Measuring flavonoid content

Flavonoid content was determined by the method described by Harbone [7]; 5 grams of the extract were heated under refluxing and mixed with 50 mL of HCl this was left to stand for 30 min. The mixture was cooled and filtered through Whatman No. 1 paper. A portion of the filtrate was then diluted with an equal amount of ethyl acetate and added, slowly drop by drop. This was then filtered into a crucible previously weighed. The filtrate was dried in the oven at 60 °C until all liquid had evaporated. The weight of the crucible after drying less than its original weight determined the flavonoid content.

$$\text{Flavonoid content (mg/100 g)} = \frac{\text{Weight of flavonoid precipitate (mg)}}{\text{Weight of } plant \text{ material used (g)}} \times 100$$

2.4.2. Measuring alkaloid content

Using Harborne's methodology [7], 5 grams of the specimen were carefully measured and subjected to a 2-hour extraction process with 200 mL of a solution consisting of 10% acetic acid in ethanol. Subsequently, the filtrated extract underwent a concentration process utilizing rotary evaporation until it reached a quarter of its original volume. A gradual introduction of ammonium hydroxide (15 drops) was carried out into the extract until the point where precipitation was deemed complete. The resultant alkaloid precipitate was then filtered, rinsed with diluted ammonium hydroxide (20 cm^3 of 0.1 M), and subsequently desiccated until reaching a consistent weight.

$$\text{Alkaloid content (mg/100 g)} = \frac{\text{Weight of } alkaloid \text{ precipitate (mg)}}{\text{Weight of plant material used (g)}} \times 100$$

2.4.3. Measuring tannins content

The tannic acid content was determined using the method outlined by Pearson [9]. A standard solution of tannic acid was prepared by dissolving 10 mg of tannic acid in 100 mL of methanol. Serial dilutions of this standard solution were then prepared to obtain concentrations of 20, 40, 60, 80, and 100 mg/L in 100 mL volumetric flasks. One gram of the extract was crushed in 50 mL of methanol and filtered. The filtrate was subsequently mixed with 0.3 mL of 0.1 N ferric chloride in

0.1 N HCl and 0.3 mL of 0.8 mM potassium ferricyanide. The absorbance of the resulting mixture was measured at a wavelength of 720 nm.

$$\text{Tannin conc.}\,(\text{mg}/100\text{g}) = \frac{\text{Absorbance of sample}}{\text{Gradient Factor(slope)}} \times \text{Dilution Factor}$$

2.4.4. Measuring glycosides content

A scientific procedure described by Harborne [7] was followed for this analysis. The sample was reduced to powder and suspended in water, then added to a lead acetate solution. After filtration, chloroform was added and the mixture was shaken. The bottom layer was collected, evaporated, and dissolved in acetic acid. Ferric chloride and sulfuric acid were added, and the solution was incubated in the dark. Finally, the absorbance was measured at 530 nm using a spectrophotometer.

$$\text{Glycosides content}\,(\text{mg}/100\text{ g}) = \frac{\text{Absorbance of sample}}{\text{Gradient Factor(slope)}} \times \text{Dilution Factor}$$

2.4.5. Measuring saponin content

A scientific procedure described by Auta [10] was followed for this analysis The sample was soaked in 10 mL of petroleum ether, then transferred into a beaker. An additional 10 mL of petroleum ether was transferred into the sample, then into the beaker again. Filtrates from the two decantations were mixed and evaporated to dryness, then the residue was diluted with 6 mL ethanol. With the help of a pipette, two milliliters of the solution that was formed were transferred into the test tube. Chromogen color reagent-was added to it. The solution was allowed to stand for 30 min. A standard (disogenin) was prepared at different dilution under identical condition.

$$\text{Saponin conc.}\,(\text{mg}/100\text{ g}) = \frac{\text{Absorbance of sample}}{\text{Gradient Factor(slope)}} \times \text{Dilution Factor}$$

2.4.6. Measuring steroid content

The presence of steroids was investigated using a method established by Edeoga [11]. In brief, a precisely measured amount of each extract was mixed with 100 mL of fresh distilled water and thoroughly blended. The resulting mixture was then filtered, and the filtrate was passed through a column containing a 0.1 Mm ammonium hydroxide solution (pH 9) to isolate specific compounds. Two mL of this isolated fraction were added to a test tube and combined with 2 mL of chloroform. The mixture was then cooled with ice and three milliliters of acetic anhydride were carefully added. Finally, two drops of concentrated sulfuric acid were cautiously introduced. A standard steroid solution was prepared and processed in the same way. The amount of light absorbed by both the standard solution and the prepared extract was measured at a wavelength of 420 nm using a spectrophotometer.

$$\text{Steriod conc.}\,(\text{mg}/100\text{ g}) = \frac{\text{Absorbance of sample}}{\text{Gradient Factor(slope)}} \times \text{Dilution Factor}$$

2.5. Measuring phenolic acid content

The Folin-Ciocalteu method [12] was used to determine the total phenolic content of the plant extract. One milliliter of the extract was mixed with Folin-Ciocalteu reagent, followed by the addition of sodium carbonate solution. The mixture was diluted to 10 mL with distilled water and incubated in the dark for 90 min. The absorbance of the solution was then measured at 725 nm using a spectrophotometer, with a blank reagent and gallic acid as a standard

$$\text{Phenolic acid conc.}\,(\text{mg}/100\ \text{g}) = \frac{\text{Absorbance of sample}}{\text{Gradient Factor(slope)}} \times \text{Dilution Factor}$$

Measuring terpenoids content

About 1 g of the extract was macerated with 50 mL of ethanol by following the method of Harborne [7], then 2.5 mL portion of the strained liquid was mixed with a 5% phosphomolybdic acid solution. Gradually, concentrated H_2SO_4 was added. The solution was incubated for 30 min before being diluted with ethanol to a volume of 12.5 mL. The measurement of absorbance was taken at a wavelength of 700 nm. The terpenoid standard was mixed with known concentrations within the range of 5–25 mg/mL, and the previously mentioned procedure was then performed.

$$\text{Terpenoids conc.}\,(\text{mg}/100\ \text{g}) = \frac{\text{Absorbance of sample}}{\text{Gradient Factor(slope)}} \times \text{Dilution Factor}$$

2.6. Determination of vitamin composition of *Dialium guineense* fruit pulp

The vitamin compositions were determined as follows using the methods according to Okwu and Josiah [13].

2.6.1. Measuring vitamin B_1 (thiamine) levels

Five grams of the specimen were homogenized with 50 mL of ethanolic sodium hydroxide. Subsequently, the mixture was filtered into a 100 mL flask. Ten mL of the resulting filtrate was carefully pipetted, and the color development ensued with the addition of 10 mL of potassium dichromate. The absorbance was measured at 360 nm. A parallel blank specimen was meticulously prepared using an identical procedure, and its absorbance was also quantified at 360 nm.

$$\text{Vit. B1 conc.}\,(\text{mg}/100\ \text{g}) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

2.6.2. Measuring vitamin B_2 (riboflavin) levels

The mixture was filtered into a 100-millilitre flask. 10 mL of the filtered solution was transferred into a 50-millilitre volumetric flask. Into the flask, 10mL of potassium permanganate at 5% and hydrogen peroxide at 30% were both added. The extract was then allowed to incubate in the water bath for about half an hour. After that, the addition of 2 mL of 40% sodium sulfate solution followed, and the solution was diluted up to the mark of 50 mL. The final solution absorbance was finally measured by the spectrophotometer at an average wavelength of 510 nm.

$$\text{Vit. B2 conc. (mg/100 g)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

2.6.3. Measuring vitamin B_3 (niacin) levels

Five grams of the extract underwent digestion with 50 mL of 1 N sulfuric acid for 30 min. The resultant digest was then neutralized using an ammonia solution and subsequently filtered. About 10mL of the liquid that passed through the filter was cautiously moved to a 50 mL volumetric flask. Afterwards, 5 mL of potassium cyanide solution (0.05 M potassium cyanide in distilled water) were added, and the solution was made acidic with 5 mL of 0.02 N sulfuric acid. The spectrophotometer measured the absorbance of the solution at 470 nm wavelength.

$$\text{Vit. B3 conc. (mg/100 g)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

2.6.4. Measuring vitamin B_6 (pyridoxine) levels

About 50 mg of pyridoxine hydrochloride was transferred into a 100 mL flask containing distilled water. Various quantities of the stock solution were then dispensed into eight 50 mL flasks. Buffer solutions and ferric ammonium sulfate were subsequently introduced. The mixture was agitated and allowed to stand for 5 min. Additional distilled water was incorporated to achieve a final volume of 25 mL, ensuring thorough homogenization of the solutions. A standard solution was prepared similarly. The absorbance was read at a wavelength of 465 nm using a spectrophotometer.

$$\text{Vit. B6 conc. (mg/100 g)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

2.6.5. Measuring vitamin B_{12} (cobalamin) levels

About 5 g of the extract was weighed, and vitamin B_{12} was extracted using 0.1 N HCl as the solvent. The mixture of sample and solvent was warmed in a water bath for 30 min, cooled down, and filtered to gather the filtrate. The filtrate was exposed to cyanocobalamin to produce a hue, which was analyzed with a spectrophotometer at 361 nm. The vitamin B12 concentration was determined by comparing the sample's absorbance to a standard calibration curve.

$$\text{Vit. B12 concentration (mg/100 g)} = \frac{\text{Absorbance of sample}}{\text{Gradient Factor(slope)}} \times \text{Dilution Factor}$$

2.6.6. Measuring vitamin C (ascorbic acid) levels

A weight of not less than ten grams of the extract was determined and mixed with eighty mL of C_2H_5OH and twenty mL of distilled H_2O.the resulting mixture was agitated for two hours, filtered and the volume ascertained. About 5 mL of the extract was introduced and 2.5 mL of 1M H_2SO_4 was added to the beaker then 1 mL of 0.05 M of iodine solution was also introduced and stored. 0.05 M of iodine solution was used as the titer. After filtration, a bluish-black color was observed.

$$\text{Vit. C conc. (mg/100 g)} = \frac{\text{vI} \times \text{mI} \times \text{MWvit c} \times 100}{\text{Weight of sample(g)}} \text{concentration of standard}$$

where:

vI = Volume of iodine used

mI = molarity of iodine

MW vit C = molecular weight of vitamin c

2.6.7. Measuring vitamin E (tocopherol) levels

A sample weighing one gram was subjected to heat in a solution containing methanol and sulfuric acid in a proportion of 90:10 for a duration of 30 min. Then, the combination was moved to a funnel and rinsed with diethyl ether three times individually. Each instance involved gathering the ether layer. The ether layer was dried for 30 min and then evaporated completely. Next, the remaining substance was mixed with ethanol. Separate tubes were used to hold 1 mL of this solution and an equivalent quantity of a standard vitamin E solution. Following the addition of additional alcohol and concentrated nitric acid, the mixtures were left to stand for a period of 5 min. In the end, the light absorption of each solution was assessed at a wavelength of 410 nm, with a blank solution serving as the baseline (with an absorbance value of zero).

$$\text{Vit. E conc. (mg/100 g)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of solution}} \times \text{concentration of standard}$$

2.7. Antioxidant potential (based on in vitro studies)

2.7.1. Antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging

This was determined according to the method described by Molyneux [14]. Plant extract samples and a DPPH solution (0.1 mM in ethanol) were mixed in a microtiter plate at various concentrations (50, 100, 200, and 400 µg/mL), and incubated in the dark for 30 min The scavenging activity of the extract was expressed as a decrease in absorbance at 515 nm, which was measured using a spectrophotometer. The cuvette filled only with ethanol was used as a blank. Ascorbic acid was used as a positive control

$$\text{Scavenging activity percentage (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times \frac{100}{1}$$

2.7.2. Ferric ion reduction test for antioxidant potential

The FRAP assay was carried out to the method of [15]. The stock solution was serially diluted to obtain 50, 100, 200 and 400 µg/mL test concentrations. 1 mL of FRAP working reagent, containing acetate buffer, 2, 4, 6-tri-(2-pyridyl)-1, 3, 5-triazine and FeCl3.6H2O in a 10:1:1 ratio, was added to two 50 µL replicate aliquots of the diluted samples. Ten minutes later, the absorbance at 593 nm was determined with the reagent blank serving as the background. All specimens were tested at standard room temperature and kept out of direct sunlight. Then, the antioxidant capacity of the sample was quantified as an EC50 value by comparing its absorbance to a standard curve of ascorbic acid.

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}}$$

2.7.3. Nitric oxide scavenging activity

According to the method delineated by Marcocci [16], a solution of sodium nitroprusside (SNP) was prepared to a final concentration of 100 mM in phosphate-buffered saline (PBS) pH 7.4. The plant extracts were prepared in PBS at the given concentrations: 50, 100, 200, and 400 µg/mL. A 2 mL mixture was prepared by adding 0.2 mL of the SNP stock solution to give a final concentration of 10 mM with 1.8 mL of either the plant extract samples or PBS (control). The mixtures incubated at 25 ℃ for 150 min. One milliliter of each mixture, after incubation, was mixed with 1 mL of Griess reagent comprising 1% sulphanilic acid in solution of 20% glacial acetic acid, and 0.1% naphthylethylene diamine dihydrochloride in the same acetic acid solution. The resultant solutions were read for absorbance at 540 nm.

$$\text{Percentage Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}}$$

2.8. Statistical analysis

The data was represented in terms of the mean and standard deviation. Subsequently, a one-way ANOVA analysis was conducted, followed by the application of Turkey's post hoc test. Discrepancies among the groups were identified through the utilization of SPSS software version 23.0. The significance threshold was set at $p < 0.05$.

3. Results

3.1. Phytochemical composition

Phytochemical compounds of *D. guineense* fruit pulp extract were screened and quantified. The presence of flavonoids, alkaloids, tannins, terpenoids, phenolic acid, saponins, steroids and cyanogenic glycosides was detected as shown in **Table 1**.

Table 1. Phytochemical analysis of *D. guineense* fruit pulp.

Phytochemical compound	Qualitative	Quantitative
Flavonoids	+++	20.28 ± 0.24(mg/100 g)
Alkaloids	+	8.00 ± 0.24 (mg/100 g)
Tannins	+	4.63 ± 0.25(mg TAE/100 g)
Terpenoids	+	1.15 ± 0.10 (mg LE/100 g)
Saponins	++	14.05 ± 0.24 (mg DE/100 g)
Phenolic acid	++	17.64 ± 0.33 (mg GAE/100 g)
Steroids	+	0.23 ± 0.05 (mg DE/100 g)
Cardiac glycosides	+	2.83 ± 0.12 (mg RE/100 g)

TAE: Tannic acid equivalent, LE: Limonene equivalent, DE: Disogenin equivalent, GAE: Gallic acid equivalent, RE: Rutin equivalent, Key: '+' Mild presence (0.1–5 mg/100 g), '++' Moderate presence (5–15 mg/100 g), '+++' Strong presence (15–30 mg/100 g).

3.2. Vitamin composition of *Dialium guineense* fruit pulp

Vitamin composition of *Dialium guineense* fruit pulp extract shows a significant ($P < 0.05$) higher concentration of vitamin C followed by vitamins: E, B_2, B_{12}, B_1 and B_6 as presented in **Figure 1**.

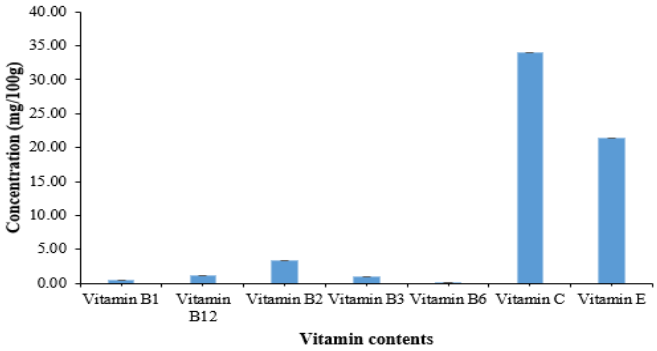

Figure 1. Vitamin concentration of *D. guineense* fruit pulp.

3.3. DPPH scavenging activity of *Dialium guineense* fruit pulp

In **Figure 2**, fruit pulp exhibited a significant DPPH scavenging activity with a percentage inhibition of 90.25% at 400 µg/mL, compared to ascorbic acid control which had a percentage inhibition of 79.66 at 400 µg/mL, compared to the ascorbic acid control, The IC50 values for the *D. guineense* fruit pulp and vitamin C are 87.85 µg/mL and 129.25 µg/mL, respectively.

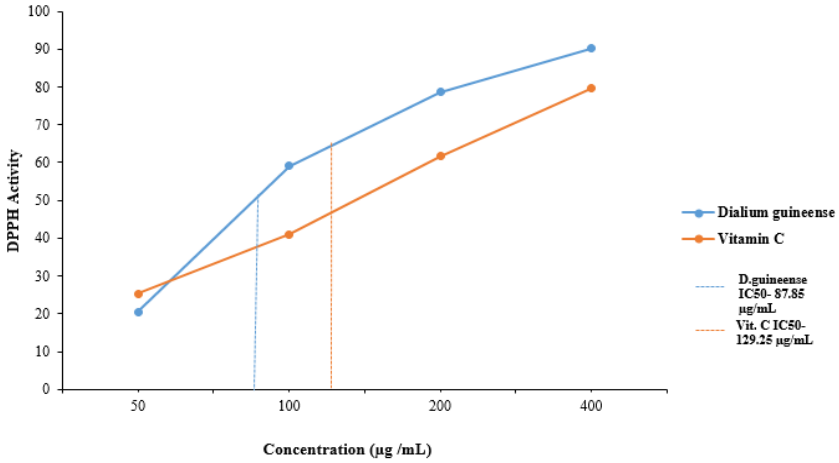

Figure 2. DPPH scavenging activity of *D. guineense* fruit pulp.

3.4. Nitric oxide scavenging activity of *D. guineense* fruit pulp

In **Figure 3**, *D. guineense* fruit pulp showed a higher nitric oxide scavenging activity with a percentage of 83.07% compared to the ascorbic acid control, which had a percentage inhibition of 75.46%. The IC50 values for the *D. guineense* fruit pulp and vitamin C are 135.50 µg/mL and 169.3 µg/mL, respectively.

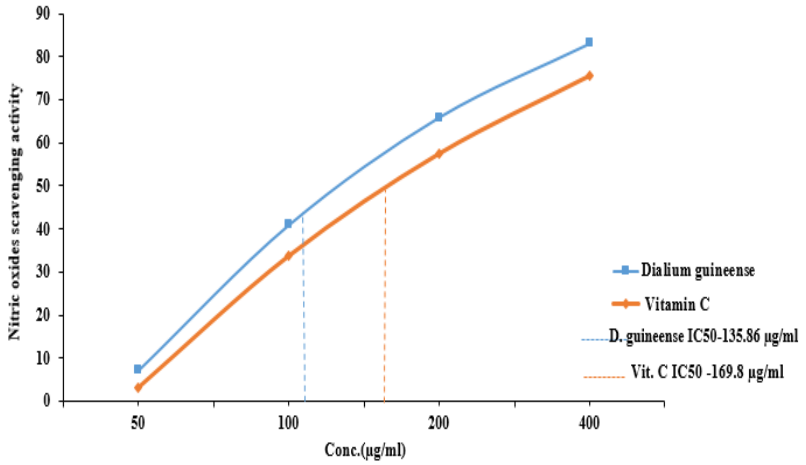

Figure 3. Nitric oxide scavenging activity of *D. guineense* fruit pulp.

3.5. Ferric reducing antioxidant power (FRAP) assay of *D. guineense* fruit pulp

D. guineense fruit pulp in **Figure 4** displays a FRAP value with a percentage inhibition of 83.07% at 400 µg/mL compared to the ascorbic acid control, which had a percentage inhibition of 75.46 at 400 µg/mL is shown in **Figure 4.** The EC_{50} values for the *D. guineense* fruit pulp and vitamin C are 333.50 µg/mL and 359.56 µg/mL, respectively.

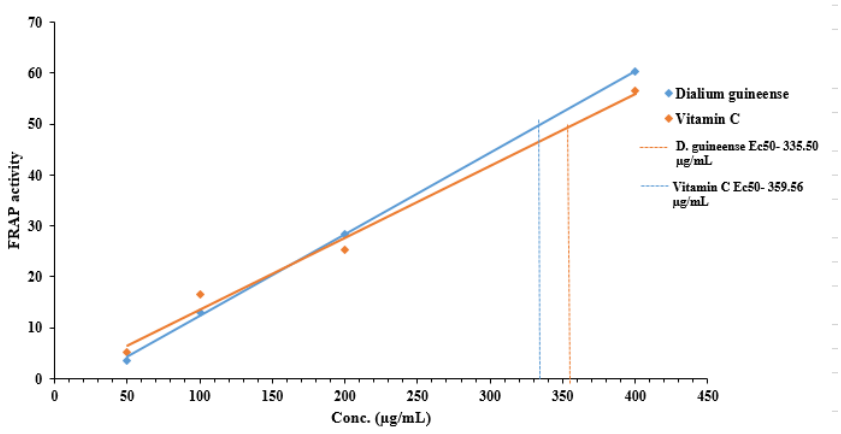

Figure 4. Ferric reducing antioxidant power (FRAP) assay of *D. guineense* fruit pulp.

4. Discussion

Preliminary phytochemical screening facilitates the detection of bioactive compounds and assists in their quantification and separation. **Table 1**. illustrates the presence of various phytochemicals in *Dialium guineense* fruit pulp as follows: flavonoids phenolic acids saponins alkaloids (tannins, glycosides terpenoids and steroids). Flavonoids had the highest concentration at 20.28 ± 0.24 mg/100 g. According to Zhang et al., flavonoids have been widely reported to show anti-oxidant, anti-inflammatory, and anti-microbial properties [17]. This compares well with nutrient-rich fruits like avocados, (14.4 mg/100 g) [17], kiwi (1.3 mg/100 g) [18]

and pineapples (10–20 mg/100 g) [18], further establishing *D. guineense* as a nutritionally important fruit. The level of flavonoids is especially of essence since it has been touted to scavenge free radicals, modulate various enzyme activities, and augment immune responses that may be associated with the fruit's application in folk medicine.

Phenolic acids, estimated at 17.64 ± 0.33 mg GAE/100 g. These phenolic compounds may have antioxidant potentials and hence would contribute very importantly to the reduction of oxidative stress, preventing cellular damage [19,20]. The value of the phenolic acid presented in *D. guineense* was within the range for other fruits, such as pumpkins (0.044–6.6 mg/100 g) [21] and cucumbers (20–29.50 mg/100 g) [22], suggested for their antioxidant properties, which therefore means that it may also be a dietary source for antioxidants. It would therefore suggest that *D. guineense* holds an equal health importance in antioxidant activity hence protection against chronic diseases like cardiovascular disorders. Oki et al. [23], found that the higher the phenolic compound content, the greater the radical scavenging activity.

This health-enhancing profile of the fruit is further added to by the presence of saponins at 14.05 ± 0.24 mg DE/100 g. These are known for their cholesterol-lowering properties, immune function, and anti-obesity and anti-hyperlipidaemic activities [24]. The occurrence of saponins agrees with the general trend in some other fruits and legumes that have been reported to modulate lipid metabolism and increase oxidative biomarkers [24,25].

Alkaloids were determined at 8.00 ± 0.24 mg/100 g, tannins at 4.63 ± 0.25 mg/100 g, glycosides at 2.83 ± 0.12 mg/100 g, terpenoids at 1.15 ± 0.10 mg/100 g, and steroids at 0.23 ± 0.05 mg/100 g. These all contribute peculiar properties in a plant [26,27]. Alkaloids are known as nitrogenous compounds with pharmacological actions that include sedative, anti-inflammatory, and antihypertensive activities [28–30]; tannins are said to contain astringent action that helps in tissue repair and reduction of inflammation [31,32]. More relevantly, cardiac glycosides are of greater importance in monitoring the contractility of heart muscles [33–35] further establishing the fruit's medicinal importance.

This result supports the previous studies that were carried out by Abu; Ajiboye; Oluwole-Banjo and Onah et al. [15,36–38] However, it is contrary to the report of Okerulu [39] that alkaloids were the most abundant phytochemicals in the *D. guineense* and also that steroids were absent. Also, the findings of Oluwole-Banjo [37] revealed the absence of saponins, phenols and alkaloids. This could be relative to the environment, like variation in weather conditions and soil quality, and the time of harvest or ripeness at which the fruit pulp was extracted. [40,41]. David et al. [42] suggest that the presence of bioactive compounds within the plant sample may account for its utilization in traditional medicinal practices.

Investigation of the vitamin concentrations of *Dialium guineense* fruit pulp revealed the presence of vitamins C, E and B (1, 2, 3, 6 and 12), in varying concentrations vitamin C (ascorbic acid) had the highest concentration (33.90 ± mg/100 g) followed by vitamin E tocopherol (21.37 ± mg/100 g) while vitamin B6 (pyrodoxine) had the lowest concentration (0.16 ± mg/l100 g).

The vitamin C and E content of *Dialium guineense* fruit pulp can be compared to common citrus fruits, nuts, and seeds, thus revealing the nutritional value.

Vitamin C is an important nutrient involved in the synthesis of collagen, immunity, and iron absorption [43], hence its occurrence at about 33 mg/100 g in *D. guineense* is comparable to citrus fruits like oranges (50-70 mg/100g) [43] suggesting its potential as a valuable dietary source, particularly in regions where access to citrus may be poor.

Vitamin E is a fat-soluble antioxidant that plays a role in the protection of cellular membranes and is found at about 21.37 mg/100 g in *D. guineense*, somewhat higher compared to peanuts (6.56 mg/100 g) [44], the vitamin E content of *D. guineense* is a much richer source of this fat-soluble antioxidant. Therefore, it can be very important as part of the diet in cell membrane protection, immune function, and preventing oxidative damage [44]. According to the NIH [45], a healthy adult needs to consume 90 mg/day of vitamin C for men and 75 mg/day for women; and 15 mg/day of vitamin E.

The vitamin C content agrees with the reports by Awotedu and Chukwudebe; Oluwole-Banjo and Asoiro et al. [17,46,47]. However, the findings of Airaodion [48] and Ramde-Tiendrebeogo et al. [49], suggested that *Dialium guineense* had much lower vitamin E content 2.58 ± 0.02 mg/100 g and 0.109 ± 0.07 mg/100 g respectively Differences in the vitamin composition of *Dialium guineense* fruit pulp in previous studies may be influenced by soil, climate, geography, humidity, genotype and cultural practices [40,41]. Although thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), and vitamin B12 were found in minute quantities in fruit pulp, they play vital roles in human health. B vitamins are indispensable for physiological functions, such as tissue regeneration and upkeep. Vitamin B1 facilitates the release of energy, enhances appetite, and sustains muscle and nerve operation. Riboflavin and niacin are imperative for oxidative phosphorylation and coenzyme synthesis. Vitamin B6 is engaged in the production of neurotransmitters and the functioning of the nervous system [50,51]. The vitamin composition extracted from plants validates its utility as a supplement among indigenous groups in the southern region of Nigeria [2].

In this study, the IC_{50} values for the DPPH radical scavenging activity of *D. guineense* fruit pulp extract was determined. The IC_{50} and a maximum percentage inhibition (dose-dependent) of *Dialium guineense* fruit pulp extract compared ($p < 0.05$) to vitamin C was found to be approximately 87.85 µg/mL; 90.25 ± 0.37% and 129.25 µg/mL; 79.6 ± 1.21% respectively. The relatively low IC_{50} value suggests that the *Dialium guineense* pulp extract possesses strong antioxidant properties, effectively neutralizing free radicals at lower concentrations. Although Vitamin C is a well-established antioxidant, its higher IC_{50} compared to *Dialium guineense* fruit pulp extract suggests that it is less effective at lower concentrations in this specific assay. Also, the higher maximum percentage inhibition of *Dialium guineense* suggests that the bioactive compounds present in the extract might be more effective in neutralizing free radicals than those in vitamin C [52]. This may be due to the presence of flavonoids and phenolic compound in the plant extracts as past studies have suggested that the antioxidant properties of many plants with medicinal benefits might stem from these natural substances. [53,54].

In the nitric oxide scavenging assay, *Dialium guineense* fruit pulp extract exhibited an IC_{50} of approximately 135.86 µg/mL, indicating a potent nitric oxide

scavenging activity. This relatively low IC_{50} value in comparison to the IC_{50} value for vitamin C (169.80 µg/mL) suggests that the extract is highly effective at neutralizing nitric oxide radicals at lower concentrations. This finding suggests that *Dialium guineense* fruit pulp extract may possess superior nitric oxide scavenging properties compared to vitamin C. This is contrary to the findings of Ogu et al. [55] who reported that vitamin C was more effective in scavenging nitric oxide (95.75%) at 250 µg/mL concentration than the leaves of *Dialium guineense.* Nitric oxide has various biological functions, including nerve communication, relaxing muscles, and fighting against microbes and tumours [56]. However, excessive nitric oxide production can lead to inflammatory disorders. Inhibitors of nitric oxide can help reduce inflammation and tissue damage in inflammatory diseases. The high level of inhibition of nitric oxide by the fruit pulp of *Dialium guineense* may likely be due to its abundance of saponins and flavonoids [15,57]. Saponins and flavonoids have been reported to inhibit nitric oxide production and scavenge free radicals, thus lessening the development of oxidative stress and inflammation [58,59]. This is in agreement with the previous findings that *Dialium guineense* being rich in bioactive compounds may be responsible for its therapeutic use in diseases associated with excessive production of nitric oxide [59,60].

In the analysis of the FRAP (Ferric Reducing Antioxidant Power) activity, the EC_{50} values for the *Dialium guineense* fruit pulp extract and vitamin C were determined. The EC_{50} of *Dialium guineense* (335.50 µg/mL) was comparatively lower ($p < 0.05$) than that of vitamin C (359.56 µg/mL). The maximum percentage inhibition of both *Dialium guineense* and vitamin C at a concentration of 400 µg/mL was revealed to be 60.30 ± 0.40% and 56.65 ± 1.03% respectively. These results show that *Dialium guineense* fruit pulp extract may be marginally more effective in its antioxidant activity at lower concentrations compared to vitamin C. The phenolic compounds, flavonoids, saponins, and tannins in plant extracts have been linked to their significant antioxidant activity. [61].

5. Conclusion

The study analyzed the phytochemical constituents, vitamin composition, and antioxidant activity of *Dialium guineense* fruit pulp. The findings revealed that the fruit pulp possesses an abundance of bioactive compounds including flavonoids, saponins, alkaloids, and high levels of essential vitamins especially vitamins C and E along with potent antioxidant properties.

The results indicated that fruit pulp of *D. guineense* is a good dietary ingredient, providing potential sources of natural antioxidants that might prevent and manage chronic oxidative stress-related diseases like heart diseases, some forms of cancers, and neurodegenerative disorders. The efficient antioxidant activity exhibited by *D. guineense* underlines its potential as a functional food ingredient in possible applications for the prevention of disorders related to oxidative damage. Phytochemicals and vitamins in the fruit pulp synergistically enhance its antioxidant activity, putting it forward as a very useful natural product for overall health promotion and the prevention of chronic diseases related to oxidative stress These are great findings, yet they only impress how high the nutritional and

medicinal values this plant, *D. guineense*, contains. It is necessary that more research be done on environmental and genetic factors that could influence the phytochemical composition of *D. guineense*. Climatic conditions, geographical location, soil composition-even the stage at which these fruits are harvested-may alter their bioactive content in the pulp. More importantly, future studies should be directed to unravel the best way of cultivation and harvesting practices to maximize the nutritional values and bioactive potential of Dialium guineense. Such an understanding shall, therefore, be important in making this fruit remain uniform in its quality and effectiveness as a natural supplier of antioxidants apart from other vitamins. The present study gives good evidence that *D. guineense* fruit pulp is a good source of bioactive compounds and essential vitamins, mainly vitamin C and E, which might account for its strong antioxidant properties. Therefore, the findings are very promising to explore great potential as a functional food providing immense health benefits due to their action against oxidative stress and its related chronic diseases. Being a native fruit with underexploited potential, *D. guineense* stands to be an important contributor toward improved nutrition and health concerns, particularly where its production occurs in regions with very limited access to other foods rich in vitamins. Further studies targeted at optimizing its cultivation and understanding of the variables affecting its phytochemical profile are paramount for tapping into its full potential for nutritional and pharmacological applications.

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