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BIOCHEMICAL CHARACTERIZATION OF SOME NIGERIAN *CORCHORUS* L. SPECIES

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ABSTRACT
Corchorus (L) is an important vegetable, grown mainly for its leaves. It contributes to the dietary requirements of the local populace as well as local economy. Five accessions, three labeled NHCO3, NHCO25 and NHCO26 obtained from the Plant Genetic Resources Unit of Nigerian Institute of Horticulture (NIHORT), Ibadan and two BN/OD/01 and ON/OD/02 obtained from home gardens were evaluated for their proximate: ash, moisture, carbohydrate, protein, lipid and fibre contents. Result showed that all biochemical parameters evaluated vary significantly (P 0.05) among the accessions. Accession NHCO25 had the highest ash (8.82 ± 0.87), lipid (10.83 ± 1.27) and moisture content (11.31 ± 0.93). Accession NHCO26 had the highest carbohydratecontent (17.94 ± 0.17) but is low in crude fibre content. ON/OD/02 had the highest protein content (62.96 ± 4.80). The highest crude fibre content was obtained from accession NHCO3. Data obtained were standardized and subjected to principal component analysis to determine which proximate parameter accounted most for the variation observed in the five accessions studied. PCA axes 1, 2, and 3 accounted for 96.25% of the total variation. At PCA I ash and lipid accounted for the variations, fibre in PCA II and moisture in PCA III. Single linkage cluster analysis was accessed to group accessions based on similarities and differences in characters. A resulting dendrogram from the data reflected a clustering pattern of three cluster groups. Cluster I contained accession NHCO26, cluster II contained accession NHCO3, BN/OD/01 and ON/OD/02; while cluster III contained accession NHCO25. Accession NHCO25 and NHCO26 were the most distinct and diverse of all the accessions and could serve as sources of variability in character for Corchorusgermplasm improvement. These results revealed that the accessions of Corchorus evaluated contain appreciable amounts of lipids, ash, fibre and moisture contents and these components could account for the variability among these accessions. This study suggests proximate analysis as another window for determining variability among crop plants.

Key words: Corchorus, leafy vegetable, biochemical characterization, principal component analysis, single cluster analysis.

INTRODUCTION

Corchorus species an indigenous crop plant belongs to the family Malvaceae (APG III, 2009). The genus *Corchorus* consists of some 40-100 species of which about 30 are found in Africa (Mbaye*et. al*., 2001). Edmond (1991) also stated that thirty species are found in Africa and four species in Nigeria. The geographical origin of *Corchorus*sp. is often disputed, because it has been cultivated since centuries both in Asia and Africa, and it occurs in the wild in both continents. Some authors consider India or the Indo-Burmese area as the origin of *Corchorus* sp However, the presence in Africa of more wild *Corchorus* species and the larger genetic diversity within *Corchorus* sp point to Africa as the first centre of origin of the genus, with a secondary centre of diversity in the Indo-Burmese region. At present *Corchorus* sp is widely spread all over the tropics and it probably occurs in all countries of tropical Africa (Khandakar and Van der Vossen, 2003).

It is of high social-economic importance especially in western Nigeria where the livelihoods of millions of local farmers depend on *Corchorus* production and utilization. The leaves are used as traditional vegetable. In Nigeria, traditional vegetables are important sources of nutrients and vitamins for rural population as many nutritional studies have shown (Mnzava*et. al.,* 1999). Farmers have cultivated and collect these vegetables for generations as an additional food source.

The composition of *Corchorus olitorius* leaves per 100g fresh edible portion is: water 80.4g(74.2-91.1), energy 243 kj (58 kcal), protein 4.5g, fat 0.3g, carbon hydrate 12.4g, fiber 2.0g, Ca 360 mg, P. 122mg, Fe 7.2 mg, β-carotene 6410µg, thiamine 0.15mg, riboflavin 0.53mg, niacin 1.2mg, ascorbic acid 80mg (Leung *et al.,* 1968). This composition is in line with other dark green leafy vegetables, but the dry matter content of fresh Jew's mallow leaves is higher than average. The compositions, especially the micronutrient content, are strongly influenced by external factors such as soil fertility and fertilization. Nitrogen fertilizer greatly improves the micro-nutrient content, example Fe, P, Ca, Carotene and vitamin C (Fondio and Grubben, 2004). *Corchorus* is mainly known for its fiber product and for its leafy vegetable. Several species of *Corchorus* are used as vegetables of which *Corchorus olitorius* is most frequently cultivated (Akoroda, 1988).

Jute fibers are obtained from the blast. The use is limited to coarse fabrics, because the length: diameter ratio of jute filaments is only 100-120, much below the minimum of 1000 required for fine spinning quality. Fiber cells are 2-2.5mm long and 15-20 µm in diameter. Fiber cells are cemented together into filaments of up to 250mm long. The tensile strength of jute is 395-775 mpa, the elongation at break is only 1-2%, jute has a low ignition temperature of 193^{0}C, posing a considerable fire hazard in warehouses (Singh, 1976).

Principal component analysis shows the pattern of co-variation of characters among the individuals (Rhodes and Martins, 1972; Jolliffe, 2002). It reduces the dimension of multivariate data by removing inter-correlation among variables and allows a multi-dimensional relationship to be plotted on two or three principal component axes (Hayman, 1967). The relative discriminating power of the axes and their associated characters are measured by eigen-values and factor scores respectively. However, PCA alone would not give an adequate character representation in terms of relative importance when numerous characters are considered simultaneously (Shalini*et. al.,* 2003). To complement the result of such multivariate analysis; single linkage cluster analysis (SLCA) are often employed to classify the variation. SLCA is an agglomerative technique which shows the pattern of relationship between individuals of a population (Ariyo and Odulaja, 1991). SLCA is generally employed to summarize the position of accessions by sorting them into distinct groups. It is often used to illustrate patterns of co-variation of characters among individuals. It is the aim of this study to examine the variation in *Corchorus* germplasm based on biochemical traits in the research institute and domestic (local market) cultivar, with a view of identifying potential cultivars to improve *Corchorus* production in Nigeria. More so, assess the genetic variability among the five accessions of *Corchorus* using Single linkage cluster analysis and principal component analysis.

MATERIALS AND METHODS

STUDY AREA: The studies of the *Corchorus* were conducted in an experimental plot garden in University of Benin, Benin City, Edo State.

PLANT MATERIALS: Three accessions labeled NHCO3, NHCO25, and NHCO26 were collected from plant Genetic Resources Unit, NIHORT, Ibadan, Nigeria and other landraces, BN/OD/01 and ON/OD/02 from Benin City, Edo State and Owo, Ondo state respectively, were used for the study (table 1). The landraces were identified based on local names at the point of collection by farmers. Herbarium samples were prepared and deposited at Plant Biology and Biotechnology Departmental herbarium.

Table 1: Accession Codes and their Sources

Serial number	Accession name	Source	Longitude and Latitude
1	NHCO3	NIHORT	7.2^{o}N and 3.52^{o}E
2	NHCO25	NIHORT	7.2^{o}N and 3.52^{o}E
3	NHCO26	NIHORT	7.2^{o}N and 3.52^{o}E
4	BN/OD/01	BENIN	6.2^{o}N and 5.73^{o}N
5	ON/OD/02	ONDO	7.05^{o}N and 4.49^{o}E

PLANT HUSBANDRY: The seed dormancy for accessionsNHC03, NHC025, NHC026, BN/OD/01 and ON/OD/02 were removed by technique described by Schippers (2000). They were raised in nursery box filled with 500g of top soil. Planting was done by broadcasting method using 30 g of seeds of each of the accessions in each box. Watering was done daily every morning and evening. After four weeks in nursery, three seedlings were transplanted in polythene bags filled with top soil. After two weeks of transplant each bag was thinned to two plants per bags.

EXPERIMENTAL SET UP: A spacing of (45 × 70) cm^2 (Remison, 2005) was used to mapout the crops in a randomized complete block design (RCBD). In each block, each accession was in four replications.

DATA COLLECTION: Ten leaves each from the 10^{th} nodes upward of each accession was washed, ground and the paste taken to the laboratory for analyses. Proximate analysis of ash content, moisture content, carbohydrate, protein, lipid and fiber content was carried out.

ANALYSIS OF THE SAMPLE PASTE: Proximate analysis of the leaf paste of the different accessions was conducted at the Pharmaceutical Chemistry Laboratory, University of Benin, Benin City, Edo State. Using procedures outlined by AOAC (1995), the moisture content was determined by air-oven drying as weight difference at 130^{O}C for 1 hr. and the crude protein by micro Kjeldahl method (% total nitrogen × 6.25; AOAC 1990). The crude fibre content was extracted by exhaustively extracting 10g of each sample in a soxhlet apparatus using dimethyl ether (boiling range, $30\text{-}60^{O}$C) as solvent. Ash content was also determined by the incineration of 10g of each sample placed in a muffle furnace maintained at 550^{O}C for 5 hrs. The total carbohydrate content was calculated by the difference: 100-(crude protein + crude fat + ash + crude fibre + moisture content).

Data Analysis

Data were analyzed using SPSS (version 20.0) and PAST (Paleontological statistics, version 1.34). Analysis of variance was done to locate significant difference among the accessions. The PCA and SLCA were used to determine the extent of genetic variation and percentage similarity among the accessions.

Eigen-values and factor scores were obtained from PCA, which were used to determine the relative discriminative power of axes and their associated characters.

K-means model clustering procedure was used to group the 5 accessions based on their genetic relationship. A dendrogram was generated from the SCLA to display position of accessions and their distance similarity.

RESULTS

Results are presented in Tables2, 3, 4, 5 and 6 as well as figure 1. Table 2 shows means and standard errors for the proximate compositions of the different accessions. Ash content was analyzed for the various accessions. NHCO25 had the highest ash content value with 8.82 ± 0.87%, while accession BN/OD/01 had the lowest ash content value with 4.22 ± 0.91%. Accession NHCO25 had the highest moisture content value of 18.18 ± 0.57 while BN/OD/01 had the lowest moisture content value with 11.31 ± 0.93%.

The result for the average carbohydrate content shows thatNHCO26 had the highest carbohydrate content value with 17.94 ± 0.17%; while NHCO25 had the lowest carbohydrate content with 6.72 ± 0.52%. The average protein content of the various accessions of *Corchorus* depict that ON/OD/02 had the highest protein content value with 62.96 ± 4.80%; while accession number NHCO25 had the lowest protein content value of 44.84 ± 3.75%.The result for average lipid content of the various accessions of*Corchorus* shows thatNHCO25 had the highest lipid content value of 10.83 ± 1.27%, while NHCO3 had the lowest lipid content value of 4.54 ± 0.41%.The average crude fibre content for the various accessions shows that accession number NHCO3 had the highest fibre content value while accession number NHCO26 had the lowest crude fibre content.

Table 2: Means Value and Standard Error for Biochemical Traits in *Corchorus* Accessions

Accessions	Ash Content (%)	Moisture Content (%)	Carbohydrate Content (%)	Protein Content (%)	Lipid Content (%)	Fiber Content (%)
NHCO3	4.43 ± 0.56^{a}	15.73 ± 0.79^{bc}	7.75 ± 1.09^{a}	56.23 ± 2.19^{b}	4.54 ± 0.41^{a}	11.17 ± 1.19^{b}
NHCO25	8.82 ± 0.87^{c}	18.18 ± 0.57^{c}	6.72 ± 0.52^{a}	44.84 ± 3.75^{a}	10.83 ± 1.27^{b}	8.86 ± 0.31^{b}
NHCO26	5.62 ± 0.70^{ab}	12.12 ± 0.65^{ab}	17.94 ± 0.17^{b}	50.59 ± 4.09^{b}	7.90 ±0.42^{a}	3.52 ± 0.54^{a}
BN/OD/01	4.22 ± 0.91^{a}	11.31 ± 0.93^{a}	8.35 ± 057^{a}	50.91 ± 3.29^{b}	8.93 ± 1.15^{ab}	9.39 ± 2.45^{b}
ON/OD/02	4.97 ± 0.97^{a}	13.38 ± 2.31^{ab}	7.12 ± 0.57^{a}	62.96 ± 4.80^{c}	5.42 ± 0.85^{a}	6.15 ± 2.96^{b}

Different alphabetic superscript shows Significance ($P<0.05$)
*Duncan multiple range tests was used to compare means

Table 3: Correlation coefficients of biochemical variables of the various accessions of*Corchorus*

	AC	MC	CC	PC	LC	FC
AC	1					
MC	0.737	1				
CC	-0.103	-0.491	1			
PC	-0.655	-0.321	-0.192	1		
LC	0.703	0.189	0.054	-0.876*	1	
FC	-0.183	0.378	-0.933*	0.340	0.326	1

Coefficient significant at* $p\geq0.05$
Key: AC- ash content, MC- moisture content, CC- carbohydrate content, PC- protein content, LC- lipid content, FC- fibre content.

Table 3 shows the correlation coefficient of the proximate composition variables. These variables include: Ash content, Moisture content, Carbohydrate content, Protein content, Lipid content, and Fibre content. The relationship between the two variables is expressed by these coefficients. For instance ash content is seen to correlate positively with moisture content (r=0.737) and lipid content (r=0.703); while it negatively correlated with fibre content (-0.830). The result of the PCA for biochemical variables of the various accessions of *Corchorus* in the first two axes accounted for 85.92% of the total variation when 0.5 is taken as Joliffe minimum. A total cumulative of 96.25% variability was observed at PCA 3. PCA I was loaded with ash content (0.53) and lipid content (0.53);PCA 2 was with fibre content (0.58); PCA 3 moisture content (0.56), while PCA 4 was loaded with protein content (0.66), giving a total of 99.99% variability. The relative discriminating power of PCA as revealed by the eigen-value was higher in PCA I (2.85) and least in PCA 4 (0.22).

Table 4: Principal Component Analysis (PCA) of proximate composition characters among five accessions of *Corchorussp*

Character	PC Axis 1	PC Axis 2	PC Axis 3	PC Axis 4
Ash Content	0.53*	0.18	0.35	0.46
Moisture Content	0.29	0.48	0.56*	-0.31
Carbohydrate Content	0.08	-0.62	0.33	-0.29
Protein Content	-0.53	0.06	0.31	0.66*
Lipid Content	0.53*	-0.05	-0.52	0.32
Fibre Content	0.23	0.58*	-0.29	-0.25
Eigen-Value	2.84	2.31	0.062	0.22
% Variance	47.41	38.51	10.33	3.74
Cumulative % Variance	47.41	85.92	96.25	99.99

*significant at each axis (above 0.50)

Table 5: The Standardized Principal Component Scores

ACCESSIONS	AXIS 1	AXIS 2	AXIS 3	AXIS 4
NHCO3	-1.2696	1.0112	0.4481	-0.64787
NHCO25	2.596	1.3176	0.1303	0.10832
NHCO26	0.5925	-2.4785	0.4915	-0.06591
BN/OD/01	-0.3131	-0.3309	-1.3854	-0.07155
ON/OD/02	-1.6057	0.4806	0.30991	0.67702

Table 5 shows the standardized principal component scores. Four characters represent 99.9% of the variance from the six biochemical characters. Accession NHCO25 was more described by characters that were significant in PCA 1 and PCA 2. These characters are reflected in ash, lipid fibre content. Significant characters in PCA 3 best describe accession NHCO26. This character is moisture content.While PCA 4 significant characters (protein content) best describe ON/OD/02.

Table 6: Clustering offive accessions of*Corchorus*species based on proximate characters using *k-means* model

CHARACTER	I	II	III
ACCESSION	NHCO25	NHCO26	NHC03, BN/OD/01, ON/OD/02
ASH CONTENT	8.829(0.87)	5.62(0.70)	4.54(0.22)
MOISTURE CONTENT	18.18(0.57)	12.12(0.65)	13.47(1.28)
CABOHYDRATE CONTENT	6.72(0.52)	17.94(0.17)	7.74(0.36)
PROTEIN CONTENT	44.84(3.75)	50.59(4.09)	56.7(3.49)
LIPID CONTENT	10.83(1.27)	7.90(0.42)	6.30(1.34)
FIBRE CONTENT	8.86(0.31)	3.52(0.54)	10.06(0.56)

Table 6 shows the clustering of accessions based on biochemical attributesusing the *K-means* model. Three clusters of one, one, and three accessions were recorded.. Cluster I was loaded with accessions with high ash content, moisture content and lipid content. Cluster II with high carbohydrate content, while cluster III contained accessions probably with high protein and fibre contents.

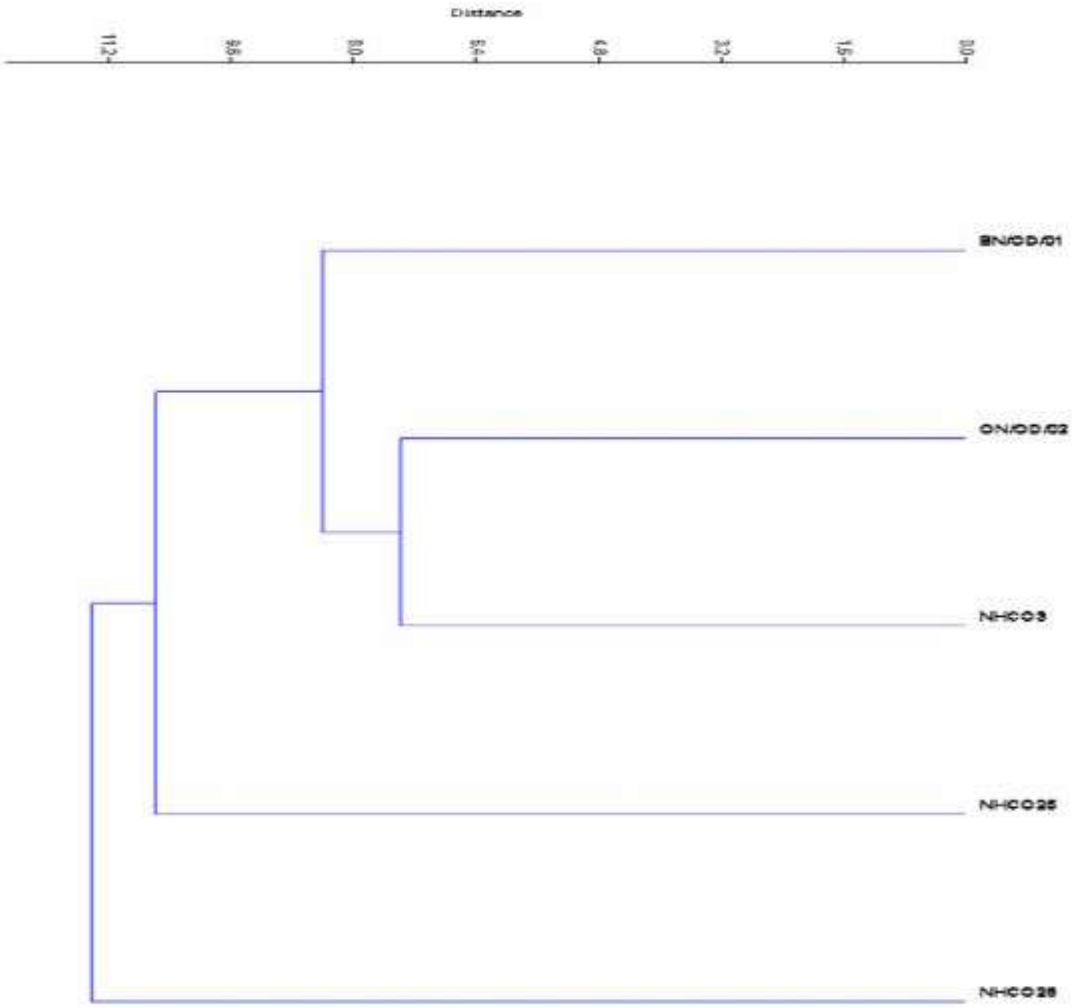

Figure 1: Dendrogram of 5 accessions of *Corhorus* species

The dendrogram reveals 3 clustersat Euclidean distances 10.5. These clusters are NHCO25, NHCO26 and (NHC03, BN/OD/01 and ON/OD/02). This clustering agreed with the proximate composition of each accession.

DISCUSSION

The relationship and distinctiveness among Nigeria *Corchorus* species have been demonstrated using morphological and protein profiling analysis (Osawaru*et al.,* 2012). Credence was ascribed to using morphological characters based on leaf features in characterizing Nigeria *Corchorus* grown in the same environmental condition. This study examined the use of biochemical features (proximate) to evaluate the distinctiveness of the crop plant. It was observed that all proximate features were statistically significant (Table 2).Udosen (1995), Isong and Idiongo (1997), Ali (2009), Iheanacho and Udebuani (2009), Asaolu*et. al.,*(2012) also confirmed and evaluated vegetable crops for their distinctiveness in proximate compositions. Thus, using proximate to classify crop plants.

Average percentage ash content (Table 2), NHCO25 had the highest value of 8.82%, while BN/OD/01 had the lowest. The proportion of highash content in NHCO25 is a reflection of the mineral contents present in the leaves. This could suggest a high deposit of mineral elements in the accession NHCO25. The moisture percentage moisture content as shown, accession NHCO25 was observed to be highly moisture filled; while BN/OD/01 had the lowest moisture content value. The high moisture content of NHCO25 provides for greater activity of water soluble enzymes and coenzymes needed for metabolic activity of this leafy vegetable.

Carbohydrate percentage content, NHCO26 had the highest carbohydrate value with 17.94%, while NHCO25 had the lowest carbohydrate content value with 6.72%. Protein percentage content, ON/OD/02 had the highest protein content value of 62.96%, while NHCO25 had the lowest protein content value with 44.84%. The high crude protein content in ON/OD/02 suggests its richness in essential amino acids. Lipid percentage content, NHCO25 had the highest lipid content value with 10.83%, while NHCO3 had the lowest lipid content value with 4.54%. This shows that accession NHCO25 contains more lipid bi-molecules than the other accessions and as such helps in the absorption of fat soluble vitamins (A, D, E and K) and improve taste (Vasudevan and Sreekumari, 2007). Lipid positively correlated with ash content; this shows that accessions with high lipid content should possess high dry matter weight. However the type of lipid was not considered in this study. However further research is needed to ascertain the composition of the different classes of lipid contained in the different accessions.Fibrepercentage content was high as compared to the work of Leung *et al*., 1968, most especially in accession HNCO3 having the highest fibre content value of 11.17%, such high fibre content of NHCO3 makes it recommended as a good laxative when cooked and eaten in large amount during constipation.

Proximate data were standardized, analyzed and subjected to principal component analysis and single linkage cluster analysis. Correlation analyses (table 3), it revealed some degree of correlation among the proximate characters. Ash content correlated with moisture content (r=0.74) and lipid content (r=0.70) and negatively correlated with protein content (r=-0.655). This is an indication that the dry ash matter content is a function of moisture and lipid content of the crop plant. Nwangburuka *et al*, (2011) stated that when one character correlates positively with the other, when the former is used to characterize and evaluate, the other should be relate redundantly for the same purpose. Hence, in this study ash content character appears to be a function of moisture and lipid content (Table 3).

Principal component analysis method shows the pattern of co-variation of characters among the individuals (Rhodes and Martins, 1972; Jollife, 2002). It tends to reduce the dimension of multivariate data by removing inter-correlation among variables and allows a multi-dimensional relationship to be plotted on two or three principal component axes (Hayman, 1967). The relative discriminating power of the axes and their associated characters are measured by eigen-values and factor scores respectively. The loading of each character on the different principal component axes is showed on table 4. It assesses therelative contribution to the variation. PCA 1 was highly affected by characters such as ash content. PCA 2 was significantly loaded with lipid content. PCA 3 was highly affected by fibre content; PCA 4 protein accounted for the dominant character reflecting the total of 99.99% variability.Ariyo (1993), Rodrigeuz 1999a, 1999b, 2001, Osawaru*et. al.,* 2012) also evaluatedcrop plants to show the genetic variability and listed characters contributing to variability in crops like cocoyam, okra, and bambara groundnut. In this study PCA using proximate data shows significant variability among Nigerian *Corchorus* accessions evaluated.

PCA alone would not give an adequate character representation in terms of relative importance when numerous characters are considered simultaneously (Shalini*et al.,* 2003). To complement such result, single linkage cluster analysis (SLCA) was employed to evaluate the variation and also show relationship pattern among the five accessions. Ariyo and Odulaja, (1991) accessed and evaluated okra using the same statistical procedures. The accessions were grouped into 3 distinct cluster groups with accession NHCO3, ED/OD/01 and ON/OD/02 all clustering in one (figure 1). Such relationship could be use to identify them as having a similar ancestral origin with a common gene for these biochemical components. NHCO25 and NHCO26 were dispersed into different cluster groups. Such accessions would be needed in breeding for improvement of the crop plant. Characters possess by this accessions may also be important for assessment of germplasm collected. The clustering and dendrogram reflected from this study depict similar patterns of accession(s) alignment (Table 6 and Figure 1). This likely suggests credence to the use of biochemical compositions to show variation crop plants grown in the same environmental condition.

In conclusion, the study depicts the importance of biochemical characters as relevant as morphological characters for crop plants evaluation. The importance of principal component analysis as an analytical tool is also significant in data analysis from morphological, biochemical as well as molecular evaluation. From principal component analysis, ash content and moisture content highly contributed to the total variation observed in the five accessions of *Corchorus;* thus, ash content and moisture content should stand out as proximate composition characters that can be used to show variation among Nigeria *Corchorus* in terms of nutritional content.Considering the important of fibre in our diet, the potentials for producing economic important fibreare also implicated by the. Furthermore, NHCO25 had high potentials for ash content, moisture content and lipid content. This would make it a good parental stock material when breeding for *Corchorus* leafy vegetable in terms of nutritional qualities.

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