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PROXIMATE AND MINERAL ELEMENTS COMPOSITION OF THREE SPECIES OF KOLANUT *(Cola acuminata, Cola afzelii and Cola nitida).*

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Abstract: Kolanut is a member of the tropical family sterculiaceace. It is a native stimulant which is commonly chewed in many West African cultures. The objective of this study is to evaluate the Proximate and Mineral Composition of three species of kolanuts (Cola acuminata, Cola afzelii and Cola nitida). Five varieties of kolanuts were collected from a local farm in Orin Ekiti. Nuts removed from the husks, dried and powdered. Three types of extraction were carried out (acetone, cold and hot aqueous extraction). Proximate and mineral composition were determined using standard analytical methods. The results showed that red cola acuminata has the highest content of moisture (10.54%) crude protein (10.11%), crude fat (2.70%), ash (4.96%) and crude fiber (7.60%) and the least carbohydrate (64.09%). White cola nitida has the highest calcium (951.56mg/100g), magnesium (48.14 mg/100g), and iron (1.85mg/100g). The results obtained indicate that these three species of kolanuts pose promising chemotherapeutic and antibacterialpotentials.

Key words: Kolanuts, Cola nitida, Cola, acuminata, Cola afzelli, Proximate analysis.

INTRODUCTION

Plants are very important in our everyday existence, they provide our foods, produce the oxygen we breathe and serve as raw materials for many industrial products such as clothes, foot wears and so many others. Plants also provide raw materials for our building and in the manufacture of dyes, pesticides and drug etc (Adesuyi *et al*., 2012). Generally plants are primary sources of medicines, fibre, food shelters and often other items used by human. Their roots, stem, leaves, flowers, fruits and seeds provide food for humans (Amaechi, 2009).
The use of plant in traditional medical practice has a long drawn history and remain the mainstay of primary health care in most of the third world population in both developing and developed countries where modern medicines are used by about 60% of the world population (Mythlypriya *et al.,* 2007).
Plants have been used since antiquity for medicinal purposes by diverse people and culture throughout the world indeed, the recorded use of natural products as a source of relief from illness dates back at least four thousand years and it can be assumed that unrecorded practices are as old as mankind (Dewole *et al.,* 2013). It is believed that crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects (Jana *et al*.,2010). Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available and with less side effects. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity (Misra, 2009). Well known examples of drugs with plant origin include; aspirin, atropine, digoxin, ephedrine, morphine, quinine, reserpine, vincristine and vinblastine as well as several plant steroidal 2 sapogenin which serve as semi-synthetic precursors to the steroidal drugs (Dewole *et al.,* 2013). Herbal medicine has used plants, plant parts, their juices or solvent extracts, essential oils, gums resins exudates or other forms of plant products to treat, cure or prevent some diseases in both humans and animals. People on all continents used hundreds to thousands of indigenous plants for food and treatment of ailments (Udoh *et al.,* 2011). Despite the existence of hospitals in developing countries and laws that consider traditional medicine as an illegal act, the valorization of the traditional medicine has become an early criterion of identity as well as rights of health and education. The use of plants for medicinal purposes continues to this day, usually in the form of traditional medicine which is now recognized by the World Health Organization as a building block for primary health care (Akerele, 1998; Dewole *et al.,*2013).

II. MATERIALS ANDMETHODS

A. SamplingProcedure

Fresh pods of kolanuts were obtained from a local farm in Orin-Ekiti, Ekiti State Nigeria. This is to ensure the nuts are free from insecticides or fungicides.
The nuts were removed from the husks using sterile knife and soaked in clean water for 3 – 4 hours to ease the removal of the coats, rinsed with distilled water after removing the coats and then sorted out according to the species. After sorting, the nuts were chopped into pieces in separate trays using grater and air dried for two weeks. The dried samples were milled using electronic blender, packed in different air-tight plastic containers and kept in the refrigerator at 4 ^{o}C prior analysis.

B. Samples ExtractPreparation

Three types of extractions were carried out. Cold aqueous extraction was done by weighing a portion of 100g of each sample of kolanut in separate conical flask. 100 ml of distilled water was added, corked and left overnight (Udoh *et al.,* 2011). Hot aqueous extraction was produced at 8 ^{o}C for 4 hours in a water bath using the same measurement (Oshodi *et al.*, 2004). Acetone extract was done using the same weight the individual measured samples were macerated in 100 ml acetone in separate conical flask for 24 hours (Udoh *et al.,* 2011). The resulting liquor was filtered through whatman no 1 filter paper. The filtrate of the aqueous extraction was concentrated under vacuum at 45 ^{o}C and that of acetone was concentrated using waterbath. The extracts were then poured into different clean sterile plastic bottles and preserved in the refrigerator at 4 ^{0}C for furtherusage.

METHODS

Proximate Analysis

Proximate analysis determines the total moisture, ash, crude fat, protein, crude fibre and carbohydrate contents of a analysis was carried out in triplicate using the method described by A.O.A.C, (2006).

Moisture Content

Cleaned and dried petri dishes were weighed and respective weight was recorded as (W1). About 3Sg of each sample was weighed into the respective dishes spreading as much as possible. The petri dish and the sample were weighed and recorded (W2). The dishes with the samples were transferred into an oven maintained at 105 ^oC and dried for 3 hours. The dishes with the samples were transferred into a desiccator to cool and then reweighed. The process of heating, cooling and weighing was continued at 30 minutes interval until a constant weight recorded as (W3) was obtained. (A.O.A.C, 2006). The loss in weight during drying in percentage was taken to be the percentage moisturecontent.

$$\% \text{ Moisture content} = \frac{\text{Weight loss}}{\text{Weight of sample}} \times 100$$

Weight loss = weight of sample before drying – weight of sample after drying

$$\% \text{Moisturecontent} = \frac{W2 - W3}{W2 - W1} \times 100$$

W1 = Weight of empty evaporatingdish

W2 = Weight of empty evaporating dish +sample

W3 = Constant weight evaporating dish and driedsample

Ash Content

About 1g each of the samples was weighed into a crucible (W2) which had been previously placed in a muffle furnace for 15 minutes at 350 ^oC, cooled in a desiccator and weighed (W1). The crucible and the samples were well labeled and placed in a muffle furnace maintained at 550^oC to ash the samples until a white or light ash was obtained. The crucibles were later cooled in a desiccator and weighed (W3). The percentage ash was calculated and the ash was used for mineral analysis (A.O.A.C,2006).

$$\% \text{AshContent} = \frac{\text{Weight of Ash}}{\text{Weight of Sample}} \times 100$$

$$\% \text{AshContent} = \frac{W3 - W1}{W2 - W1} \times 100$$

W1 = Weight of empty crucible W2 =
Weight of crucible +Sample

W3 = Weight of crucible + ashsample

Crude Fat

Soxhlet extraction method was used to determine the crude fat. A previously dried filter papers were weighed (W1). 3g of each sample was added and weighed (W2). The filter paper was tightened very well with white thread and transferred into a thimble. A 500 ml round bottom flask was filled with n-hexane up to two – third of the flask. The soxhlet extractor was attached to the round bottom flask and was also fitted with a reflux condenser. The set up was placed on electron – thermal hot plate which was adjusted so that the solvent boils gently and the solvent was allowed to siphon over several times for 8 hours. After the extraction the defatted samples were air dried for the solvent to evaporate which after were dried in an oven at 50 ^0C, cooled in a desiccator and weighed (W3) (A.O.A.C,2006).

$$\% \text{ Crude Fat} = \frac{W2 - W3}{W2 - W1} \times 100$$

W1 = Weight of the filterpaper

W2 = Weight of the filter paper +sample

W3 = Weight of filter paper + deflatedsample

Crude Fibre

The crude fibre content is a measure of the structural carbohydrate in a sample (Banjo, 2006; Chris. 2010). About 3g

of each sample was weighed (W_1) into different 250 ml conical flasks. 200ml of 1.25% H_2SO_4 was added into each flask and the solution was brought to boiling point within one minutes. The mixtures were then boiled gently for 30 minutes with constant rotation of the flask every minute so as to mix the content and remove particles from the sides. The mixture was allowed to cool for one minute and then filtered through muslin cloth stretched over Buchner tunnel. The residue was thoroughly rinsed with distilled water and then scrapped back into the flask with spatula and 200 ml of 1.25% of NaOH was added then allowed to boil for 30 minutes. The resulting solution was then filtered. The residue was thoroughly rinsed with distilled water, later rinsed once with 10% HCl and twice with methylated spirit and petroleum ether. The residues were then drained and scrapped into a crucible that the weight had beennoted and dried to a constant weight at 105^0C, cooled in a desiccator and weighted (W_2). The residues were then transferred into a muffle furnace at 450^0C for ninety minutes to ash, later cooled in a desiccator and weighed (W_3). (A.O.A.C, 2006).

$$\% \text{ Crude Fibre} = \frac{\text{WeightLoss}}{\text{Weight of Sample}} \times 100$$

$$\% \text{ CrudeFibre} = \frac{W_2 - W_3}{3g} \times 100$$

Crude Protein

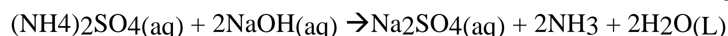
The protein content of foods can be determined on the basis of total nitrogen content, while the kjedahl method has been almost universally applied to determine nitrogen content. (A.O.A.C, 2000). Nitrogen content is then multiplied by a factor to arrive at protein content (A.O.A.C, 2000).

1g of the sample was weighed (W_1) into a kjeldahl flask and a tablet of kjeldahl catalyst was dropped into the sample flask along with 15 ml of concentrated H_2SO_4. The mixture was then swilled together and the flask was placed in a preheated digester in a fume cupboard. The mixture was then heated until clear solution was obtained. The clear solution was cooled, then poured into a 50ml volumetric flask and made up to the mark with distilled water(V_1).

Sample + Conc. $H_2SO_4 \rightarrow (NH_4)_2SO_4$ + Excess Conc. H_2SO_4.

ii. Distillationstage

The second step was the distillation stage. This involves steam distillation of the cooled, diluted digested sample. 15ml of diluted solution was measured and transferred into a distillation apparatus, and then 25ml of 40% NaOH was added.



$(NH_4)_2SO_{4(aq)} + 2NaOH_{(aq)} \rightarrow Na_2SO_{4(aq)} + 2NH_3 + 2H_2O_{(L)}$

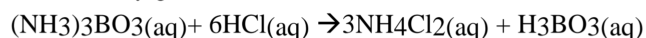
5ml of 2% boric acid was pipetted into a conical flask with 2 drops of mixed indicator (0.016g of methyl red and 0.08g of bromocresol green were dissolved in 100ml of alcohol) to produce a pink colour solution and placed under the condenser outlet. The conical flask was put in such away that the delivery tube was immersed into the boric acid inside the conical flask. As the distillation proceeds, the pink colour solution of the receiver turned light green, indicating the presence of NH_3. About 50 ml of the solution was collected into the flask.

The receiving NH_3 forms a complex with boric acid as H_3BO_3 +

$3NH_3 \rightarrow NH_4 + HBO_3$

iii. Titrationstage

The third stage which was the final stage involves titration in which NH_3 received in the boric acid solution was titrated against standard 0.1M HCl. A colour change of this solution from bluish back to pink due to the presence of the mixed indicator that was earlier introduced in the distillation stage indicated the end point. The titre value was noted and recorded. The % Nitrogen in the sample was estimated and the crude protein was determined by multiplying that value by general factor6.25.



$(NH_3)_3BO_{3(aq)} + 6HCl_{(aq)} \rightarrow 3NH_4Cl_{2(aq)} + H_3BO_{3(aq)}$

$$\% \text{ Nitrogen} = \frac{(T - B) \times 14 \times 0.1 \times V_1 \times 100}{\text{Weight of sample} \times V_2}$$

Where T = The titre value

B = Blank

V_1 = Volume of Digest

V_2 = Volume of Digest Used

% = Crude Protein = % Nitrogen x 6.25

The amount of crude protein contained in the sample is obtained by multiplying the nitrogen content of the food by 6.25. The factor 6.25 owes its origin to the assumption that all food protein contains 16% nitrogen, and all nitrogen in a food is present asprotein.

Determination of Carbohydrate Content

Carbohydrate is the most abundant constituent of plants and animals. The most common approach for the determination of carbohydrate content of food is the difference between thtotal predominant contents in percentage (ash, moisture, fat, crude fibre, crude protein) and one hundred (A.O.A C.,2010)

% Carbohydrate = 100 – (% ash + % moisture + % Fat + % crude fibre + % crude protein)

Mineral Analysis

The minerals were analyzed from solution obtained by first dry – ashing. The ashed content of the samples were dissolved with 20 ml 10% HCl solution, and filtered into 100 ml volumetric flask, and made up to 100 ml with distilledwater.

The sodium and potassium contents were determined using TENWAY Flame Photometer while other minerals were determined using Atomic Absorption Spectrophotometer (AAS).

RESULTS AND DISCUSSION

The results of the proximate composition and mineral analysis of the different species of kolanuts (Red and White *Cola acuminata* (A_1 & A_2), Red and White *Cola nitida* (B_1 & B_2) and *Cola afzelii* (C) respectively are represented in each table. The analysis was carried out in three replicates. The results were expressed as $\pm$ Standard Deviation (SD) of the three replicates. Data obtained were subjected to Analysis of Variance (ANOVA). Table 1 shows the proximate composition of red and white *C*ola *acuminata* (A_1 & A_2), red and white *C*ola *nitida* (B_1 & B_2) and *C*ola *afzelii* (C) in terms of a moisture, crude protein, ash, crude fat, crude fiber and carbohydrate.The moisture content revealed that red *Cola acuminata* (A_1) has the highest value of 10.54% and the lowest value is from the white species of *Cola acuminata* (A_2) 8.91%. The values for red *Cola nitida,* white *Cola nitida* and *Cola afzelii* are 9.52, 10.01 and 9.66% respectively. The values are comparable with 9.73% *Cola acuminata* and 9.81% for *Cola nitida* reported by Dewole *et al*., (2013) but lower than 20.62 and 22.50% reported for *C*ola *acuminata* and *C*ola *nitida* respectively by Ajai *et al*., (2012). The protein content of red *acuminata* (A_1), white *acuminata* (A_2) red *nitida* (B_1) white *nitida* (B_2) and *Cola afzelli* (C) are 10.11, 10.06, 9.95, 8.75 and 9.63% which revealed that red *Cola acuminata* has the highest protein value and the white of this same species has the lowest protein value. The results are higher than 8.65% (*Cola acuminata)* and 8.68% (*Cola nitida)* reported by Ajai *et al.,* (2012) but lower to 19.14% (*C*ola *acuminata)*, 15.24 (*Cola nitida)* reported by Dewole *et al,* (2013). Jayeola, (2001) also reported that protein content of *Cola nitida* was 8.90% which was within the range of this research result for *Cola nitida*.

Table 1: Proximate composition (% Dry Weight) of three species of kolanuts

Parameter	A1	A2	B1	B2	C
Moisture	10.54±0.12	8.91±0.08	9.52±0.13	10.01±0.21	9.66±0.11
Crude protein	10.11±0.10	10.06±0.50	9.95±0.11	8.75±0.05	9.63±0.11
Fat	2.70±0.12	2.41±0.02	1.15±0.17	2.24±0.21	1.31±0.16
Ash	4.96±0.15	4.08±0.12	4.22±0.14	4.90±0.10	4.02±0.10
Crude fiber	7.60±0.00	7.32±0.07	7.50±0.10	7.00±0.01	7.23±0.15
Carbohydrate	64.09±0.14	67.22±0.67	67.66±0.36	67.10±0.14	68.05±0.34

Results are presented as mean ±standard deviation

A1 – Red *Cola acuminata* A2 –
White *Cola acuminata* B1 – Red
Cola nitida
B2 – White *Cola nitida*
C – *Cola afzelii*

The fat content of red *acuminata,* (A1) white *acuminata* (A2), red *nitida* (B1), white *nitida* (B2) and *Cola afzelii*. (C) are 2.70, 2.41, 1.15, 2.24 and 1.31% respectively. The fat content shows that the red *acuminata* has the highest value (2.7%) and the least value for the red *nitida*(1.15%).

Red *acuminata* show the highest value for crude fiber content (7.60%) and the least value is for the white *C*ola *nitida* (7.00%). The proximate composition of kolanuts varies relatively from different researchers.The crude fiber content of red and white *acuminata* (A1 & A2), red and white *C*ola *nitida* (B1 & B2) and *C*ola *afzeli* are 7.60, 7.32, 7.50, 7.00 and 7.23% respectively. This indicates that red acuminata has the highest content (7.60%) and the least value is for the white *Cola* nitida (7.00%). These values are lower than what had previously been reported by Abulude (2004) who reported 17.5% for *Cola nitida* and 12.00% for *Cola acuminata,* higher than 4.25% for *Cola nitida* and 3.38% for *Cola acuminata* reported by Ajai *et al.,*(2012).

However, the result was similar to 7.13% fo However, the result was similar to 7.13% for *Cola nitida* reported by Odebunmi *et. al.*, (2009). Ash content of red *acuminata* (A1), white *acuminata* (A2), red *Cola nitida* (B1), white *Cola nitida* (B2) and *Cola afzelli* are 4.96, 4.08, 4.22, 4.90 and 4.02% respectively. These values are of the same range with 4.2% and 3.1% reported for *Cola acuminata* and *Cola nitida* by Abulude, (2004) but higher than 2.72% for *Cola acuminata* and 2.21% for *Cola nitida* reported by Dewole, (2013). *Cola afzelli* has the highest carbohydrate value (68.05%) followed by red C*ola nitida,* (67.66%), white *Cola acuminata* (67.22%), white *C*ola *nitida* (67.10%) and red *Cola acuminata* (64.09%), respectively. The carbohydrate values are of the same range with what had previously been reported for *Cola nitida* and *Cola acuminata*. Ajai *et. al.,* (2012) reported 61.11% for *Cola nitida* and 64.05% for *Cola acuminata,* Dewole, (2013) reported 66.45% for *Cola nitida* and 58.09% for *Cola acuminata*. However, these values are higher than 38.81% and 33.84% reported for *Cola nitida* and *Cola acuminata* respectively by Odebunmi *et. al.,* (2009) and lower than 84.43% and 85.94% reported for red and white *Cola nitida* respectively by Ugioro *et.al.,*(2016).

The varying values reported may be due to different environment where the plants were being planted, seasons and climatic conditions as reported by Dewole *et al*., (2013).

The relationship between percentage Proximate Compositions and percentage of different body constituents is presented in Table 2. Moisture, crude protein, fat, ash, crude fibre and Carbohydrate showed positive relationship and positive correlation. All these relationships were found to be highly significant at $P < 0.05$.

Table 2: Summary Statistics of Proximate Compositions relationship

Relation	R	A	B	SE(b)	t value when b=0	Sig.
PC A1	0.96	-4.77	5.2854	0.02	456.91	0.000
PC A2	0.095	0.58	4.96	0.05	104.38	0.000
PC B1	0.82	0.58	4.96	0.05	149.84	0.000
PC B2	0.95	1.458	4.9	0.03	93.47	0.000
PC C	0.59	0.34	4.97	0.05	312.89	0.000

r = Correlation coefficient; a = intercept; b = slope; S.E= standard error; *** P<0.05.

Table 3: Mineral Composition of three species of kolanuts

		A1	A2	B1	B2	C
Calcium (mg/100g)	(Ca)	469.56±0.099	634.64±0.016	530.96±0.785	951.56±3.995	485.47±0.721
Magnesium (mg/100g)	(Mg)	37.26±0.081	44.99±0.016	41.34±0.001	48.14±0.001	38.69±0.733
Potasium (mg/100g)	(K)	90.00±0.049	87.24±0.028	85.36±0.021	74.59±0.014	80.13±0.035
Sodium (mg/100g)	(Na)	16.83±0.007	18.83±0.028	16.13±0.014	17.83±0.021	15.74±0.035
Iron (mg/100g)	(Fe)	1.35+0.014	1.35+0.014	1.24+0.014	1.85+0.007	1.34+0.007

A1 – Red *Cola acuminata* A2 – White *Cola acuminata* B1 – Red *Cola nitida*
B2 – White *Cola nitida*
C – *Cola afzeli*

Mineral Composition.
Minerals are important component of diet because of their physiological and metabolic functions in the body. Table 3 shows the mineral composition of the different species of kola nut. The mineral composition in the three species (Red and white *C. acuminata,* (A1&A2), red and white
C. *nitida* (B1 & B2) and *Cola afzelii* (C) varied according to species. Calcium (ca) value rangebetween 469.55 – 951.56 mg/100g. White *C. nitida* had the highest concentration (951.56 mg/g) and red *C*ola *acuminata* had the least value (469.56) mg/g. Though the value of calcium (ca) is very high in this study compared to Dah Novvlessonon *et. al.,* (2015) but the result revealed that white *C*ola *nitida* had the highest value which is comparable to the result of the work of Dah Novvlessounon *et al.,* (2015) which also revealed the *C. nitida* has the highest value of calcium. Contrary to the result showed by Mustapha *et. al.,* that both *C*ola *nitida* (15.6 mg/100g) and *C*ola acuminata (13.3 mg/100g) are not good sources of calcium because of low value of the calcium in the kolanuts. The recommended dietary intake of calcium per day is between 360-1200 mg (Mustapha*etal*.,2009).The results of this research showed that kolanut is a good source of calcium. The element (Ca) is an important mineral required for bone formation and neurological function of the body.
The values obtained from magnesium, potassium, calcium and sodium were high and varied according to the species. Iron content was low and varied according to species. The result is comparable with that of Mustapha *et al.,*(2009) in which the result of iron was found to be considerably low in *Cola nitida* (1.1 mg/100g) and 0.8 mg/100g *Cola acuminata* respectively. It was revealed in the results that all the species are rich in major elements such as calcium, potassium, sodium and magnesium which is comparable to the work reported by Agbeniyi and Ayodele, (2010) that health kolanuts is rich in major elements such as calcium, potassium, phosphorus, sodium and magnesium. (Agbeniyi and Ayodele, 2010).

CONCLUSSION

The use of traditional remedies and the belief in their effectiveness are apparently steeped in treatment experiences of relatives and in traditional and cultural health care practices. The results of proximate analysis and mineral composition reported in this study disclosed the specific basis for the usefulness of kolanuts. It can also be depicted by the results that red *Cola* acuminata has the highest composition except in some of few minerals. On the other hand, *Cola afzelii* that is underutilized has almost the same composition with other species used in this research.

From the findings, it can be suggested that kola nuts including the *Cola afzelii* can act as a source of some of the vital nutrients in order to complement their deficiency in our diet.

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