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(Research Article)

Phytochemistry and Proximate composition of *Dalium guineense* pulp and seed

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Abstract

Dalium guineense, commonly referred to as "African velvet tamarind" (AVT) or "Black velvet tamarind" is a medicinal plant that belongs to the Fabaceae family and subfamily *Caesalpinioideae*. Therefore, this study aimed to investigate and compare the phytochemical constituents and proximate composition of *D. guineense* fruit pulp and seed. Phytochemicals were measured using GC-MS-FID, and Proximate analysis was carried out using standard AOAC methods. The phytochemical constituents were more abundant in the pulp compared to the seed. The pulp showed higher levels of flavonoids such as kaempferol (29.60 ± 0.00 ppm), catechin (18.19 ± 0.00 ppm), vanillic (19.28 ± 0.00 ppm) and daidzein (6.91 ± 0.00 ppm) when compared with the seed (0.28 ± 0.00 ppm), (0.57 ± 0.00 ppm), (5.50 ± 0.00 ppm) and (1.25 ± 0.00 ppm) respectively. However, the proximate analysis results revealed that the seed had higher ash content (2.85 ± 0.00%), fibre content (12.23 ± 0.00%), crude fat (9.24 ± 0.00%) and crude protein (9.80 ± 0.00%) when compared to the pulp (1.15 ± 0.00%), (7.18 ± 0.00%), (6.10 ± 0.00%) and (4.55 ± 0.00%) respectively. Also, the pulp showed higher moisture content (16.88 ± 0.00%) and carbohydrate content (71.32 ± 0.00%) when compared to the seed (10.89 ± 0.00%) and (67.23 ± 0.00%) respectively. The results suggest that the pulp and seeds of *D. guineense* can serve as a good source of essential nutrients for humans and livestock due to its nutritional composition.

Keywords: *Dalium Guineense*; Proximate Analysis; Phytochemical Constituent; Seed; Pulp

1. Introduction

Edible plant elements such as seed-bearing structures, flowers, buds, leaves, stems, shoots, and roots that are grown or harvested wild and consumed raw or with little processing are known as fruits and vegetables (1). Similar to vegetables, fruit eating has been strongly advised by researchers and was underlined once more as a crucial step in enhancing health during the 2021 International Year of Fruits and Vegetables. Around 2.8 percent of deaths globally and around 1 percent of disability-adjusted life years were attributed to inadequate fruit and vegetable eating (2). Inadequate consumption of fruits and vegetables has been linked in studies to gut health, chronic lung diseases, poor diabetes control, and some sensory organ dysfunction (3,4). Nigeria has a wealth of different fruits with a range of health and nutritional advantages; yet some, like the black velvet tamarind, are underutilized and could become extinct (5).

Dalium guineense, a legume genus that belongs to the Fabaceae family and subfamily of the Caesalpinioideae, is commonly referred to as African velvet tamarind (AVT) or black velvet tamarind (BVT). The fruit's pulp is sweet and edible, with relatively low quantities of tannin and ascorbic acid. It is a reasonably good source of minerals and protein (6). It is a large tree found in many parts of Africa, such as West Africa, the Central African Republic and Chad. *Dalium guineense* has a thickly leafy crown and is 30 meters high, though it is frequently shrubby. The leaves are leathery, recessed at the midrib, roughly elliptic, blunt at the tip, and coarsely hairy. It has whitish-looking blooms and horizontally arranged branches (7). *Dalium guineense*, also known as velvet tamarind, is found in West African nations like Ghana, where it is called Yoyi, Sierra Leone, Senegal, and Nigeria, where it is called Awin in Yoruba, Icheku in Igbo,

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and Tsamiyar kurm in Hausa (8). Generally, it is fondly referred to as licky-licky in pidgin English. The fruits are popular in local markets and are eaten as a snack by people of all ages when they are fresh. A non-alcoholic beverage produced from the fruit is consumed by certain elderly people.

The physicochemical and sensory qualities of *Dalium guineense* pulp flour have been shown to be favorable, potentially making it a valuable raw material for the food industry, particularly for the manufacturing of highly sought-after confectionery (9). It is used in beverage industries as a sweetener (10) or infused and consumed hot or chilled as a drink. *Dalium guineense* contains a significant amount of protein for a fruit, a large amount of carbohydrate, and relatively little fat (11). *Dalium guineense* pulp's high ash content is a sign of its mineral richness. In addition to being high in carbohydrates, fruit pulp is also high in vitamins and minerals. Because *Dalium guineense* fruit pulp has a low moisture content and is within the safe moisture ranges stated for the majority of fruits (12), it may be less perishable than most fruits.

Dalium guineense may have an anti-ulcer effect via increasing the secretion of stomach mucus (13). Generally, fruits are vitamin and mineral-dense, and they also have phytochemicals (14). As a result, it has therapeutic potential. The phytochemical and nutritional content of medicinal plants make it useful for the treatment and management of various diseases (15-19). The pulp of AVT is abundant in iron, calcium, magnesium, and potassium (20). According to earlier research, the plant contains saponins, which are thought to improve teeth cleaning while also preventing plaque and cavities (21). The aim of this study, therefore, is to evaluate the phytochemistry and potential proximate composition of pulp and seed of *Dalium guineense*.

2. Methods

2.1. Plant Collection and Identification

The *Dalium guineense* seeds were sourced from Eke market, Awka, Anambra State. It was authenticated by a taxonomist in the Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University, Awka. The herbarium number as deposited in the Department of Botany was NAUH-70.

2.2. Sample Preparation

Dalium guineense seeds were dried at room temperature for a period of two weeks. It was thereafter pulverized into powder using a corona manual grinding machine and used for phytochemical and proximate analysis. *Dalium guineense* pulp was separated from the fruit, dried at room temperature for a period of two weeks, and ground into powder using a corona manual grinding machine. It was thereafter used for phytochemical and proximate analysis.

2.3. Phytochemical Analysis

2.3.1. Extraction of phytochemicals

One (1) gram of the sample was weighed and transferred to a test tube, and 15ml of ethanol was added. The test tube was allowed to react in a water bath at 60 °C for 60 mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water and 3 ml of hexane, which was all transferred to the funnel. These extracts were combined and washed three times with 10 ml of 10%v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent evaporated. The sample was solubilized in 1000ul of hexane, of which 200ul was transferred to a vial for analysis.

2.3.2. Quantification by GC-MS and FID

The analysis of phytochemicals was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15-meter MXT-1 column (15 m x 250 um x 0.15 um) was used. The injector temperature was 280 °C with splitless injection of 2 ul of sample and a linear velocity of 30 cms^{-1}, Helium 5.0 pa.s was the carrier gas with a flow rate of 40 $mlmin^{-1}$. The oven operated initially at 200 °C, it was heated to 330 °C at a rate of 3 °C min^{-1} and was kept at this temperature for 5min. The detector operated at a temperature of 320 °C.

Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals expressed in ug/g.

2.4. Proximate Analysis

2.4.1. Moisture Content

Procedure

- A petri dish was washed and dried in the oven
- Approximately 1-2 g of the sample was weighed into petri dish
- The weight of the petri dish and sample was noted before drying
- The petridish and sample were put in the oven and heated at 105 °C for 2 hrs. The result noted and heated another 1hr until a steady result is obtained and the weight was noted
- The drying procedure continued until a constant weight was obtained

$$\% \text{ moisture content } = \frac{W1 - W2 \ x \ 100}{\text{Weight of sample}}$$

Where W_1 = weight of petridish and sample before drying
W_2 weight of the petridish and sample after drying.

2.4.2. Carbohydrate Determination

(Differential method)
100 – (%Protein + %Moisture + %Ash + %Fat + %Fibre)

2.4.3. Ash Content

Principle: The ash of foodstuff is the inorganic residue remaining after the organic matter has been burnt away. It should be noted, however, that the ash obtained is not necessarily of the composition as there may be some from volatilization.
Procedures

- Empty platinum crucible was washed, dried and the weight was noted.
- Approximately 1- 2 g of sample was weighed into the platinum crucible and placed in a muffle furnace at 550 °C for 3 hours.
- The sample was cooled in a dessicator after burning and weighed.

Calculations

% Ash content =

$$\frac{W_3 - W_1}{W_2 - W_1} \ x \ \frac{100}{1}$$

Where
W_1 = weight of empty platinum crucible
W_2 = weight of platinum crucible and sample before burning
W_3 = weight of platinum and ash.

2.4.4. Crude Fibre

Procedure:

- Defat about 2 g of material with petroleum ether (if the fat content if more than 10%)
- Boil under reflux for 30 minutes with 200 ml of a solution containing 1.25 g of H_2SO_4 per 100ml of solution
- Filter the solution through linen
- Wash with boiling water until the washing is no longer acidic.
- Transfer the residue to a beaker and boil for 30 minutes with 200ml of a solution containing 1.25 g of carbonate free NaOH per 100 ml
- Filter the final residue through a thin but close pad of washed and ignited asbestos in a Gooch crucible
- Dry in an electric oven and weigh
- Incinerate, cool and weigh

The loss in weight after incineration x 100 is the percentage of crude fibre.

$$\% \text{ crude fibre } = \frac{\text{Weight of fibre}}{\text{Weight of sample}} \ x \ 100$$

2.4.5. Crude Fat

Soxhlet Fat Extraction Method

This method is carried out by continuously extracting food with non-polar organic solvent such as petroleum ether for about 1 hour or more.

Procedure:

- Dry 250 ml clean boiling flasks in oven at 105 – 110 °C for about 30 minutes.
- Transfer into a dessicator and allow to cool
- Weigh correspondingly labeled, cooled boiling flasks.
- Fill the boiling flasks with about 300 ml of petroleum ether (boiling point 40 – 60 °C)
- Plug the extraction thimble lightly with cotton wool
- Assemble the soxhlet apparatus and allow to reflux for about 6 hours
- Remove thimble with care and collect petroleum ether in the top container of the set – up and drain into a container for re – use.
- When flask is almost free of petroleum ether, remove and dry at 105 °C – 110 °C for 1hour.
- Transfer from the oven into a dessicator and allow to cool; then weigh.

$$\% \text{ fat} = \frac{\text{weight of flask + oil - wt of flask}}{\text{weight of sample}} \times 100$$

2.4.6. Crude Proteins

Principle: The method is the digestion of the sample with hot concentrated sulphuric acid in the presence of a metallic catalyst. Organic nitrogen in the sample is reduced to ammonia. This is retained in the solution as ammonium sulphate. The solution is made alkaline and then distilled to release the ammonia. The ammonia is trapped in dilute acid and then titrated.

Procedures

- Exactly 0.5 g of sample was weighed into a 30ml kjehdal flask (gently to prevent the sample from touching the walls of the side of each and then the flasks were stoppered and shaken. Then 0.5 g of the kjedahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appeared.
- The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling was made up to 100ml with distilled water was added to avoid caking and then 5ml was transferred to the kjedahl distillation apparatus, followed by 5 ml of 40% sodium hydroxide.
- A 100 ml receiver flask containing 5ml of 2% boric acid and indicator mixture containing 5 drops of Bromocresol blue and 1 drop of methlene blue was placed added under a condenser of the distillation apparatus so that the tap was about 20 cm inside the solution and distillation commenced immediately until 50 drops gets into the receiver flask, after which it was titrated to pink colour using 0.01N hydrochloric acid.
- Calculations

$$\% \text{ Nitrogen} = \text{Titre value} \times 0.01 \times 14 \times 4$$
$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25$$

2.5. Statistical Analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences software for Windows version 25 (SPSS Inc., Chicago, Illinois, USA). All the data collected were expressed as Mean ± SEM. Statistical analysis of the results obtained were performed by using ANOVA Tests to determine if a significant difference exists between the means of the test and control groups. The limit of significance was set at $p<0.05$.

3. Results

3.1. Phytochemical analysis of *D. guineense* pulp and seed

Figure 1 represents the result of Quantitative Phytochemical analysis of *D. guineense* pulp and seed. The results showed that phytochemicals such as kaempferol, catechin, vanillic, and daidzein are statistically higher in the pulp compared to the seed. The results also showed that apigenin, naringenin, and artemetin are statistically higher in the seed compared to the pulp.

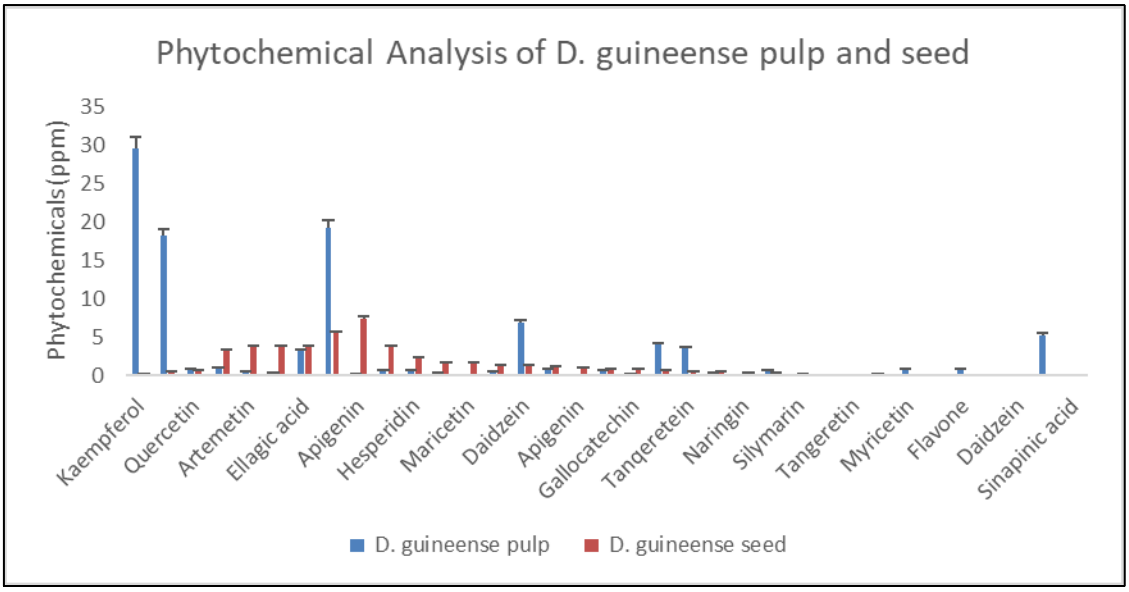

Figure 1 Quantitative phytochemical analysis of *D. guineense* pulp and seed

3.2. Proximate analysis of *D. guineense* pulp and seed

Figure 2 represents the result of proximate analysis for the whole seeds and pulp of *Dalium guineense.* The results showed that the ash, crude fibre, crude fat, and crude protein values are statistically higher in the whole seed as compared to the pulp. The results also showed that the moisture content and carbohydrate values are statistically higher in the pulp as compared to the seed.

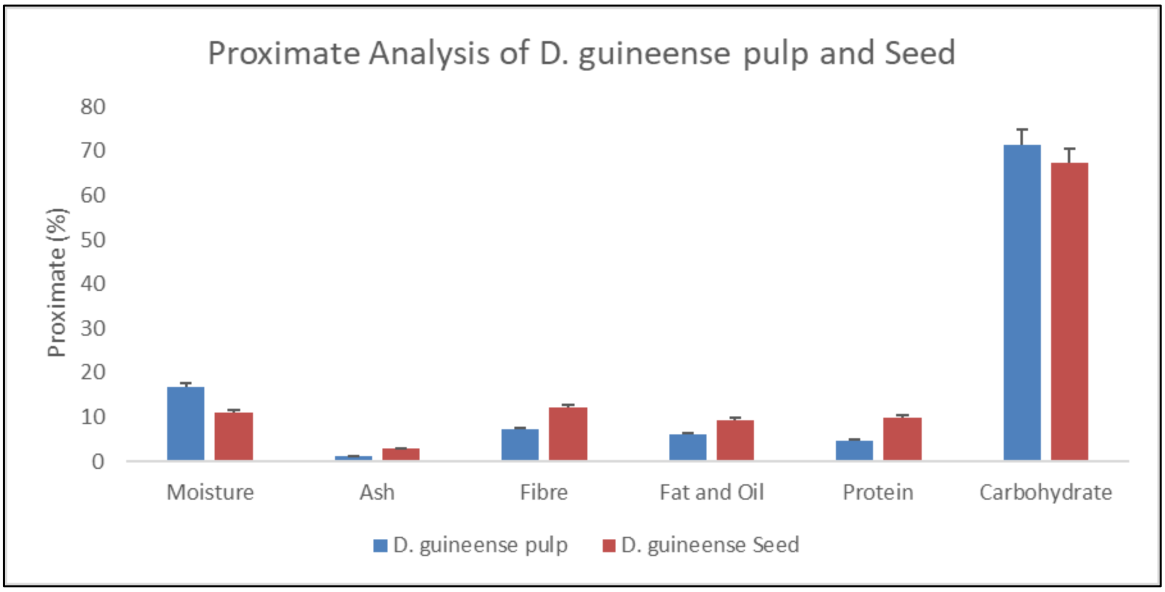

Figure 2 Proximate analysis of *D. guineense* pulp and seed

4. Discussion

In this study, a quantitative comparative screening for the presence of phytochemicals was performed on the pulp and seed extract of *Dalium guineense*. The results revealed that the pulp had greater levels of flavonoids such as Kaempferol (29.60 ± 0.00 ppm), catechin (18.19 ± 0.00 ppm), vanillic (19.28 ± 0.00 ppm) and daidzein (6.91 ± 0.00 ppm) when compared with the seed which has (0.28 ± 0.00 ppm), (0.57 ± 0.00 ppm), (5.50 ± 0.00 ppm) and (1.25 ± 0.00 ppm) respectively. Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables, and certain beverages. Their numerous beneficial biochemical and antioxidant properties are linked to a number of illnesses, including cancer, Alzheimer's disease (AD), and atherosclerosis (22,23). Flavonoids are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. Kaempferol, a class of flavonoid and a naturally occurring flavonol, is extensively present in various vegetables, fruits, and herbal medicines (24). Kaempferol exhibits various bioactive functionalities, encompassing antioxidant, anti-inflammatory, anti-apoptotic, and anticancer properties (25). A common flavonoid, kaempferol has a high level of antioxidant activity because of the large number of phenolic hydroxyl groups in its structure (26). Kaempferol's antioxidant activity has been demonstrated in a number of studies by its capacity to scavenge radicals in oxygen-damaged erythrocytes, which results in the production of reactive oxygen species (27). A recent study suggested that catechins suppressed amyloid-β and neurofibrillary tangles, showing potential prevention and treatment of Alzheimer’s Disease (28). According to Fernando *et al*. (29), catechins can improve patients' cognitive function and affect the production of amyloid-beta. Vanillic acid is a benzoic acid derivative that is used as a flavoring agent. Vanillic acid inhibited the expression of cyclooxygenase-2 and the activation of transcription nuclear factor-B p65 in DSS-treated colon tissues, according to the findings (30).

In contrast, the results also revealed that the seed extract had higher levels of apigenin (7.35 ± 0.00 ppm), naringenin (3.65 ± 0.00 ppm) and artemetin (3.71 ± 0.00 ppm) when compared with the pulp (0.21 ± 0.00 ppm), (0.62 ± 0.00 ppm) and (0.60 ± 0.00 ppm) respectively. Apigenin has been used to treat or prevent amyloid-related disorders (31), limit viral infection of foot-and-mouth disease, enhance the susceptibility of head and neck squamous cell carcinoma to 5-fluorouracil and cisplatin (32), improve pathologic parameters of inflammation, decrease MPO activity in an experimental model of rat pancreatitis (33), and inhibit viral internal ribosome entry site-driven translational activity (34). Mostly found in citrus fruits, naringenin is a flavanone that belongs to the flavonoid family (35). With its antiviral, anti-inflammatory, and antioxidant properties, naringenin reduces the risk of cancer, metabolic syndrome, and cardiovascular disease (36,37).

The result of the proximate analysis of the pulp and seed extract of *Dalium guineense* showed that both the pulp and seed extract contain moisture, ash, crude fat, protein, crude fibre and carbohydrate in varying proportion. The results showed that the pulp extract had higher moisture content (16.88 ± 0.00%) and carbohydrate content (71.32 ± 0.00%) when compared to the seed (10.89 ± 0.00%) and (67.23 ± 0.00 %). A key factor in food products' shelf stability is their moisture content. Foods with a lower moisture level typically last longer on the shelf than those with a greater moisture content. The fruit pulp's high moisture content could be caused by the simple sugars that give it its sweet flavor (38), which could also explain why it is hygroscopic, meaning it absorbs water from its surroundings (39). Since fruit pulp has a shorter shelf life due to its high moisture content, it cannot be stored for as long as the seed. The value of the moisture content recorded by the seed (10.89%) is in line with that obtained by Okegbile *et al*. (40), who reported (10.96%) wet weight. The high carbohydrate content of pulp indicates that it is a good source of energy.

In contrast, the seed of *Dalium guineense* showed higher levels of ash content (2.85 ± 0.00%), crude fiber (12.23 ± 0.00%), crude fat (9.24 ± 0.00%), and crude protein (9.80 ± 0.00%) when compared to the pulp (1.15 ± 0.00%), (7.18 ± 0.00%), (6.10 ± 0.00%) and (4.55 ± 0.00%) respectively. Ash contents indicate the degree of mineral content that has been retained in the food materials. Total ash is crucial for determining whether foreign organic matter, such as silica or metallic salts, is present in a medicine, which is one of the ways of measuring its purity (41). The value obtained for the ash content of the seed (2.85%) is in line with the value reported by Okegbile *et al*. (40) that recorded 2.86%. The seeds' high fiber content increases the bulk of the diet, which promotes healthy bowel movements. Additionally, it might help prevent illnesses like colon cancer (42). This outcome is in line with other studies, such the one conducted by Abdualrahman *et al*. (43), which examined the crude fiber content of the pulp and seeds of similar wild edible fruits. Dietary fats work by absorbing and holding onto flavors, which makes meals more delicious and palatable (44). The high fat content of seeds makes it a good source of vegetable oil which can be used for cooking, medicine cosmetics etc. (45). High fat content of seeds also indicates that they could serve as high source of energy when eaten (46). The high crude protein content of seeds makes it a good body building food for growth and repair of worn-out cells and tissues (in children most especially) (47).

5. Conclusion

In this study, *Dalium guineense* fruit pulp showed high levels of phytochemicals in comparison to its seed. The seed showed high level of proximate composition when compared to the pulp. The high levels of phytochemicals such as kaempferol, catechin, vanillic and daidzein, supports its antioxidant activities. Whereas the seed is high in crude protein, fat, crude fibre, ash content and phytochemicals such as apigenin and naringenin, which support its potential in food fortification and nutraceuticals.

This finding supports the importance of using both the pulp and seed of *Dalium guineense* in food and pharmaceutical applications. However, further research to know the mechanism of action of this phytochemical is recommended to fully harness its therapeutic potential.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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