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A Study on the Nutritional and Antimicrobial Properties of *Dialium Guineense* (Velvet Tamarind) PULP and Seed Coat

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Abstract

The fruit pulp and seed coat of *Dialium guineense* (Velvet Tamarind) from two different locations was analyzed for their chemical composition, amino acids, vitamins and minerals using standard methods. The anti-nutritional properties of the plant materials were analyzed to determine the phytochemical components using standard methods. The antimicrobial properties of the methanolic and ethanolic extracts of the fruit pulp and seed coat were examined using dehydrogenase (DHA) assay with 2,3,5-triphenyltetrazolium Chloride (TTC) as the artificial electron acceptor. Bacterial isolates, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi* and *Candida albican* were exposed to various concentrations of the extracts ranging from 0-1800 µg/ml in 4 ml volume of nutrient broth-glucose-TTC medium. The inhibition of DHA activity in the bacterial isolates by *D. guineense* extracts were calculated relative to the control and the toxicity threshold concentrations (IC_{50} and IC_{10}) were determined from the linear regression plots. The plant materials contain crude protein, ether extract, Ash, Crude fibre, moisture and NFE; minerals such as, sodium, iron, zinc, calcium, magnesium, copper and selenium as well as vitamin A and C in different quantities based on the location. The fruit pulp contain both essential and non-essential amino acids. The phytochemical screening showed the presence of oxalates (3.065-14.775 mg/100 g), Tannins (0,085-0.606 mg/100 g), Phytic acids (1.65-3.68 mg/100 g), Alkaloids (2.405-3.345 mg/100 g), Phytates (0.365-0.535 mg/100 g) and cyanides (4.045-7.02 mg/100 g). The extracts demonstrated good antimicrobial activities with broad spectrum though not better than the conventional antibiotics. The rich nutritional composition of the fruit makes it an excellent dietary supplement for both infants and adults.

Keywords: *Dialium guineense*; Anti-nutritional; Nutritional and antimicrobial.

1. Introduction

Medicinal plants have been used for centuries as agents to combat diseases which could be dated to the origin of man [1]. Medicinal plants are the richest bio-resources of remedies for human diseases and offer a new source of drugs of traditional medicinal systems, modern medicines, biologically active chemical compounds as antimicrobial, nutraceuticals, food supplements, traditional medicines, pharmaceuticals, intermediate and chemical for synthetic drugs [2]. It has been estimated that 14 – 28 % of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethno-medicinal use of the plants [3]. Fruits are known as excellent source of mineral and vitamins [4] and may permit to increase rural population food quality [5].

Plants have demonstrated their capacity to treat a wide range of infections and diseases and have been quite promising recently [6]. The capacity of herbal plants to demonstrate medicinal values has been attributed to some phytochemicals present in them [1, 7, 8]. One importance of medicinal plants is that they also have additional nutritional benefits they confer. This nutrients include macro and micro-nutrients, amino acids, carbohydrates, oils, etc [9-11].

Dialium guineense is commonly called black velvet or velvet tamarind (English), *Icheku* (Ibo, Eastern Nigeria), *Awin* (Yoruba, Western Nigeria), *Tamarinier noir* (French) [12]. The pulp of the fruit is edible and sweet, fairly low levels of ascorbic acid and tannin are present. The fruit pulp of velvet tamarind is red with astringent flavour and is eaten raw when dry by man and animals [13]. It is a fairly good source of protein, minerals and reported to possess antimicrobial activities in the cure of diarrhoea, palpitations as well as fever [14]. Okwu and Okeke [15], reported that the plant contains saponins which are presumed to add to the cleaning effect of teeth and at the same time prevent caries and plaques on the teeth of the users. The ripe fruits of the plant are chewed among some women in southeast Nigeria to improve lactation and check genital infection [16]. Among the Esan tribe of Edo State in Nigeria, the twig or bark is chewed for oral hygiene and stomach ache [17]. Traditionally, *D. guineense* leaves and stem bark are used as remedies for diarrhoea, severe cough, bronchitis, wound, stomach aches, malaria fever, jaundice, antiulcer and haemorrhoids [18].

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Studies had been reported on the fruit of *Dialium guineense* in Nigeria [14, 19, 20] and Ghana [21]. However, literature on the proximate composition of velvet tamarind is scanty. Therefore, scientific evaluation of this fruit is important to elucidate its chemical composition, nutritional properties as well as its antimicrobial potentials in order to support its use as snacks and as alternative medicine in the treatment of some infections, especially enteric diseases.

This study reports on the nutritional properties and antimicrobial potentials of the fruit pulp and fruit coat.

2. Materials and Methods

2.1. Sample Collection

Well ripe velvet tamarind (*D. guineense* fruits) were collected from two locations, Awo-Omamma (A) and Ahiazu Mbaise (B) in Imo state, Nigeria between the months of February to March, 2015. The fruits were identified and authenticated by a Plant Science at the Biotechnology Department, Imo State University, Owerri. The voucher specimen was deposited at Imo State University herbarium with herbarium number 0411. The fruits used in this research were without any visible identifiable blemish.

2.2. Sample Preparation

The fruit pulp was dehulled from the seed coat. The pulps were dried and blended into powdery. The fruit coats were sun dried and blended into fine powder and stored in dry plastic container prior to analysis.

2.3. Chemical Composition

The moisture, crude protein, crude fat, total ash and crude fibre contents of the sample was determined using standard methods of the Association of Official Analytical Chemists [22].

2.4. Determination of Amino Acid Profile

The amino acid profile in the sample was determined using methods described by Benitez [23]. The sample was defatted using chloroform methanol mixture of ratio 2:1 and hydrolysed to cleave the peptide bonds between the amino acids contained in the sample before being subjected to amino acid analysis. Amino acid values were calculated from the chromatogram peak. The Norleucine Equivalence (NE) for each amino acid in the standard mixture was calculated using the formula

$$NE = \frac{Area\ of\ norleucine}{Area\ of\ each\ amino\ acid}$$

A constant S_{std} was calculated\ for each amino acid in the standard mixture:

$$where\ S_{std} = NE_{std} \times molecular\ weight\ \times \mu MAA_{std}$$

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using the formula

$$Concentration\ \left(\frac{g}{100g} protein\right) = NH \times W@NH/2 \times S_{std} \times C$$

$$where, C = \frac{dilution \times 16}{smple\ wt(g) \times N\% \times 10 \times Vol.\ loaded} \div NH \times W(nleu)$$

$$where\ NH = net\ height;\ W = width\ @\ half\ height\ and\ nleu = Norleucine$$

[22].

2.5. Mineral and Vitamin Analysis

Prior to the mineral analysis, samples were digested with a tri-acid mixture (concentrated nitric acid-perchloric acid and sulphuric acid, 4.0:5.0:0.5 v/v. Phosphorus was analyzed by spectrophotometer based on the reaction of phosphorus with molybdovanadate complex. Sodium and potassium were determined by flame photometry. Iron, Copper, Manganese, Zinc, Calcium and Magnesium were analyzed by Perkin Elmer, Model 403 atomic absorption spectrophotometer. Ascorbic acid (Vitamin C) was estimated by 2, 6-Dichlorophenol-indophenol visual titration according to the method described by Onwuka [24]. Vitamin A Retinol was determined using the method of [22] and DeVries, *et al.* [25].

2.6. Quantitative Phytochemical Screening of Plant Materials

Phytochemical analysis was carried out on both the fruit pulp and seed coat of the samples collected from the two locations. Tannin was determined using methods employed by Ejikeme, *et al.* [26]. Phytic acid (inositol hexaphosphate) content of each sample was determined by the standard method of Association of Official Analytical Chemists, AOAC [27]. Oxalate was determined according to methods described by [22]. The gravimetric method of Harborne [28] as employed by Ejikeme, *et al.* [26] was adopted for alkaloid determination.

2.7. Crude Extraction of Fruit Pulp and Fruit Coat

A 15 g portion of the fruit pulp and the fruit coat were extracted with 75 ml of ethanol and methanol respectively and shaken for 48 hrs. Soluble extract from filtration in a whatman No1 filter paper was concentrated by distillation under reduced pressure at 49°C in a Buchi rotavapour (Switzerland). The extract was then dried to solid form in a rotary evaporator (CNS Simax), and stored in a freezer (<4.0°C) before use.

2.8. Authentication and Standardization of the Isolates

Authentic clinical isolates of *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Candida albicans* were obtained from the Microbiology unit of the Federal Medical Centre, Owerri. The bacterial isolates were grown to mid exponential phase in nutrient broth (Lab M) on a rotary incubator (150 rpm) at room temperature (28 ± 2°C). The cells were later harvested by centrifugation at 4000 rpm for 10 min and washed thrice in deionised distilled water and re-suspended in water. The washed cells were re-suspended in peptone water and the turbidity adjusted to an optical density of 0.5 at 540 nm. An aliquot of 0.1 ml of the cell suspension was used as analyzer in the dehydrogenase assay [29].

2.9. Extract Preparation for Inhibition of Dehydrogenase Activity (DHA) Assay

One-fifth gram (0.2 g) of each of the extracts (toxicants) was dissolved in 40 mls of Dimethyl Sulphoxide (DMSO).

2.10. Media Preparation for DHA Assay

Nutrient broth was prepared in Phosphate buffer pH 7.0 in order to achieve four times strength (which signifies four times the manufacturer's specification) and autoclaved.

2.11. DHA Assay

Total dehydrogenase assay method as described by Alisi, *et al.* [30] was employed to determine the antimicrobial activity of the extracts. The total dehydrogenase activity was assayed using 2, 3, 5-triphenyltetrazolium chloride (TTC) (BDH England) as the artificial electron acceptor in reduction process, which was reduced to the red-coloured 1,3,5-triphenyl-formazan (TPF). The assay was carried out in 4 ml volumes of nutrient broth-glucose-TTC medium supplemented with varying concentrations (0– 1800 µg/ml) of extract in separate screw-capped bottles. Portions of 0.5 ml of the buffered medium, 0.1 ml (w/v) TTC in deionised distilled water, and varying concentrations of the extracts were added into the screw capped bottles respectively. One-tenth milliliter (0.1 ml) of the different isolates suspension were then inoculated into the medium and the volumes were made up to 4 ml with water. The reaction mixtures in the sample bottles were agitated and incubated for 36 hrs at room temperature. The negative controls consisted of the media, isolates, TTC and water without any extract while the positive control consisted of the media, isolates, TTC, Ciprofloxacin or Fluconazole and water with no extract. The reaction mixtures were also agitated and incubated at room temperature for 36 hrs. The TPF produced were extracted in 4 ml of butanol and determined colorimetrically at 540 nm.

Protocol Table

TUBE	1	2	3	4	5	6	7	8	9	10
CONCENTRATION (µgml)	50	100	200	400	600	800	1000	1400	1800	2000
EXTRACT (ml)	0.04	0,08	0.16	0.32	0.48	0.64	0.80	0.96	1.12	1.44
INOCULUM (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TTC (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BUFFERED MEDIUM(ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
VOLUME OF WATER (ml)	3.26	3.22	3.14	2.98	2.82	2.66	2.50	2.34	2.18	1.86
FINAL Volume	4	4	4	4	4	4	4	4	4	4

2.12. Determination of Dehydrogenase Activity

Dehydrogenase activity was expressed as mg of 1, 3, 5-triphenyl-formazan (TPF) formed per mg dry weight of cell biomass per hour. Inhibition of dehydrogenase activity in the isolates by *D. guineense* extract was analyzed relative to the control. The percentages of inhibition of each of the test organisms were linearized against the concentrations of the extracts using gamma parameter described by Kim, *et al.* [31].

$$\%\ inhibition = \left(1\ \frac{OD_{test}}{OD_{control}}\right) \times 100$$

$$\gamma = \frac{\%\ Inhibition}{100 - \%\ Inhibition}$$

The toxicity threshold concentrations (IC_{50}) were then determined from the linear regression plots.

3. Results

Figure 1 show results of the proximate composition of the fruit pulp of velvet tamarind. Values of the proximate analysis shows that there was significant difference between the values using the student t-test at $p<0.05$. Sample A had higher values of crude protein, Ether extract, Ash and Crude fibre while Sample B had higher values of Moisture and Non-fatty extract (NFE).

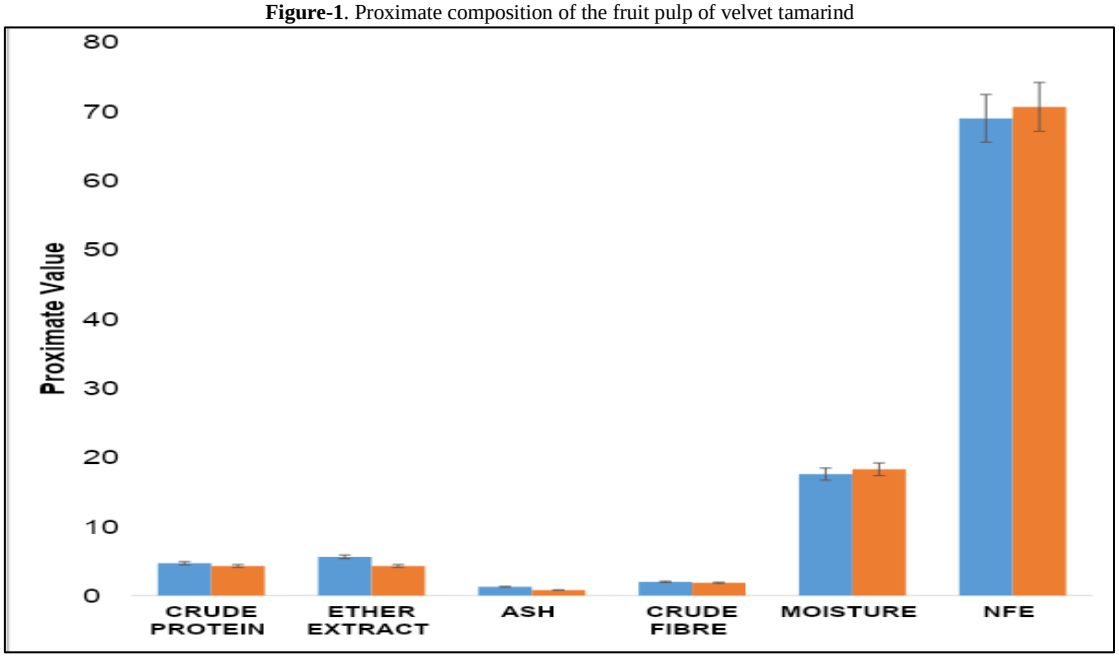
Figure 2 show results for the amino acids composition of the fruit pulp. Results show that Velvet tamarind are rich in amino acids. They contain both essential and non-essential amino acids. Specifically, results revealed that sample A had higher values of most amino acids except arginine and phenylalanine. Values of the amino acids obtained from samples A and B were significantly different from each other at $p<0.05$ using the Paired T-Test for significant differences except for threonine, cystine and isoleucine.

Table 1 show results for mineral and vitamins composition of the fruit pulp from the two locations. Results subjected to ANOVA at $p<0.05$ shows that there was significant difference in values obtained from different locations. Sample A was considerably higher in concentrations of Na, Fe, Zn, Ca, Mg, vitamin A and vitamin C with values of 1.134, 0.84, 0.0648, 147.4, 15.4, 0.2084 and 4.12 respectively (all in mg/kg SI units). Sample B had the highest concentrations for K and Se with values of 80.144 mg/kg and 0.0202 mg/kg respectively. Overall, samples obtained from location A is richer in vitamins and minerals compared to sample B.

Figure 3 shows the results obtained for phytochemical analysis of fruit pulp and seed coat .Samples A and B are the fruit pulp while Samples D and E are fruit coat. The result shows that the samples contained oxalates (3.065-14.775 mg/1 g), tannins (0.085-0.606 mg/100 g), phytic acids (1.65-3.68), alkaloids (2.405-3.345 mg/100 g), phytates (0.365-0.535 mg/100 g) and cyanide (4.045-7.02 mg/100 g). Higher concentrations of the phytochemicals was recorded in some specific samples such as Sample A with oxalate and phytic acid values of 14.775 mg/100 g and 3.68 mg/100 g respectively; Sample B with alkaloid value of 3.345 mg/100 g, Sample D with tannins value of 0.605 mg/100 g; and sample E with phytates and cyanide value of 0.535 mg/100 g and 7.02 mg/100 g respectively. This implies that the fruit coats had higher values of tannins, phytates and cyanides while the fruit pulp had higher concentrations of oxalates, phytic acids and alkaloids.

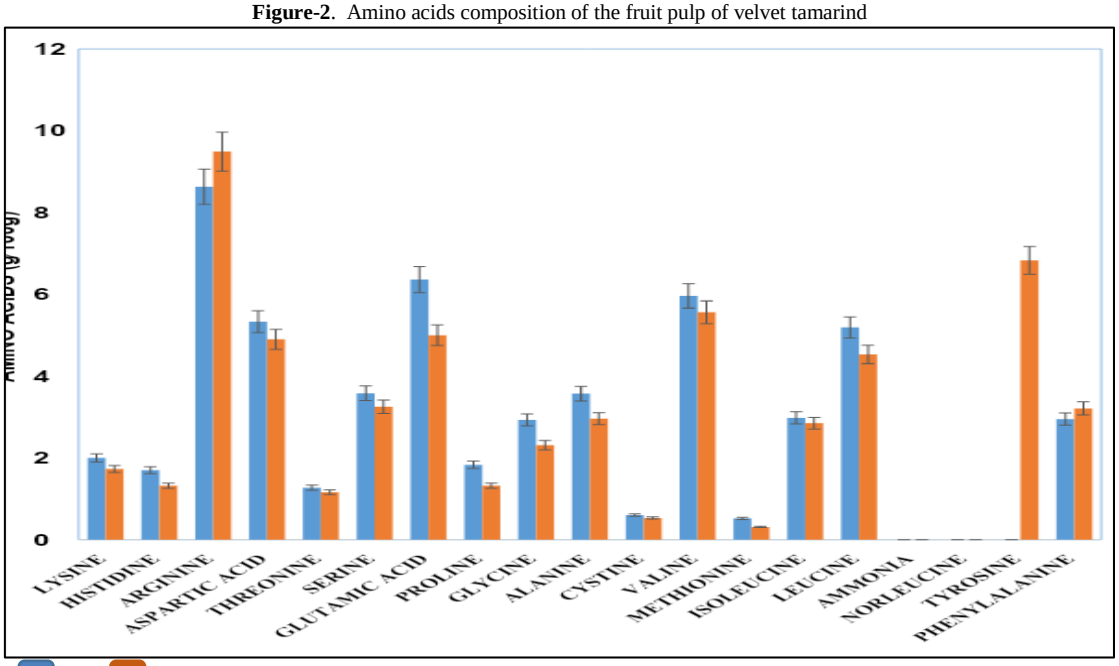
Table 2 shows the inhibitory activities of fruit pulp and seed coat extracts against *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans*. Results are presented by their linear models, R^2 values depicting goodness of the fit, and their IC_{10} and IC_{50} values [32].

Figure-1. Proximate composition of the fruit pulp of velvet tamarind



A B "NFE = 100–(Crude protein+ Ether Extract + Ash + Crude Fibre) AVE: Average value."

Figure-2. Amino acids composition of the fruit pulp of velvet tamarind



A B

Table-1. Minerals and Vitamins values of Two Fruit Pulp

PARAMETER	SAMPLE A	SAMPLE B	ANZFSC
K (mg⁄Kg)	44.737	80.144	***
Na (mg⁄Kg)	1.134	0.335	10 mmol/L
Fe (mg⁄Kg)	0.84	0.461	030 mg
Se (mg⁄Kg)	0.0162	0.0202	17.5 µg
Cu (mg⁄Kg)	0.0259	0.0317	***
Zn (mg⁄Kg)	0.0648	0.0233	0.8 mg
Ca (mg⁄Kg)	147.4	55.5	200 mg
Mg (mg⁄Kg)	15.4	6.4	22 mg
Vitamin A (mg⁄Kg)	0.2084	0.1883	200 µg
Vitamin C (mg⁄Kg)	4.12	3.947	40 mg

ANZFSC= Australia New Zealand Food Standards

Figure-3. Phytochemical composition of fruit pulp and seed coat of velvet tamarind

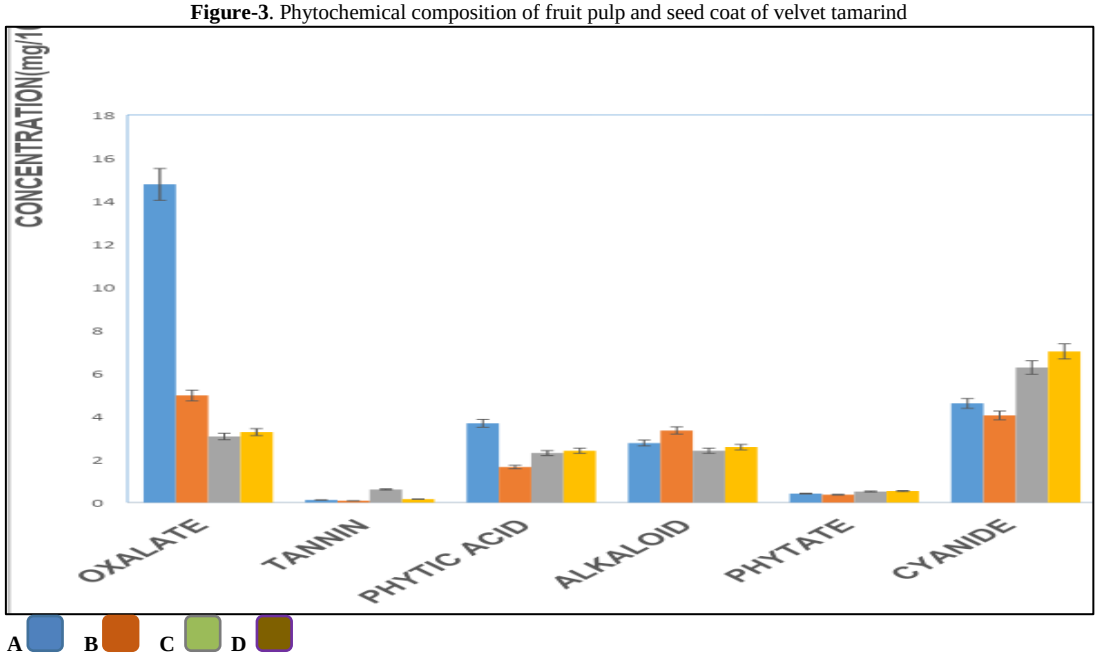


Table-2. IC_{10} and IC_{50} values of Pulp and Seed Coat of Tamarind Extract against Test isolates

Sample code	Linear model	R^2 values	IC_{10} and IC_{50}
PMP (methanolic pulp extract against *Pseudomonas*)	y = 0.006x - 0.1761	0.9675	47.87, 196.02
PMC (Methanolic coat extract against *Pseudomonas*)	y = 0.0125x - 0.7765	0.9571	71.01, 142.12
PEC (Ethanolic coat extract)	y = 0.005x - 0.316	0.9311	85.42, 263.2
PEP (Ethanolic pulp extract)	y = 0.005x - 0.4314	0.9444	108.50, 286.28
EMC (Methanolic coat extract against *E. coli*)	y = 0.004x - 0.2951	0.9175	101.55, 323.76
EEC (Ethanolic coat extract)	y = 0.0153x - 1.2309	0.8937	87.71, 145.81
EMP(Methanolic pulp extract)	y = 0.0127x - 0.7257	0.9477	65.89, 135.88
EEP (Ethanolic pulp extract)	y = 0.01x - 0.757	0.9293	86.81, 175.7
SaEP (Ethanolic pulp extract against *Salmonella typhi*)	y = 0.0058x - 0.1591	0.9513	46.59, 199.84
SaMP (Methanolic pulp extract)	y = 0.0075x - 0.6133	0.9207	96.58, 215.11
SaEC (Ethanolic coat extract)	y = 0.0107x - 0.5611	0.9278	62.82, 145.90
SaMC (Methanolic coat extract)	y = 0.0085x - 0.549	0.9125	77.66, 182.24
CEP (Ethanolic pulp extract)	y = 0.0033x - 0.1782	0.9234	87.67, 357.03
CMC (Methanolic coat)	y = 0.0073x 0.5575	0.9299	91.59, 213.36
CEC (Ethanolic coat extract)	y = 0.0092x - 0.48	0.9274	64.25, 160.86
CMP(Methanolic pulp extract)	y = 0.012x - 0.208	0.9624	26.59, 100.67

4. Discussion

The result of this study has shown that velvet tamarind is a good source of vitamins and minerals which include vitamin A and C; Na, Fe, Zn, Ca, Mg, Cu and Se with slight variations from the locations. This is consistent with reports by Besong, *et al.* [33]. Several factors have been reported to affect the abundance of nutritional variables in plants such as temperature, time of harvest, soil nutrients, maturity, genetics of parent plant and much more [1].

The proximate composition of the plant material reported crude protein, ether extract, ash, crude fibre, moisture and NFE. The plant contained relatively low moisture compared to other fruits. Relatively low moisture content could imply long shelf life [24, 34]. Proteins are important source of diets to living things. As chemical compounds, they repair and replace worn out cells, form structural and globular materials that holds the body, form blood proteins, boost immune system, etc [35]. Dietary fibres alter the colonic environment in such a way as to protect against colorectal diseases. It provides protection by increasing faecal bulk, which dilutes the increased colonic bile acid concentrations that occur with a high fat diet [36]. Ash constituents of the investigated samples could be related to their mineral contents which play numerous functions towards the improvement of health in the body of organisms [24, 35]. Carbohydrates (NFE) are related to energy generation [35]. Carbohydrates produce energy to power the cells and tissues of the body on consumption. Results showed that the samples of velvet tamarind are rich in amino acids. They contain both essential and non-essential amino acids. However, results showed that sample A had higher values of most amino acids except arginine and phenylalanine. The presence these amino acids makes it an excellent raw material for the production of pharmaceuticals and diet supplements. Particular focus is given to the lysine requirements of adults, since this indispensable amino acid is most likely to be limiting in the cereal-based diets characteristic of populations in large areas of the developing world [37, 38]. *D. guineense* fruit pulp can serve as a good food supplement especially for the obese because of its low fat content.

Phytochemicals are important chemicals found virtually in different parts of plant. Phytochemicals are secondary metabolites of plants and confers variable functions to the plants that houses them [1]. These phytochemicals also have important medicinal properties having wide applications in phytomedicine [1, 39-41]. The plants contained oxalates (3.065-14.775 mg/100 g), tannins (0.085-0.606 mg/100 g), phytic acids (1.65-3.68), alkaloids (2.405-3.345 mg/100 g), phytates (0.365-0.535 mg/100 g) and cyanide (4.045-7.02 mg/100 g).

Alkaloids are important secondary metabolites. Isolated pure form of alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic and bactericidal effects. Alkaloids, saponins and tannins are known to have antimicrobial activities as well as other physiological activities [42].

This study showed that *D. guineese* has great potentials as antimicrobial agents against the selected pathogens; *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans*. It has been reported that the lower the IC values, the higher the antimicrobial activities of the plant materials [43]. Results showed that methanolic extracts of the pulp and fruit coat of velvet tamarind had better activity on *Pseudomonas aeruginosa* than the ethanolic extracts which had IC_{50} values of 196.02 mg/ml and 142.12 mg/ml respectively for methanolic pulp and fruit coat and IC_{50} values of 286.28 mg/ml and 263.2 mg/ml for ethanolic pulp and fruit coat extract respectively. Similarly, methanolic and ethanolic extracts of the fruit coat had better activity than extract of the fruit pulp.

Considering the antimicrobial activity of the plant extracts on *Escherichia coli*, the methanolic extracts of the fruit coat had lower activity than the ethanolic extracts with IC_{50} values of 323.76 mg/ml and 145.81 mg/ml respectively. On the contrary, methanolic seed pulp extract had better activity than the ethanolic extracts with IC_{50} values of 135.88 mg/ml and 175.7 mg/ml respectively. Comparatively, methanolic extracts of the fruit pulp had better activity than that of the fruit coat. The reverse was the case with ethanolic extract.

Results showed that the ethanolic extract had better activity than the methanolic extracts for both the fruit pulp and the fruit coat with IC_{50} values of 199.84 mg/ml and 145.90 mg/ml for ethanolic extracts; and 215.11 mg/ml and 182.24 mg/ml for methanolic extracts respectively for *Salmonella typhi*. The ethanolic extracts of the fruit coat showed better activity than that of the fruit pulp.

Results for the antifungal activity against *Candida albicans* showed that the IC_{50} values of the methanolic pulp extract recorded better activity than methanolic seed coat extract with values 100.67 mg/ml and 213.36 mg/ml respectively. On the other hand, the ethanolic pulp extract had lower activity than the seed coat extract with IC_{50}values of 357.03 mg/ml and 160.86 mg/ml respectively. Comparatively, the methanolic extract of the seed coat had lower activity than the ethanolic extract while the methanolic extract of the pulp had higher activity than the ethanolic extracts of the pulp. Comparing the results with the inhibition of the dehydrogenase activity by standard antibiotics reported by Ukaoma, *et al.* [44], the plant material had lower activities than Gentamicin and Fluconazole.

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