



ANALYTICAL EVALUATION OF FOUR ACCESSIONS OF *DIALIUM GUINEENSE* (L.) LEAVES

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ABSTRACT

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The study evaluates the diversity of accessions of *Dialium guineense* collected from four different eco-geographical zones of Nigeria. Physicochemical composition was undertaken using the parameters recommended by WHO. The physicochemical parameters evaluated in this study include; the ash values (Loss on drying, Water-soluble Ash, Total ash content, acid insoluble ash, Nitrated ash, Sulphated ash), the exhaustive extraction was done using various solvents: Ethyl-acetate, Methanol and Aqueous with increasing polarity using soxhlet apparatus. Proximate composition which includes; crude fibre, crude protein, crude fat and total carbohydrate were investigated and organoleptic characters and the PH of the plants across the accessions were also investigated. Physicochemical and Proximate parameter investigated shows a reasonable amount of the nutritional composition of the plants for all the accessions. Organoleptic characters evaluated include; taste, odour, colour, sand and silica, insect infestation and rodent infestation, while the PH for all the accessions ranges from 6.0-6.5. Significant differences were observed between the studied parameters across the accessions. Variations observed in the results of this physicochemical evaluation could be pioneered by several factors such as environment, habitat, age of plant, season and chemical race of plants. Thus, from the observed result, it is expected that the physicochemical and proximate properties of this genotype will be very useful in the quality of the plant and also in the nutritional composition of the plant.

Contribution/Originality: This study is one of the very few studies which have investigated the variation in the analytical evaluation of *D. guineense* across different accessions, knowing fully well that several factors such as environment, climate, habitat and chemical nature of plant could pioneer the genotypic behaviour of the plant.

1. INTRODUCTION

A medicinally proven and scientifically inclined plant with a technically reported biological activity is usually termed medicinal plant (Elujoba, 1997). A research made by the World Health Organization emphasized the importance of scientific research into herbal medicine. Lots of the world developing countries have shifted to medicinal plant as an alternative to orthodox medicine and also to the essential drug so far the plant is clinically proven. Our existence as humans depends solely upon the ability with which man harness and utilizes the plants

and plants product (Ayoka, Akomolafe, Akinsomisoye, & Ukponmwan, 2008). One of trending and important plant of interest is *Dialium guineense*, a medicinal plant that belongs to Fabaceae family. It is commonly called Black velvet tamarind. In Africa, so many plants are known to be of high medicinal value but only a few of them are scientifically proven and studied (Rabe, Taylor, McGaw, Jäger, & Van Staden, 2001). The tree is 30m in height with a dense and leafy crown but at times shrubby. The bole are without buttress, it has smooth bark, reddish slash that yields little red gum (Hutchinson & Daniel, 1958). The leaves are hairy with a long stalk of 5-13cm, leaflets are usually opposite or alternate. It has white flowers with large terminals, at times axillary (Szolnok, 1985). The entire inflorescence is covered with short and brown hairs. Fruits are less circular, abundant and almost flattened, the globose are up to 2.5cm in diameter, and it is embedded in a dry brownish sweet, edible and acidic pulp (Hong & Daniel, 1958). The pulp is orange in colour with a sweet and sour taste commonly taken as food by man and animal (Matsuda, 2006). Southern eastern Nigeria eats the pulp because of its refreshing and scorching taste.

The thirst-quenching and refreshing pulp can also be soaked in water and drunk as a beverage and also provides jam and jellies (FAO, 2004). It could also be used as flavour in snacks and non-alcoholic beverages (Adamec, 2002; Effiong, Ibia, & Udofia, 2009). Location and provenance have a major role to play in the genetic make-up and nutritional composition of a plant, and since little information has been recorded for the physicochemical properties of *D. guineense* accessions, hence, the present study was therefore embarked on to evaluate the effect of different accessions on the physicochemical, proximate and organoleptic properties of *D. guineense*.

2. MATERIALS AND METHOD

2.1. Collection and Sample Preparation

The leaves of *D. guineense* was collected from different locations across four geo-political zones of the country. The different accessions to be investigated includes; FCT (Abuja), Oyo State, Edo State, Abia State. After the collection, the leaf samples were identified and then authenticated at the taxonomy section of Forestry Research Institute of Nigeria, Ibadan, Oyo State, Nigeria. It was air-dried, pulverized and later stored in a closed container until it is ready for analysis.

2.2. Preparation of Extract

50g of each of the *D. guineense* leaves sample was soaked in 250ml of methanol (95%, boiling point; 64.6°C) for three days during which it was placed on a mechanical shaker at 220rpm. After the three days, the plant mixture was filtered and the filtrate was concentrated using a rotary evaporator. The crude extract recovered was put in a petri dish, and then kept in a desiccator under a normal temperature to remove available residual solvent before analysis was done (Awotedu, Okeke, Ogunbamowo, Ariwoola, & Omolola, 2020).

2.3. Physicochemical Evaluation

The physicochemical parameters evaluated in this study include the following; the ash values (loss on drying, total ash, Water-soluble Ash, Acid insoluble ash, Nitrated ash, Sulphated ash). The solvents used were ethyl-acetate (EA), Methanol (ME), and Water (AQ). All these parameters were determined using WHO guidelines (World Health Organization, 1998).

2.4. Ash Values

2.4.1. Loss on Drying

10 g of plant sample was placed (without preliminary drying) after accurately weighing it in a tarred evaporated dish. This was dried at 105°C for 5h and weighed. Drying and weighing were continued at 1hr interval

until we got the constant weight. Constant weight was reached when two consecutive weights, after drying for 30min. and cool for 30min. in a desiccator, showed not more than 0.1 g difference.

2.4.2. Total Ash Value

About 2-3 g of ground plant material was incinerated in a silica crucible at a temperature of 450°C and not exceeding that temperature, until free from carbon. Then it was cooled and then weighed. The ash percentage was calculated with reference to the air-dried plant sample.

2.4.3. Acid Insoluble Ash Value

25ml of dilute hydrogen chloride was added to a crucible containing the total ash. The insoluble matter was placed on ashless filter paper and then washed with hot water until the filtrate collected was neutral. The filter paper containing the insoluble matter was then transferred to the original crucible and it was then dried on a very hot plate, ignited to constant weight. The residue was left to cool in a desiccator for 30 min. and then weighed immediately. The insoluble ash was calculated with reference to the air-dried plant sample.

2.4.4. Water Soluble Ash Value

The ash sample was boiled for 5 min with 25mL of water; the insoluble matter was then collected in an ashless filter paper or a crucible, it was washed with hot water, and then ignited for 15min at 450°C temperature not exceeding that. The water-soluble ash is calculated usually by the difference in the weight of the insoluble matter and the weight of the ash. The water soluble ash percentage was calculated in references to the air-dried plant sample.

2.4.5. Sulfated Ash Test.

A platinum/silica crucible was heated to redness for 10min, it was allowed to cool in a desiccator, and then weighed. Sensitive weighed 1-2 g of the plant sample was weighed and put in a crucible, it was gently ignited, until the substance was charred thoroughly. The residue was left to cool, moistened with 1mL of H₂SO₄, and it was gently heated until a white fume disappears, and it was ignited at 800°C ± 25°C until all black particles disappeared. The ignition was done in a place carefully protected from air currents. The crucible was allowed to cool down and little drops of H₂SO₄ were added and the crucible was heated. Then it was ignited, and allowed to cool, and weighed. The operation was repeated until two successive weighings did not differ by more than 0.5mg.

2.5. Solvent extractive Values

2.5.1. Extractive Values (Successive)

A definite amount of plant sample was taken and all the sugars leached out with cold water, dried thoroughly in a desiccator till the plant weight was constant, it was then extracted successively with Ethyl-acetate, Methanol, and Aqueous (water) in a Soxhlet extractor for complete extraction and different extracts were then weighed and percentage with respect to the weight of the plant sample taken was calculated.

2.6. Determination of Proximate composition

Proximate parameters (protein, ash, moisture, fats, fibre, and carbohydrate,) of the powdered sample were done using standard procedures as determined by the AOAC (2005). The protein content of the samples was described by the micro-Kjeldhal method, moisture content was done by oven drying to constant weight, ash content was determined using muffle furnace, fat content was extracted with ether using soxhlet apparatus while carbohydrate was estimated by difference.

2.6.1. Determination of Crude Fat

Two (2) gram each of moisture-free samples was extracted using petroleum ether (60-80°C) in a Soxhlet apparatus for 6 hours to 8 hours. The extract was then evaporated in a pre-weighed beaker. The increased in the weight of the beaker gave the crude fat content of the samples.

2.6.2. Determination of Crude fibre

1 g each of moisture and fat void material of sample each was treated with 100ml of 0.255 ± 0.05 N H_2SO_4 and the mixture was boiled for 30 min. After filtration and washing, the residue was treated and boiled with 100ml of 0.313 ± 0.005 N NaOH solution. The filtrate was cleansed with very hot H_2SO_4 , water and alcohol. The residue gotten was then ignited and the ash was weighed. Loss in weight gave the weight of the crude fibre.

2.6.3. Determination of Crude protein

The crude protein was determined following micro Kjeldahl method. The total protein was determined by multiplying the evaluated nitrogen by a constant value of 6.25.

2.6.4. Determination of Total Carbohydrate

The percentage of carbohydrate was calculated using the formula: $100 - (\text{percentage of ash} + \text{percentage of moisture} + \text{percentage of fat} + \text{percentage of protein})$.

2.6.5. Determination of Nutritive Value

Nutritive value of the samples was determined by multiplying the values gotten fat, protein and carbohydrate by 4.00, 9.00 and 4.00, respectively and then the values are then added up.

2.7. Organoleptic Evaluation

Organoleptic characters were determined according to the taste, colour, odour, Insect Infestation, Rodent Infestation, Sand & Silica parameters.

2.8. pH Evaluation

These were determined using a pH meter (Jenway 3505).

2.9. Statistical Analysis

All the values were expressed as mean \pm standard deviation (S.D.) and analyzed for ANOVA. Differences between groups were separated using least square differences (L.S.D)

3. RESULTS

3.1. Physicochemical Analysis

In the present study, the ash values of *D. guineense* of the accessions studied had significantly different ash value. The analytical result of the ash constants is depicted in Table 1. From the results, total ash, acid-soluble ash, acid insoluble ash, water-soluble ash, water-insoluble are all expressed in varying quantities.

The soluble extractive values in methanol, ethyl-acetate and aqueous (water) shows variable concentrations and all the soluble extractive values were very high in FCT (Abuja); (11.02, 9.11 and 10.66 respectively) while the lowest values gotten for methanol was for Edo State, lowest ethyl-acetate value was for Oyo State and the lowest value for aqueous value was for Edo State. All the results were summarized in Table 2.

Table-1. Ash values of different accessions of *D. guineense* leaves.

Accessions	Loss on Drying (LOD)	Total Ash (% value (w/w))	Acid Insoluble Ash (% value (w/w))	Water Soluble Ash (% value (w/w))	Nitrated Ash (% value (w/w))	Sulphated Ash (% value (w/w))
FCT(Abuja)	14.12±0.20 ^a	17.86±0.59 ^a	9.24±0.10 ^a	1.86±0.02 ^a	13.45±0.21 ^a	18.54±0.43 ^a
Oyo State	13.32±0.31 ^b	22.72±1.64 ^b	6.82±0.01 ^b	1.71±0.10 ^a	11.52±0.23 ^b	19.92±0.21 ^b
Edo State	15.21±0.34 ^c	19.64±0.55 ^c	7.34±0.05 ^c	2.10±0.09 ^b	10.01±0.32 ^c	18.92±0.32 ^c
Abia State	12.43±0.25 ^d	18.55±0.21 ^d	8.71±0.13 ^d	1.88±0.17 ^a	8.90±0.04 ^d	20.22±0.41 ^d

Note: *Data were expressed as mean ± standard error done in triplicate. Alphabets with the same letter are not significantly different.

Table-2. Soluble extractive values.

Accessions	Methanol (mg)	Ethyl-acetate (mg)	Aqueous (Water) (mg)
FCT(Abuja)	11.02±0.08	9.11±0.11	10.66±0.04
Oyo State	9.24±0.11	5.23±0.23	9.82±0.14
Edo State	4.91±0.43	5.81±0.12	6.55±0.02
Abia State	8.54±0.12	6.91±0.05	7.34±0.12

Note: Data were expressed as mean ± standard error done in triplicate.

Table 3 shows the mean values obtained for the proximate evaluation of *D. guineense* across all the accessions and all the values recorded are significantly different from each other. Oyo accession shows the highest value for protein, while, Abuja accession shows the highest accessions for fat. Meanwhile, the highest value for carbohydrate was for Edo state, while the crude fibre highest was found at Abuja and the nutritive value was very high at Abuja accession.

Table-3. Proximate evaluation of Accessions *D. guineense* leaves.

Parameters	Locations			
	Abuja	Oyo	Edo	Abia
Crude Protein (%)	16.03±0.81 ^a	18.72±0.32 ^b	12.64±0.13 ^c	15.31±0.89 ^d
Crude Fat (%)	1.31±0.02 ^a	1.29±0.11 ^b	1.02±0.01 ^c	1.06±0.01 ^d
Carbohydrate (%)	7.21±0.03 ^a	6.46±0.24 ^b	8.24±0.21 ^c	9.39±0.53 ^d
Crude fiber (%)	14.29±0.54 ^a	13.34±0.38 ^b	7.42±0.18 ^c	1.01±0.29 ^d
Nutritive Value (cal Kg ⁻¹)	1263±7.51 ^a	1038±4.71 ^b	1175±6.61 ^c	1055±4.82 ^d

Note: Data were expressed as mean ± standard error done in triplicate. Alphabets with the same letter are not significantly different

The organoleptic features of *D. guineense* leaves shows similar characteristics for sand and silica, odour, taste, insect infestation and rodent infestation except the colour that gave pale green and deep green for Abuja and Edo state respectively while Oyo and Abia state gave light green. All these features are expressed in Table 4.

Table-4. Organoleptic evaluation of accessions of *D. guineense* leaves.

Organoleptic Parameters	Abuja	Oyo	Edo	Abia
Colour	Pale Green	Light Green	Deep green	Light Green
Sand & Silica	Absent	Absent	Absent	Absent
Odour	Faint	Faint	Faint	Faint
Taste	Bitter	Bitter	Bitter	Bitter
Insect Infestation	Absent	Absent	Absent	Absent
Rodent Infestation	Absent	Absent	Absent	Absent

Note: Primary source (Laboratory analysis).

The results expressed in Table 5. shows that *D. guineense* leaves collected across all the four locations are all acidic in nature having a pH range of 6.0-6.5.

Table-5. pH of different Accession

Accessions	Acidity (pH)
FCT(Abuja)	6.5
Oyo State	6.0
Edo State	6.1
Abia State	6.0

Note: Primary source (Laboratory analysis).

4. DISCUSSION

The evaluation of the physicochemical properties is important since they help in identifying adulterated and unstandardized plants. Such parameters include ash value (Total ash, acid-soluble ash, acid insoluble ash, water-soluble ash, water-insoluble ash, nitrated ash, sulphated ash), moisture content, and soluble extractive values (Methanol, Ethyl-acetate and Aqueous). Moisture content is an imperative parameter that measures the efficiency of plant drying process which indicates the stability of the drug during storage time (Mukherjee, 2002). Micro-organism growth is slowed down because the phyto-constituents present in plant can be stored for a long period of time due to high drying time of the plant (Sani, Agunu, Danmalam, & Hajara, 2014). The loss on drying obtained in this study in Table 1 for *D. guineense* accessions for Abuja, Oyo, Edo and Abia state are 14.12%, 13.32%, 15.21% and 12.43% respectively which is higher than the value reported by Awotedu and Ogunbamowo (2019) in its report for *Synsepalum dulcificum* leaves which gave 11.49%, which indicates an efficient and fast drying time. The result gotten in this study is higher than the value (10.8%) also reported for *Diplazium esculentum* by Dash, Khadidi, and Shamsuddin (2017) and for *Achyranthes coynei*, (10.23%) by Gireesh, Pai, Upadhyya, Hurkadale, and Hegde (2015). The result shows that the plant may not decay very fast when stored, instead it will dry up easily and lose its moisture content. The extractive soluble values gotten for a particular solvent gives an idea, insight and indicate the nature and amount of chemical constituents available in the crude drugs (Shah & Chanda, 2011). The highest amount of the soluble extractive values (11.02mg) for methanol gotten in this study is for Abuja accession. The highest to the lowest range of extractive values in Table 2 for all the accessions evaluated are (Abuja, 11.02mg-Abia, 4.91mg). The minimum to maximum extractive values (6.55-10.66mg) recorded for water is lower than that reported in similar studies; Karthika and Manivannan (2018) reported (13.50mg) for *Wedelia trilobata* leaves, while Gireesh et al. (2015) reported (15.24) for *Achyranthes coynei*. The purity and quality of plants are determined by the ash value, because they indicate diverse impurities like oxalate, silicate, carbonate. Water-soluble ash gives an estimate or exact idea of the inorganic compound present in the plant (Sumitra, 2014). The acid-insoluble ash is made up of silica and contaminants of earthy materials. Higher total ash values have been reported by different authors in various plants. The total ash value (6.40%) gotten from the findings of Ibrahim, Makinde, and Ibekwe (2012) for *Crotolaria lachnosema* is very low compared to the value reported for all the accessions in this study. Also, there are similar reports by Kumar, Mondal, Borah, and Mahato (2013) who reported (16.34, 17.34 and 17.00) for *Lasia spinosa* leaves in triplicates for total ash which is very close compared to the value reported for all the accessions in this study. Vinitha and Ashalatha (2019) reported lower total ash values for all the ten (10) accessions studied for *Glycosmis pentaphylla* compared to all the accessions reported too for *D. guineense*. The acid-insoluble ash values recorded across all the accessions are significantly different from each other. Abuja has the highest acid insoluble ash content of (9.24%) followed by Abia State (8.71%), then Edo State (7.34%) and Oyo State which has the lowest value of (6.82%). A study undertaken by Mushtaq et al. (2014) reported 8.826% for *Eremurus himalaicus* leaves which is comparably similar to Abia accession (8.71%), higher than Oyo (6.82%) and Edo (7.34%) accessions and lower than Abuja accessions (9.24%). Barus, Sitorus, and Masfria (2018) in his findings reported 1.97% acid insoluble ash content for *Tarenna polycarpa* leaf fractions which is very low compared to all the values detected for the *D. guineense* accessions across the locations. In addition, water-soluble ash content for all the accessions are very low and are comparably not significant for all the locations except Edo state that is significantly different. Edo accession has the highest water-soluble ash (2.10%) while Oyo accession has the least accession (1.71%). Karthika

and Manivannan, [20] reported (0.85%) for water-soluble ash for *Wedelia trilobata* leaves which is lower than the value reported in this study for all the accessions. A higher water-soluble ash value (2.83%) was reported by Kanakiya, Padalia, Pande, and Chanda (2018) for *Limonium stocksii* leaves. The nitrated ash values gotten in this study for all the accessions are all significantly different from each other. It was very high in Abuja accession with the value of 13.45% and very low in Abia accession with the value 8.90%. The result gotten in this experiment for nitrated ash is lower than the value (16.67%) obtained in the findings of Karthika and Manivannan (2018) for *Limonium stocksii* leaves. However, Awotedu. and Ogunbamowo (2019) in their findings for nitrated ash also gave 15.76% for *Synsepalum dulcificum* leaves which is higher than the value detected in our findings. The value (20.22%) obtained for sulphated ash was very high in the Abia accession and has a lower value (18.92%) in Edo accession. The proximate composition of the four accessions evaluated in Table 3 revealed that protein was found to be the highest (18.72%) in Oyo state and was the lowest (12.64%) in Edo accessions. This suggests that the plant organs (especially the leaves) may be a good source of protein and may also possess rich immunological protection, enzymatic catalysis and regulation of growth. The values recorded for Abuja and Abia are not significantly different from each other. Proteins are known to have diverse pharmacological and physiological roles in the biological systems. The low crude fat value observed in all the accessions suggests that *D. guineense* may not be a very good energy-yielding source and may be ideal for people suffering from obesity. Carbohydrates acts usually as a major source of energy for humans and add up to the bulk of the diet. It is essential for the nourishment of plants and animals which aids the growth of plants and animals by providing energy (Edeoga, Okwu, & Mbaebie, 2005). The moderate values gotten for carbohydrates across all the accessions in Table 3 shows that the plant is not a good source of energy. On the other hand, the higher fibre content (14.29% and 13.34%) obtained for Abuja and Oyo respectively Table 3 suggests they will help reduce constipation and therefore enhance frequent elimination of bowel content. If a diet that contains little fibre are eaten, the faeces may be hard, dry and concentrated. Besides these crude fibres, crude fat, crude protein and total carbohydrates, the energy values of *D. guineense* species have also been regarded as important to human health. Moreover, apart from their roles as flavouring agents, a study has showed that plant organ (leaves) under investigation is rich nutritionally and also contain very important nutrients in terms of proximate principles. The organoleptic evaluation of the accessions obtained across different location as expressed in Table 4. Shows that the colour of *D. guineense* leaves vary across the locations with pale green (Abuja), light green (Oyo State), deep green (Edo State), Light green (Abia) while sand and silica was absent in all the accessions. The odours observed across the accession are all faint. Bitter taste was observed for all the accessions. Insect and Rodent infestation are all absent across the locations. The pH values for *D. guineense* fruits pulp is usually very acidic in nature because the main content is ascorbic acid, however, the leaves are also reported to be acidic in nature usually having a pH of 5.5-7.0, but in this study an acidic pH range of 6.0 -6.5 in Table 5 was detected across the four accessions investigated.

5. CONCLUSION

The plant *D. guineense* having a pertinent role in the traditional health and herbal medicine has been confirmed to have a nutritional and leaf development phenology variation that is widely different according to the eco-geographical region where the species is present. In the present study, aerial part (leaves) was thoroughly investigated for their physicochemical characters to analyze their nature, amount, impurities, quality, safety and standardization of their use. The information from the present study provides background data which is helpful in the correct identification, authentication and nutritional composition of these medicinal plants and may help in preventing its adulteration.

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