Bayero Journal of Pure and Applied Sciences, 5(1): 26 – 32
Received: February 2012
Accepted: May 2012
ISSN 2006 – 6996

MORPHOLOGICAL EVALUATION AND PROTEIN PROFILING OF THREE ACCESSIONS OF NIGERIAN CORCHORUS LINN. SPECIES

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ABSTRACT
Vegetative characters and protein profiles of three accessions of Corchorus species were investigated at the experimental plot of the Department of Plant Biology and Biotechnology, University of Benin, Benin City and the Biotechnology Laboratory, University of Agriculture Abeokuta respectively. Corchorus seeds labeled NHCO3, NHCO25 and NHCO26 were obtained from Nigerian Institute of Horticulture (NIHORT), Ibadan. The morphological evaluation was conducted by studying leaf shapes, leaf margin, leaf base, leaf apex, venation, leaf color, leaf texture, plant height, branching, stem type, stem pubescence and stem color. Results show that leaf margin was the most important in distinguishing cultivated Corchorus species. Plant height varied from 16.00 – 68.40 cm for NHCO25, 16.40 – 63.60 cm for NHCO3 and 16.10 – 69.50 cm in NHCO26. Protein profiling was done through SDS-PAGE. No band was shown, signifying that the three accessions are likely to be related. It is suggested from this study that foliar characters are more important in characterizing Nigerian Corchorus species.
Keywords: Vegetative characters, SDS-PAGE, Corchorus accessions

INTRODUCTION

Corchorus L. species commonly referred to as Jute belongs to the family *Tiliaceae*. Mbaye *et al*. (2001) reported that the genus contains forty species throughout the tropics. Edmonds (1991) stated that thirty species are found in Africa and four species in Nigeria.The species found in Nigeria include *Corchorus aestans, Corchorus tridens, Corchorus fascicularis* and *Corchorus olitorius*. These four species are well distributed in the country and are popularly called Ewedu in the south western region.

These various species are usually annual or perennial woody herb that grows to about 0.75 – 2.00 m tall with procumbent or erect stem of about 2.00 – 5.00 m, glabrous sometimes pubescent especially the cultivated species. The leaves are simple ovate, elliptic lanceolate or oblong, margin serrated or crenate often with a pair of basal setae, usually rounded or cruneate at the base rarely truncate. The apex is acute to accumulate or rounded, the petioles are pubescent more or so on one side. The stipules are subulate, pubescent, glabrous or ciliate. The inflorescence is acyme of up to six flowers. Flowers are bisexual, bilaterally symmetrical, with numerous stamens and single hairy pistil. Sepal is 4 - 5, free acuminate, often torulose, cucuilate. Petals 4 - 5, ovate or oblanceolate, rarely spathulate, free, yellow in color. The fruits are 3-6 locules, capsule, solitary or in 2s or 5s, cylindrical, straight or slightly curved, highly ridged and sometimes up to 3 - 7 cm long and 2 – 4 cm in girth, green when fresh and dark brown when fully matured. The seeds are brown to blackish brown up to 5 mm in diameter and usually irregular in shape. *Corchorus* species have been reported to be extremely variable morphologically, especially in the vegetative parts like the leaves (Edmonds, 1990).

It is of high socio-economic importance especially in western Nigeria where the livelihood of millions of local farmers depends on *Corchorus* production and utilization. Various species of this genus have been reported to be of economic and systematic importance (Mbaye *et al*., 2001). Economically, they are regarded as weed although cultivated (Dania-Ogbe and Osawaru 1988, Mbaye, 1999). It is rich nutritionally and serve as a source of nutrients for those that include it in their diet. The leaves are rich in essential amino acids except methionine (Fafunso and Bass, 1975). The leaves of cultivated *Corchorus olitorius* are used as leafy vegetable in food. The leaves have mucilaginous and emollient properties (Edmonds 199 and Pursglove 1968). These properties of the leaves and succulent stem when used in soup help to facilitate the swallowing of coarse starchy and texture African foods like "Eba", "Amala", and "Fufu". The rod contains fibers that can be used in manufacture of ropes for fishing lines, cloth packing and in garment bag. In traditional medicine, infusion of *Corchorus olitorius* leaves are used to cure constipation, scrapping from the roots are used as a cure for toothache while leaves of *Corchorus tridens* are used as plaster to reduce swellings (Edmonds, 1990). *Corchorus olitorius* fruit and seeds can be used as tonic and diuretic. *Corchorus aestans* leaves and roots are being used as cure for gonorrhea while fruits and seeds of the same species are used to treat colic and pneumonia (Ayensu, 1978). Ethnobotanically, it is painted on the outside of calabash and red clay to preserve milk inside. Stems are used to make string and cord, fibers are used in fishing lines and long stems are for horizontal ties of conical roof huts (Edmonds, 1991).

In regions like China, India, Nepal and Bangladesh *Corchorus* species is of great economic importance and bulk of the world supplies are grown there. In these regions it constitutes a major source of employment and foreign exchange.

Corchorus belongs to the genus currently supported as a transition group between two tribes Tiliae and Grewiae. Consequently, identification of the group species is still difficult. Controversies also occur at the family category, taxonomist like Olorode (1984) grouped all taxa of *Corchorus* in the family Tailiaceae. Judd and Manchester (1998), Baum *et al*., (1998), Judd *et al*., (1999) related *Tailiaceae* to *Malvaceae* and concluded that *Malvaceae* was broadly circumscribed and traditionally distinct among the four families: Tailiaceae, Sterculiaceae, Bombaceae and Malvaceae. This work aims at using morphological characters and presence of soluble protein to determine the relationship among some *Corchorus L*. species in Nigeria. This information is an essential requirement to facilitate the systematics of this crop.

The theoretical basis for the use of proteins in evaluating phylogenetic and taxonomic relationship has been documented in literature cited by Yaakov *et al*., (1974) as well as the significant role that gel electrophoresis of protein can play in systematics has also been stressed. Gottlieb (1971) observed that variation in banding pattern could be equated to variations in genes coding for various protein. A number of works have been carried out that utilized protein analysis in the delimiting taxa as exemplified by the researches carried out by Akpabio (1988), Akinwusi and Illoh (1995) and Folorunso and Olorode (2002).

MATERIALS AND METHODS

Study Area

The morphological characterization of the *Corchorus* samples was conducted in the experimental plot of the Department of Plant Biology and Biotechnology, University of Benin, Benin City (6.20N, 5.73E) located within Tropical Rainforest (TRF) zone. The climate includes high rainfall up to 2000mm - 3000mm of biomodal pattern with peaks at July and September respectively, high temperature ranging between 20^0C-40^0C and high atmospheric humidity (Omuta, 1980). Radiation is fairly high and varies according to different periods of the year; above 1,600 hours per year have been reported (Onwueme and Singh, 1991).

Plant Materials

Corchorus seeds labeled NHCO25, NHCO3 and NHCO26 were collected from Plant Genetic Resources unit, NIHORT Ibadan (7.23N; 3.52E). The collections were made in June 2011.

Plant Husbandry

The seeds were drilled in nursery using nursery box filled with 500g topsoil obtained from Capitol-a refuse dump site in the University of Benin. Planting was done the following morning using 20g each of the sun dried seeds by slightly broadcasting the seeds on top of the soil. On the third day after planting, watering was done using 75 cl rain water. Thereafter watering was done every other five day. The plants were transplanted 30 days from date of sowing (DOS) into polythene bags filled with watered topsoil. A plant per bag was transplanted. The plants were grown under the same conditions using a randomized block design.

Data Collection

Quantitative and qualitative morphological characters of the leaves of the plant were from three randomly selected plants. Morphological data on leaves were recorded as follows:

1. Leaf shape: After Olorode (1984)
2. Leaf length (cm)
3. Leaf width (cm)
4. Leaf color: (i) Green, (ii) Green with red patches, (iii) Red
5. Leaf pubescence: (i) Glaborous, (ii) Slightly prickly, (iii) Conspicuous
6. Nature of base: After Olorode (1984)
7. Nature of margin: (i) Smooth, (ii) Serrated, (iii) Deeply toothed
8. Nature of leaf apex: After Olorode (1984)

Soluble Protein

This study was carried out in the Biotechnology laboratory, University of Agriculture Abeokuta using the SDS-PAGE technique.

Extraction: Fresh leaves for the study were carried in McCartney bottle to Abeokuta and labeled as Sample A, B and C. For each sample, 0.3 g of each sample was measured and ground to powder. To this 80µl of 0.1 M Tris-HCl pH was added and vortex for 1 minute.This was followed by spin down at 1000 rpm for 5 minutes in a micro centrifuge. The supernatant was collected into a micro centrifuge tube according to the label and kept at 4^0C in the refrigerator. The resultant supernatant was subjected to SDS-PAGE (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis). The gel used consist of two portions which was prepared using 1.0M Tris-HCl pH 8.8 which is the Resolving gel and 1.0 Tris-HCl pH 6.8 which is the Stacking gel. The gel plates were set up before the mixing of the gel mixes in a Thin spacers.

Resolving gel (12.5%) was prepared using 3.1ml of 30:0.8 % w/v acrylamide:bisacrylamide, 3 ml of 1.0 M Tris-HCl pH 8.8, 38 µl of 20 % SDS and 1.3 ml of distilled water which were mixed together, then 36 µl of 10 % APS (Ammonia per Sulphide) and 10 µl of TEMED was added. The Resolving gel was then poured into the thin spacers. Stacking gel (6 %) was prepared using 1ml of 30:0.8 % w/v acrylamide:bisacrylamide, 630 µl of 1.0 M Tris-HCL pH 6.8, 25µl of 20% SDS and 3.6 ml of distilled water which were mixed together, then 36 µl of APS and 10 µl of TEMED added just before pouring the gel on top of the Resolving gel in the Thin spacer.

After preparation of the gels 12 µl of the sample and 6 µl of mercaptol ethanol was added into a micro centrifuge tube on a fume cupboard and denatured for 3 mins on boiling water at 100 ^{0}C and then allowed to cool. 10 µl of each sample and 10 µl of Ladder or Protein Standard were loaded in the designated well. The gel was allowed to run at 250 mA for a minimum of 105 minutes.

In this process, the gel staining was done overnight using commassie blue. After 8hours, the gel was destained with a solution consisting of acetic acid, methanol and distilled water, and then it was taken to orbital shaker. After staining and destaining, the gel with bands was stored in other to prevent the bands from disappearing, in a solution of 25ml acetic acid in 475ml of water.

RESULTS

Tables 1, 2 and 3 summarised the observations on growth habit, plant height and leaf characteristics after 12 weeks of transplant. These features (growth habit and stem features) appear uniform in all accessions except in stem color. All are woody herb, determinate in growth with erect stem and medium in their branching. Stem color among the accessions is ratio 1:2, green-red. Leaf length varied from 5-20 cm in length and 2-7 cm in breadth. All accessions were pubescent, but accessions NHCO25 was lanceolate in shape, accessions NHCO3 was ovate/lobed and accessions NHCO26 was ovate/glossy. Characters are summarized in table 3 and plates 2 and 3, these features appear to be uniform among the accessions.

Table 1: Characteristics of Growth Habit and Stem features of Three Accessions of *Corchorus* L species transplanted 12 weeks from the date of sowing

Accession	Habit	Growth Habit	Branching	Stem Type	Stem Pubescence	Stem Color
NHCO25	Woody herb	Determinate	Medium	Erect	Slightly prickly	Red
NHCO3	Woody herb	Determinate	Medium	Erect	Slightly prickly	Green
NHCO26	Woody herb	Determinate	Medium	Erect	Slightly prickly	Red below and Green towards the tip

Table 2: Mean weekly Plants Heights of three Accessions of *Corchorus* L. Species

Weeks	NHCO25	NHCO3	NHCO26
1	16.44 ± 0.12	16.40 ± 0.12	16.62 ± 0.20
2	16.72 ± 0.11	16.88 ± 0.18	16.90 ± 0.161
3	17.10 ± 0.2.1	17.28 ± 0.13	17.26 ± 0.24
4	18.28 ± 0.12	18.88 ± 0.15	18.92 ± 0.11
5	35.16 ± 0.62	32.12 ± 0.37	31.44 ± 0.84
6	39.38 ± 1.08	37.72 ± 0.60	37.10 ± 1.27
7	40.60 ± 0.84	37.60 ± 0.66	38.52 ± 0.63
8	42.42 ± 0.33	42.13 ± 0.49	39.60 ± 0.51
9	42.46 ± 1.04	35.76 ± 0.62	41.50 ± 1.68
10	63.24 ± 1.08	64.72 ± 0.93	58.68 ± 2.63
11	65.29 ± 1.88	69.08 ± 1.70	63.06 ± 2.06
12	66.24 ± 1.38	63.84 ± 2.49	69.00 ± 8.55

In the three accessions plant increased in height progressively with NHCO26 being the most robust (Table 2).

Table 3: Leaf Characteristics of Three accessions of *Corchorus* L species transplanted 12 weeks from the date of sowing

Accessions	Shape	Margin	Apex	Base/Setae		Venation	Color	Texture
NHCO25	Lanceolate	Highly serrated	Acuminate	Acute, Setae	2	Pinnately veined	Green	Slighly prickly
NHCO3	Ovate/Lobed	Deeply toothed	Acuminate	Truncate, Setea	2	Pinnately veined	Green	Slighly prickly
NHCO26	Ovate	Serated	Mucronate	Round, Setea	2	Pinnately veined	Green	Slighly prickly

Distinctly, the margins of the leaves are interesting. All appear serrated but the degree of serration is important (Plates 1, 2 and 3).

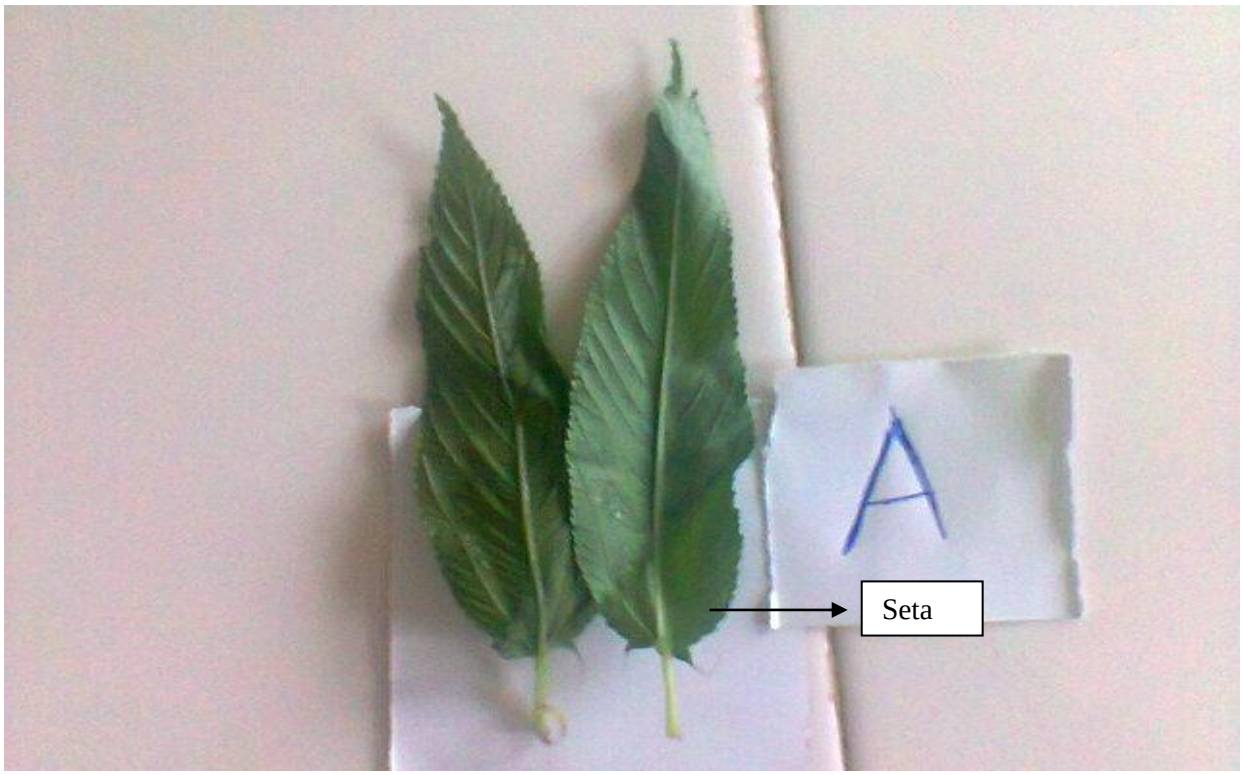

Plate 1: Abaxial Surface of leaf of NHCO25

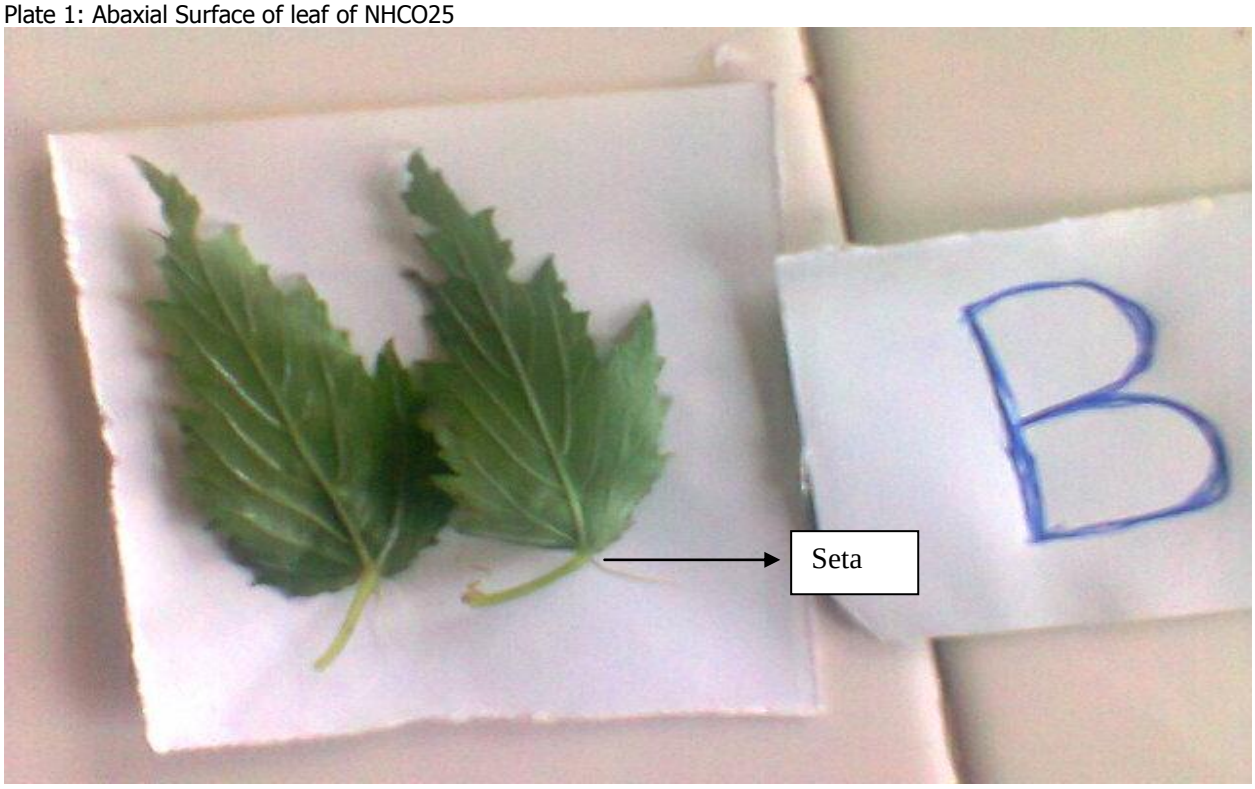

Plate 2: Abaxial surface of leaf of NHCO3

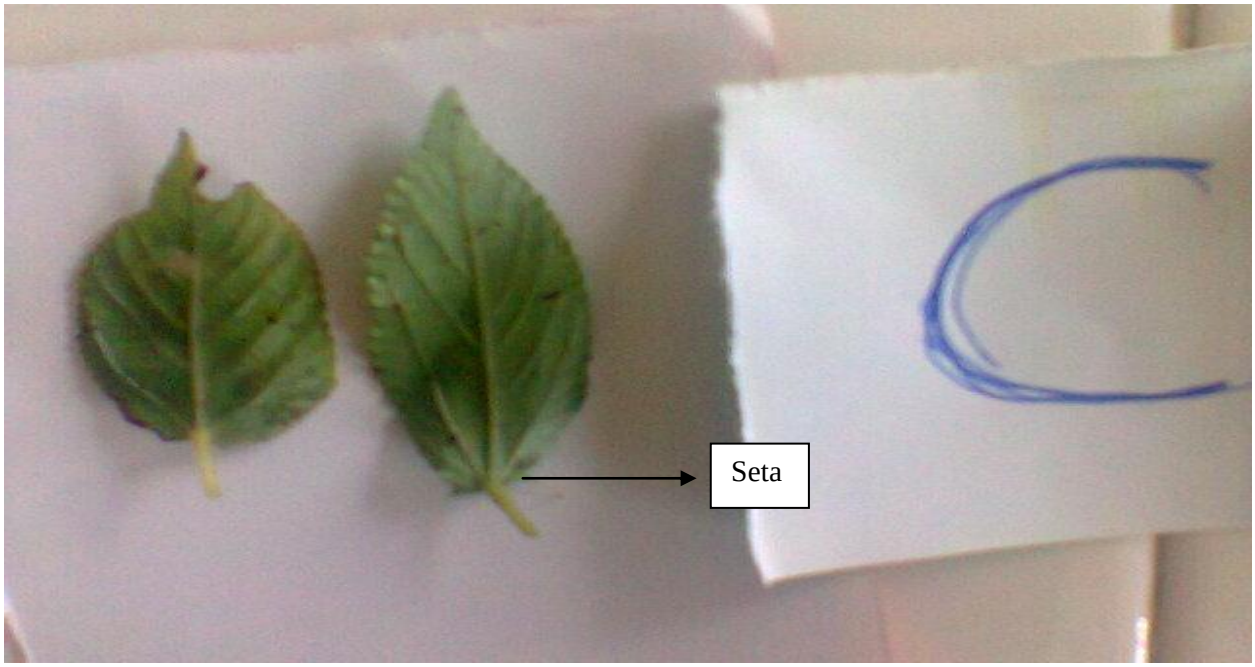

Plate 3: Abaxial surface of leaf of NHCO3

Plate 4 shows the protein profiling bands photographed using 16 mega pixel Sony digital camera and soluble protein present.

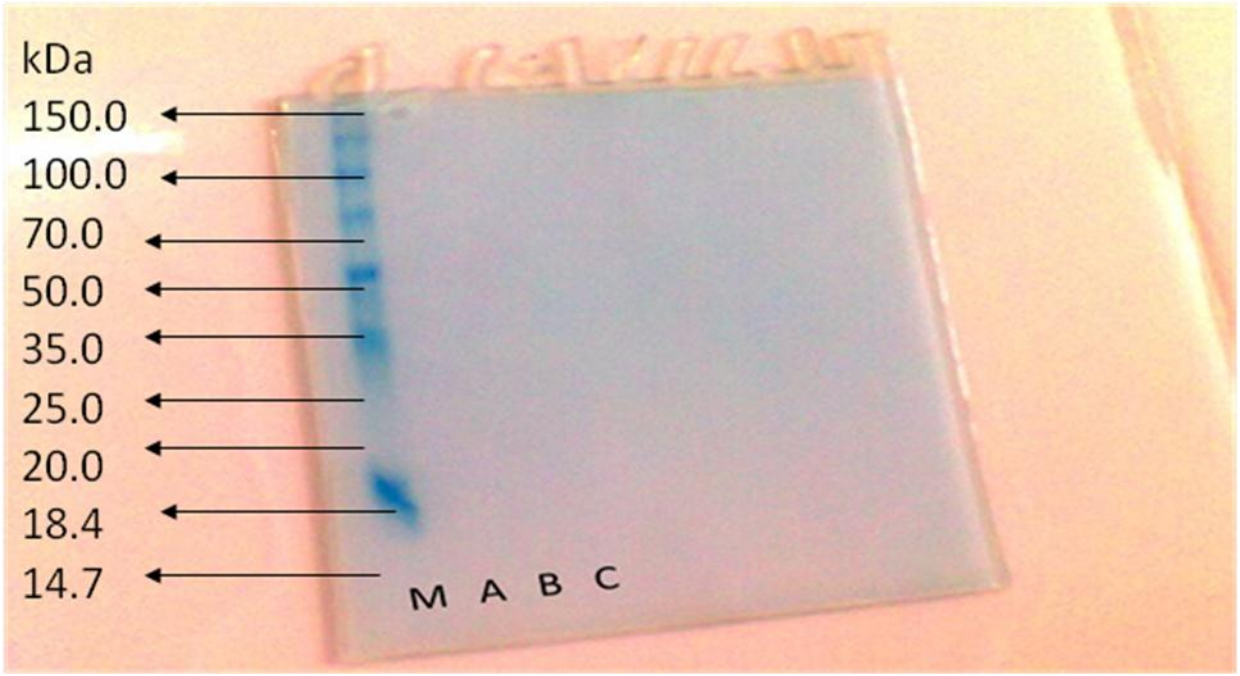

Plate 4: Protein profiling of marker and three accessions of *Corchorus* species
M = Standard protein, **A** = NHCO25, **B** = NHCO3, **C** = NHCO26

DISCUSSION

In recent times, several attempts have been made in many fields of Biology to place the taxonomic relationships of species on a firm physio-chemical foundation. The vegetative architectural and foliar characters of three *Corchorus L*. species were investigated in this study. These qualitative traits/characters namely grown habit and stem features (branching and stem type) are uniform in all accessions. This crop plant is an important leafy vegetable in Nigeria especially south western Nigeria (Akorode, 1988). This vegetative architecture is significant for leaf production. Determinate and medium branching with erect stems are likely to increase the leaves which are essential parts needed by farmers. Omonmhinmin and Osawaru (2005) have shown the significance of these qualitative traits in a leafy/fruit vegetable- *Abemoschus caillei*.

The quantitative features (plant height) showed a gradual growth from when it was transplanted. Ranges 16.00 - 68.40 cm for NHCO25, 16.40 - 63.60 cm for NHCO3, 16.10 -69.50 cm for NHCO26 were recorded, this showed a similar trend in plant height. However, the first four weeks after transplant showed slow growth in height, this is likely attributed to stress and shock during transplant. Stress and shock have been reported to be associated with plants by Vwioko *et al.* (2008). This may be due to the gradual adaptation to the new environment.

The qualitative traits expressed by leaves among accessions are significant. The serration on the margin and the setae on the leaf base may be diagnostic features of Genus *Corchorus* in the family *Tiliaceae*. Accessions NHCO3 are deeply toothed serrated. This is a distinction for this accession among the others studied. Edmonds (1991) in keys to vegetative leaf structures of the species of *Corchorus* in Nigeria – *Corchorus olitorius*, *Corchorus aestuans*, *Corchorus fasicularis* and *Corchorus tridens* stated that;

Leaf margin serrated, leaves without basal setae..... *Corchorus fascularis*

Leaf margin serrated with two basal setae…… *Corchorus olitorius*

Leaf margin serrated with at least a set on one side or on some leaves…… *Corchorus aestuans*

Leaf margin serrated with two basal setae one on each side…. *Corchorus tridens*

From these keys, the accessions in this study using leaf qualitative features could either be *Corchorus olitorius* or *Corchorus tridens*. But since *Corchorus tridens* is not edible, therefore the accessions are *Corchorus olitorius.* Color of plant, serration and presence of setae are suggested diagnostic feature of Nigeria *Corchorus.* However, the degree of serration needs to be defined.

Proteins are considered to be direct products of genes and can be taken as markers of these genes (Ladizinsky, 1983). As such protein can be taken as additional means for characterizing systematic categories. Ladizinsky (1983) reported that seed protein profile often shows genetic affinities within a taxon or between different biological entities and that seed protein profile is species specific. If seed which is a product of fertilization of gametes could result to this specificity then the leaf which is a vegetative part of the plant, is supposed to be more specific. But there were no bands of soluble protein recorded. SDS-PAGE is a dependable method for determining presence of soluble protein. The evidence resulting from the absence of protein bands suggests that the three accessions used in the study may be related. This goes to give credence to using morphology to distinguish these accessions.

The absence of protein bands maybe due to the high water content because of the fresh leaves used for the study. Hames (1998) enumerated the likely limitations to this production of protein bands in SDS Electrophoresis system to include the absence of reducing agents (dithiothreitol or β-mercaptoethanol buffer) that breaks disulfide bonds, protein structure, post transitional modification and amino acid composition which minimizes the effect of secondary structure on migration. It may also because multiple data points (at least three gels) were not generated. Trials using dried leaves or fruits with the presence of dithiothreitol or β-mercaptoethanol buffer in the plants are suggested for clarity.

In conclusion, the relationship and distinctiveness among Nigerian *Corchorus* species have been demonstrated using morphological and biomolecular analysis. From the result, credence is ascribed to using morphological characters for plants grown in the same environmental condition.

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