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Morpho-Statistical Variation and Taxonomic Characterization of *Andrographis paniculata* (Burm. F.) Nees Population obtained from Selected Locations within Southern Nigeria

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Abstract
Study of variations in plants has been identified as one successful strategy for obtaining cultivars with superior features for breeding, improvement and conservation purposes. In situ analysis of morphological variation of Andrographis paniculata (Burm. F.) Nees found in three states of Southern Nigeria was carried out using qualitative and quantitative morphological markers. Morphological traits studied included features of the stem, leaf, inflorescence, flower, fruit and seed. Qualitatively, accessions from Rivers State differed from those in Akwa Ibom and Cross River States in that the former had green stems, dark green leaves and light green unripe fruits. Of the fourteen quantitative morphological features studied, significant differences ($p < 0.05$) were recorded for five features (stem height, number of leaves per plant, number of fruits per plant, day to first flower and harvest age). Cluster analysis based on the quantitative morphological attributes produced a dendrogram which regrouped the twenty one accessions into three distinct cluster groupings indicating strong species intersections with higher similarities between Cross River and Akwa Ibom States compared to those obtained from Rivers State. Generally, this technique imprinted more or less overlapping semblances in species assortment to discrete cluster groups unrelated to locations thus implicating a continuum in persistent genetic components trivially swayed by location-based environmental influences. This observation lends credence to the fields of genetics, plant breeding and taxonomy.

Keywords: Accessions, *Andrographis paniculata,* Dendrogram, Morpho-statistical, Variation

INTRODUCTION

Andrographis paniculata (Burm. F.) Nees is an annual herb belonging to the family Acanthaceae (Minz *et al.,* 2013). Though native to India and Sri Lanka, the herb exhibits a wide global distribution hence its multiple appellations (Sabu, 2001). In Ibibio, which is one of the ethnic groups in Nigeria, the plant is called 'Fineka' while one of its English names is vinegar. The

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herb is considered medicinal because of the presence of chemical components with diverse medical applications found in all parts of the plant (Prathanturarug *et al.*, 2007). These chemical components include terpenes (andrographolide, deoxyandrographolide, neoandrographolide, saponins, steroids, noriridoides); nitrogen containing secondary metabolites (alkaloids, glycosides, glucosides, quinone, quinic acid); phenolic compounds (tannins, flavonoids, phenols, poly-phenols, xanthones, coumarin) (Ameh *et al.*, 2010; Niranjan *et al.*, 2010; Subramanian *et al.*, 2012; Hossain *et al.*, 2014 and Okhuarobo *et al.*, 2014). These active components differ widely depending on the part of plant used, genotype, location, season, time of harvest, age of plant and processing method (Maison *et al.*, 2005; Pandey and Mandal, 2010; Hossain *et al.*; 2014 and Raj and Sani, 2016).

Moreso, the presence of diverse active components in *Andrographis paniculata* contribute to its extracts having wide range of biological activities. In Nigeria, the plant is employed for the therapeutic management of HIV/AIDS (Wannang and Sokomba, 2011). According to the study conducted by Popoola *et al.* (2017) in Ogun State of Nigeria, *Andrographis paniculata* exhibited a higher level of fidelity in the treatment of various health conditions such as malaria, diabetes, high blood pressure, cancer and tumours. In Akwa Ibom State, utilization of the herb has been observed in the treatment of malaria, a prevalent ailment in the area. Since the quantity and quality of active component of *Andrographis paniculata* depend on environment and genotype (Maison *et al.*, 2005; Pandey and Mandal 2010), it is pertinent to carry out morphological study of the herb in order to ascertain the level of variability of different accessions found in the Zone as this may help in identification of superior genotype, if any, and also facilitate improvement and conservation purposes. The study will further enhance well documented identification and diversification of *Andrographis paniculata* found in parts of Southern Nigeria.

MATERIALS AND METHODS

Study Area

This study covered three states (Cross River, Akwa Ibom and Rivers) within southern Nigeria which also form part of the Niger Delta Region which is characterized by high humidity levels and temperature ranges all year round (Ojo, 2015). Its natural vegetation falls within the Tropical Rainforest belt which experience both Wet and Dry season. The area records an annual rainfall ranging between 2000 – 3000mm and its topography consists of flat plains, riverine zone and mild undulating hills (FAO 2017; NMA, 2020).

Collection of Plant Materials/Planting

Whole plants of *Andrographis paniculata* and their seeds were collected from Akwa Ibom, Cross River and Rivers States of Southern Nigeria. Those states were chosen for the study because they consist of tropical rain forest, fresh water swamp forest and salt water swamp forest. Sources of accessions from different states are shown in Fig. 1. Plants were identified in the Herbarium of the Department of Botany and Ecological Studies, University of Uyo, Nigeria where the voucher specimens are also deposited and preserved. Seeds were sown simultaneously in different plastic bags containing sawdust in each of the locations where they were collected for *in situ* study. Seedlings were transported in the field layout of each location at 30 x 30 cm spacing following the cultivation practice of Pandey an Mandal (2010) and Datta *et al.* (2012).

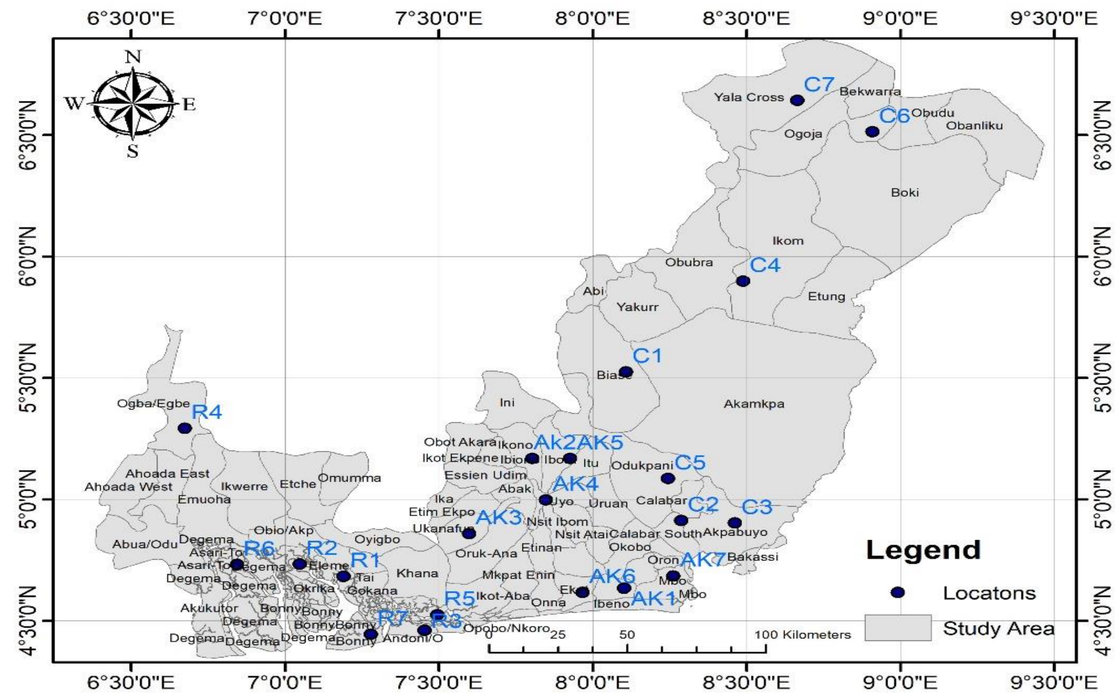

Figure 1: Map of Study Area Showing the Locations

Location Keys: AK1= Akpautong; AK2 = Mbiabong Ikon 1; AK3= Afaha Obo Ata Essien; AK4= Nung Oku;AK5= Ikot Esifa; AK6 = Afaha Eket; AK7= Enwang;CR1=Ehom; C2 = .N0 5 Efionwan Street; C3 = Behind Police Secondarry School, Atimbo; C4 = German Street, Abakpa Area; C5 = Okoyong-Along Calabar Itu Highway; C6 = Adagun Village(Mbube Tribe) C7 = Okpoma, Yala; R1 = New Layout Abuloma Town; R2 = Police Station- Marine Base; R3 = Behind St. Barnabas Anglican Church-Ngo Town; R4 = Ahoada Road, Omoku; R5 = Minima Town-Nkoro Zone; R6 = Ibalama Village; R7 = Community Sec. Sch. Burukiri.

Morphological Analysis

Morphological variations were measured from 15 plants of each accession. Fourteen quantitative features which are of agronomic and economic importance were measured at the flowering time. Those include stem height, shoot width, number of nodes per plant, number of leaves per plant, number of fruits per plant, internode length, number of branches per plant, branch length, leaf length, leaf width, flower length, flower width, day to first flower and harvest age. Also, qualitative features were observed. In stems, features observed include habit, type, colour and texture of the bark. In leaves, features observed included texture, arrangement, type, shape, margin, venation, apex, base, stipule, colour and duration. Features observed in flowers include types, arrangement, position, sex and presence of bract. Moreover, floral parts were observed in details using hand lenses and a dissecting microscope. Number, colour, shape, aestivation and duration of sepals and petals were observed. Features of androecium studied include type of anther, shape of anther, pattern of dehiscence of anther, number of filaments. Features of gynoecium studied included number and type of carpel, position of ovary, placentation, number of style, and number of stigma. Also type, shape, colour and dispersal method of fruits were observed. Features observed in seeds included colour, shape, texture and number of seeds per fruits.

Statistical Analysis

Means (± standard errors) were generated from the quantitative morphological data obtained all the accessions. Analysis of variance was to test if there be any significant variation in morphological parameters across the locations Ezekiel *et. al.* (2023) and Bassey *et. al.*. (2023). The least significant different (LSD) test procedure was used to identify significant means at 5 % level probability according to the methods of Mbong *et. al.*. (2020) and Mbong *et. al.* (2023).

Morphological data was used to generate dendrogram (UPGMA) was sketched using computer package PAST Version 4.4 (Hammer *et. al.*, 2001 and Anwana, *et. al.* 2020).

RESULTS

Results obtained from analysis of plants stem, leaves and flower features of accessions of *Andrographis paniculat*a from the three States showed significant variations and marked similarities.

Table 1 : Morpho-Variations in Plants Stem, Leaves, Inflorescence and Flower across study area.

Characters	Akwa Ibom	Cross River	Rivers
Stem			
Height (cm)	49.85 - 87.03	40.88 - 53.10	42.83 - 58.92
Colour	Dark green	Dark green	Green
Node number	15.00-17.00	14.00 – 15.00	13.00 – 17.00
Leave			
Length (cm)	5.43 – 7.26	4.53 – 5.62	3.40 – 6.67
Width (cm)	1.05 – 1.76	0.92 – 1.22	0.63 – 1.31
Leaf number	216 – 266	228 – 270	210 – 286
Colour	Dark but shiny green	Dark but shiny green	Dark green
Flower			
Length (cm)	1.43 – 1.51	1.40 – 1.52	1.43 – 1.52
Width (cm)	0.46 – 0.61	0.48 – 0.54	0.50 – 0.57
Fruits			
Colour of unripe fruit	Green	Green	Light green
Fruit number	195-221	201 – 253	195 – 239
Number of seeds per fruit	10 – 12	9 – 12	9 – 11

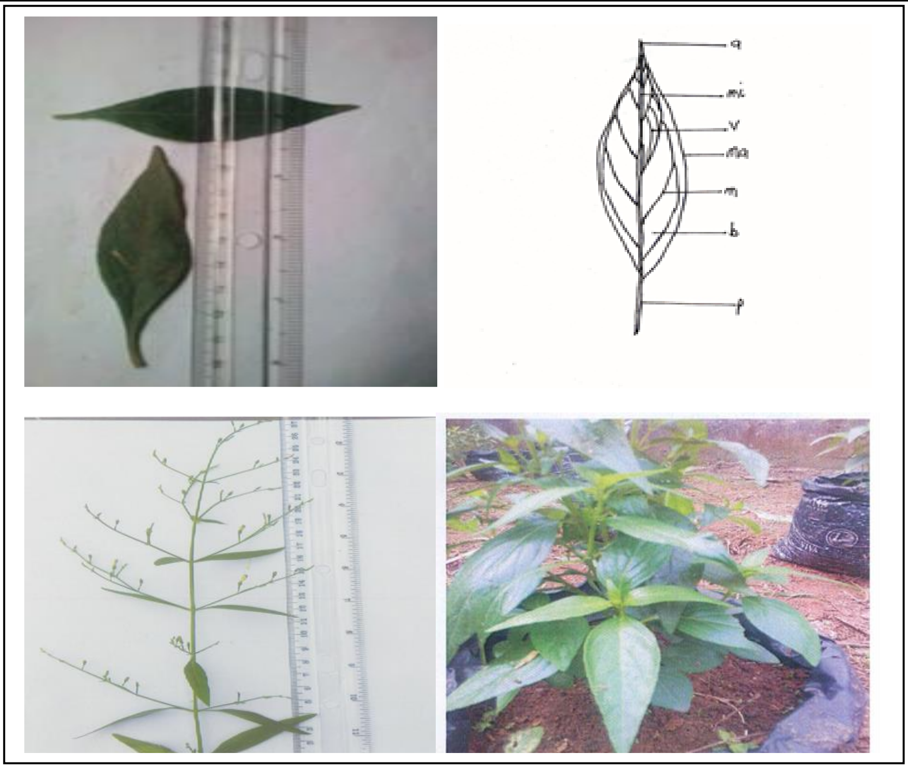

Figure 2: (a) *Andrographis paniculat*a Leaf (b) Leaf Sketch work: a- apex; mi-midrib; ma-margin; b- blade; m-main vein; p- petiole (c) The Inflorescence showing phyllotaxis (d) picture of whole plant in pot.

Table 2 : Morpho-Similarities in Plants Stem and Leaves attributes across study area.

Characters	Description
Stem	
Habit	Erect, quadrangular, annual herb
Type	Lateral branching (Racemose type)
Bark	Smooth
Node	Produces adventitious roots
Leave	
Texture	Glabrous
Arrangement	Opposite decussate
Type	Simple
Shape	Lanceolate
Margine	Entire
Venation	Reticulate
Apex	Acuminate
Base	Cuneate
Stipules	Exstipulate
Leaf duration	Persistent

Table 3: Morpho-Similarities in Inflorescence, Flower, Fruit and Seeds across Study Area

Character	Description
Inflorescence	
Peduncle	Paniculate inflorescene tapering at the apex
Flower	
Type	Zygomorphic
Arrangement	Solitary
Position	Terminal and Axillary
Sex	Bisexual
Bract	Bracteole
Sepals	
Number	5.00
Colour	Green
Freedom/ Fusion	Fused
Shape	Linear
Aestivation	Valvate
Duration	Persistent
Petals:	
Numbers	4.00
Colour	White with rose purple spots
Freedom/Fusion	Fused
Shape	Bilabiate
Aestivation	Contorted
Androecium:	
Anther type	Basifixed (Innate)
Anther shape	Bilobed
Dehiscence	Longitudinal
Filament number	2
Gynoecium:	
Carpel number	1
Overy position	Superior
Placentation	Axile
Style number	1
Stigma	2 and non-persistent
Carpel type	Syncarpous
Fruits:	
Type	Bilocular capsule
Shape	Elongated with tapering end
Colour of ripe fruit	Brown
Dehiscent/Indehiscent	Dehisces loculicidally
Dispersal method	Explosive Mechanism/Man
Seeds:	

Colour	Yellow brown
Shape	Rugose
Texture	Glabrous

Table 3: Quantitative plant features of *Andrographis paniculata* accessions in study Area

Characters	Akwa Ibom	Cross River	Rivers
Stem height (cm)	59.63±4.76^a	47.52±1.81^b	51.43±2.32^b
Shoot width (cm)	41.55±3.26^a	38.46±1.72^a	35.10±3.12^a
Node number per plant	15.71±0.29^a	14.57±0.20^a	15.14±0.51^a
Leaf number per plant	234.29±6.55^b	246.86±6.12^a	242.00±9.19^a
Fruit number per plant	206.57±3.76^b	226.43±7.89^a	212.86±6.30^b
Branch number per plant	18.14±0.34^a	18.14±0.26^a	16.14±0.40^a
3rd internode length (cm)	4.24±0.13^a	3.93±0.08^a	4.15±0.13^a
3rd branch length (cm)	20.83±2.14^a	16.09±0.47^a	18.34±1.99^a
3rd leaf length (cm)	6.11±0.23^a	5.03±0.15^a	5.25±0.49^a
Flower length (cm)	1.58±0.11^a	1.48±0.02^a	1.49±0.01^a
3rd leaf width (cm)	1.32±0.09^a	1.06±0.04^a	1.02±0.12^a
Flower width (cm)	0.53±0.02^a	0.51±0.01^a	0.53±0.01^a
Day to 1st flower	188.86±6.72^b	188.00±2.58^b	206.86±7.00^a
Harvest age (day)	204.00±6.75^b	215.71±4.50^a	223.86±6.19^a

Values are means ± Standard error. The mean values followed by different superscripts in the same row are significantly different at 0.05 levels of probability.

($p < 0.05$) recorded for five features (stem height, number of leaves per plant, number of fruits per plant, day to first flower and harvest age) while the remaining nine features were statistically similar ($p > 0.05$) in all the States (Table 3). For stem height, accessions from Akwa Ibom had the highest stem height of 59.63 cm which was significantly different ($p < 0.05$) from others while accessions from Cross River and Rivers regarding stem height was not significant ($p > 0.05$). Accessions from Akwa Ibom had significant ($p < 0.05$) lower number of leaves per plant than others with the mean value of 234.29 while accessions from Cross River had the highest mean number of leaves (246.86) per plant. No variation regarding number of leaves per plant existed between accessions from Cross River and Rivers States (Table 3).

Result showed that the highest number of fruits per plant (226.43) was found in accessions from Cross River and was significantly different from Akwa Ibom and Rivers accessions. No variation regarding number of fruit per plant was observed in accessions from Akwa Ibom and Rivers States (Table 3). The highest number of days to first flower (206.86) was observed in accessions from Rivers which differed significantly ($p < 0.05$) from others. The lowest number of days to first flower (188.00) was observed in accessions from Cross River but did not differ significantly ($P>0.05$) from those from Akwa Ibom State. Also, accessions from Akwa Ibom had the lowest harvest age with mean value of 204.00 days which was significantly different ($P<0.05$) from others while accessions from Rivers had the highest harvest age of 223.86 days (Table 3). However, variation between accessions from Cross River and Rivers regarding harvest age was not significant ($p >0.05$)

To further determine the genetic distance and identify variations among accessions of *Andrographis paniculata* from Akwa Ibom, Cross River and Rivers States, mean values produced from quantitative morphological features (Table 3) were used to generate similarity matrices which subsequently led to development of a dendrogram (Figure 5) with two clusters (A and B) at a similarity coefficient of 0.9991 (i.e 99.91%), indicating a low level of diversity among accessions. Cluster A contained accessions from Akwa Ibom and Rivers States. Those accessions appeared similar at 99.93 % similarity level, indicating high level of relatedness between those two States. Clusters B contained accessions from Cross River State and had

99.87 % similarity level with accessions from other States indicating low level of variability with those States.

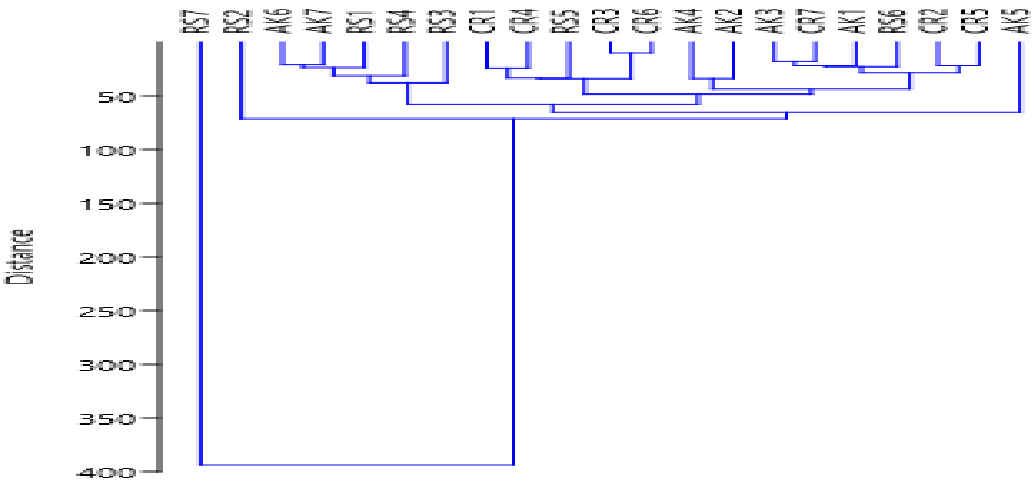

Figure 5: Dendrogram for fourteen morphological characters among accessions of *A. paniculata* from three States.

DISCUSSION

Quantitative morphological analysis of *Andrographis paniculata* accessions from Akwa Ibom, Cross River, and Rivers states in Southern Nigeria revealed notable diversity, especially in traits such as stem height, number of leaves, and number of fruits per plant. This disparity highlights significant phenotypic diversity within *A. paniculata* populations across different environmental conditions, thus yielding valuable insights that are essential for plant breeding and conservation (Akinwusi & Illoh, 1996; Anwana et al., 2020).

Clearly, significant differences ($p < 0.05$) were noted across key morphological traits across states. Accessions from Akwa Ibom displayed the tallest stem height, suggesting that specific genetic or environmental factors favor stem elongation in this region, potentially enhancing light acquisition and photosynthetic efficiency. Height advantages, as seen in other relevant studies on plant growth, mmsst often than not favour increased biomass production serving as a clear advantage for agro-medicinal purposes (Pandey & Mandal, 2010; Pham et al., 2011). Again the high leaves count observed in accessions reared in Cross River State aligns with findings that larger leaf area contributes to greater photosynthetic capacity and, consequently, enhanced growth and yield (Benoy et al., 2012; Subramanian et al., 2012). The increased fruit production in Cross River, in particular, suggests an advantageous reproductive capacity that could be beneficial for both natural propagation and cultivation aimed at medicinal utilization. High fruit yield as an agronomic trait is valued for seed production, which is crucial for medicinally significant species like *A. paniculata* due to their phytochemicals or bioactive principles (Okhuarobo et al., 2014; Raj & Sani, 2016). The variation in harvest age and flowering time across regions is well noticed and undercores plant's adaptability to different environmental conditions. The shorter time to first flowering noted in Cross River accessions suggests a potential for earlier maturity. This is a trait beneficial for breeding since early-maturation varieties could align with reduction in risks associated with prolonged growth periods, such as pest exposure or unfavorable weather events (Ezekiel et al., 2023; Popoola et al., 2017). Furthermore, early maturity is also much useful in multi-harvest systems where quick growth cycles are required for optimal production (Datta et al., 2012).

Significant differences in fruit number among states further confirm that accessions from Cross River may possess inherent advantage in reproductive capacity and so higher fruit yield, as observed in this study, suggests that these accessions may be better appropriate for breeding programs aimed at increasing seed availability and preserving phytochemical diversity. This aligns with findings from Maison et al. (2005) which emphasized the agronomic importance of selecting genotypes with high fruit yields for medicinal plant breeding.

Concerning the qualitative traits, the study recorded strong uniformity across states, except for the green stems and light green unripe fruits in Rivers State accessions. This observation corroborates the findings of Pino-Nunes et al. (2009) that qualitative traits are often genetically controlled, with minimal influence from environmental conditions. This suggests that some morphological features in *A. paniculata* may be strongly genetically stable and so making them reliable targets for selection in breeding programs. This is in agreement with similar research findings of Mallet (1996) who reported that quantitative variations exhibited by many *Piper spices* in their leaves, flowering habit and fruiting spikes are not influenced by environment but genetic divergence. Pham *et al*. (2011) who worked on the comparative analysis of genetic diversity of *Sesamum indicum* from Vietnam and Cambodia also reported that qualitative variations observed in sesame have genetic influence.

The use of cluster analysis in assessing relatedness and dissimilarities among plant group is well noted in literature (Anwana, *et. al.*. 2020). In this study, cluster analysis regrouped accessions into two main clusters: one combining Akwa Ibom and Rivers, and another grouping Cross River separately. Tis pattern indicates potential genetic distinctions among these populations. The similarity coefficient of 0.9991 reflects low genetic diversity among accessions. This is consistent with the findings of Sabu (2001) and Pham et al. (2011). These researchers reported close genetic relationships in plant accessions across similar regions. Cross River accessions distinct grouping implies trivial genetic divergence, which is likely influenced by unique environmental pressures or historical isolation. This finding corroborates the clustering patterns observed in *A. paniculata* populations from contrasting geographic regions and highlights the importance of local environmental factors in shaping genetic structure (Markert et al., 2003; Minz et al., 2013)

CONCLUSION

The study has found that accessions of *Andrographis paniculata* in Southern Nigeria are not completely phenotypically and genotypically similar. Accessions found in Rivers State are quite different from those in both Akwa Ibom and Cross River States. Since the plant is an exotic species and has more than one center of origin, the seeds could have been sourced from different ancestral origin and possibly transferred for cultivation in the different locations in the zone. Therefore, it is necessary to investigate the likelihood and levels of a possible variation in the phytochemical constituents of the medicinal herb and to create awareness to citizens on such in order to guide plant exploitation for its medicinal potentials since the quality and quantity of bioactive components of plants could be influenced by both genotype and environment. The observed morphological diversity has important implications for the conservation and breeding of *A. paniculata*. The phenotypic variation, particularly in traits such as stem height and leaf number, emphasizes the need to preserve diverse accessions, as each may possess unique traits valuable for breeding programs aimed at improving resilience, yield, and medicinal quality (Niranjan et al., 2010). Conservation of these genetic resources is essential for maintaining adaptability, especially given the increasing environmental pressures on medicinal plant populations (Hossain *et al.*, 2014).Additionally, the phenotypic diversity seen across regions supports the potential to select location-specific genotypes that are adapted to their respective environments. This approach could enhance both cultivation success and resource conservation, ensuring that genetically diverse populations of *A. paniculata* are available for future use (Borokini and Ayodele, 2012).

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