LEAF EPIDERMAL STUDIES IN THE GENUS MILLETTIA WIGHT & ARN. (FABACEAE - PAPILIONOIDAE) IN NIGERIA

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ABSTRACT

In view of the controversies in the taxonomy of the genus Millettia (Fabaceae-Papilionoideae), epidermal characteristics that are revealed through light microscopy was conducted on some indigenous Nigerian species. Qualitative characters were noted and recorded while the quantitative features were measured with calibrated ocular under microscope. All the data were recorded and subjected to appropriate statistical analyses. Results from the principal component analyses (PCA) were used to explore variations in the quantitative characters among Millettia species, and cluster analyses were used to ascertain systematic groupings of the taxa. The epidermal cells varied from polygonal to irregular with straight and undulating wall patterns. The stoma types were mainly anomocytic and paracytic. Among the ten species, *M. barteria* and *M. zechiana* were amphistomatic while the remaining eight species were hypostomatic. The frequency of stomata varied within the genus with the highest frequency observed in *M. dinklagei*. The similarity in morphology indicates interspecies relationships, which justify their groupings together in the same genus while the differences confirm their delimitation as distinct species.

Key words: Millettia, epidermal, Principal Component Analysis, stomata, species-specific, delimitation

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INTRODUCTION

Millettiais a genus in the legume family Fabaceae (Leguminosae) and the tribe Millettieae. It is a genus of multipurpose species (Banzouzi *et al.*, 2008). They are used mainly for their wood and in the traditional Pharmacopeias. Traditionally, Millettia is of great therapeutic importance, and for many centuries it has appeared in African Pharmacopeia. The genus is reported to have products with commonly wide range of biological properties such as antitumoral, anti-inflammatory, antiviral, bactericidal, insecticidal, piscicidal and pesticidal activities (Banzouzi *et al.,* 2008; Karunamorthi *et al.,* 2009). Besides this, they are useful in agroforestry in that the tree has nodulation and nitrogen-fixing ability which help in improving soil fertility and in turn lead to increase in crop yield (Egbe *et al.,* 1998; Hailu *et al.,* 2000; Orwa *et al.,* 2009). Certain species of the genus are traditionally used as windbreaks and can be planted along the grazing grounds as shelter for the animals or as natural fence.

Furthermore, Banzouzi *et al.* (2008) established that more than 60% of Millettia species are integrated in the Pharmacopeias in almost all the countries. They reported that the genus presents nearly 150 distinct therapeutic indications in the African Pharmacopoeias. It covers many important pathologies such as intestinal parasites hernias, stomachic and intestinal pains, regulation of menstrual cycles, feverish pains, odontology in general, wounds, broncho pulmonary infections,

coughs and colds, headaches. Frequently, they are also used as purgative, diuretic or laxative, also used as fishing or hunting poison.

Inspite of the great importance of Millettia, this genus is still confronted with the problem of misidentification. The various species of Millettia are sometimes difficult to recognize by the local populace (Aubreville, 1950; Hu *et al.,* 2000). Although some morphological, phytochemical and molecular studies have been conducted on Millettia, many issues still remain unresolved. A problematic example is found in the tribe Millettieae, of which the relationship among genera is notoriously difficult to unravel based on morphological evidence (Geesink, 1984; Schrire, 2005). The circumscription of the genus Millettia is very complicated, and their classification at species level has troubled many taxonomists (Hu *et al.,* 2000). In effect, many specimens have been misidentified. Apparently, some difficulties of identification occur between Millettia and related genera (Banzouzi *et al.,* 2008). Taking into account its great diversity of uses and the taxonomic confusion among its species, Millettia deserves a detailed attention.

The present study therefore aims at resolving some of these confusions and controversies surrounding the delimitation of Millettia species, and giving some resultant taxonomic changes if necessary.

MATERIALS AND METHODS

SAMPLE COLLECTION

Dried herbarium specimens of ten species of *Millettia* (*Millettia aboensis* (Hook.f.) Bak., *M. barteri* (Benth) Dunn., *M. chrysophylla* Dunn, *M. drastica* Welw. ex Bak., *M. dinklagei* Harms, *M. griffoniana* Baill., *M. macrophylla* Benth, *M. pilosa* Hutch & Dalz, *M. thonnningii* (Schum. &Thonn.) Bak. and *M. zechiana* Harms) were obtained from Forest Research Institute of Nigeria, Ibadan (FHI). The fresh specimens were collected from the University of Ibadan, Oyo State.

MICROSCOPIC EXAMINATION

The epidermal preparation followed the procedures used by Ayodele and Oluwokudejo, (2006) with little modification. Both fresh and herbarium specimens were used for this study. The specimens were examined using light microscopes. To examine the cells the herbarium specimens were irrigated by boiling in water for one hour. Sample of each species was then macerated in concentrated nitric acid for one hour under sunlight. The sample was then transferred to water in Petri-dish.

A gentle use of carmel hair brush was employed to separate the adaxial and abaxial epidermises from the mesophyll layers. The mesophyll tissues were carefully cleared with a carmel hair brush and the isolated adaxial and abaxial layers were rinsed in water before being transferred to 50% alcohol for hardening. The peeled were stained in Safranin O between 5-10 minutes. The excess staining was washed off from the tissues. The epidermal strips were mounted on glass slide in glycerol with edge of cover slip ringed with nail varnish to prevent dehydration and seal the cover slips to the slides. The slides were labeled appropriately and examined under light microscope. The leaf epidermal characters: both the qualitative and quantitative that have taxonomic significance were assessed. The upper (adaxial) and lower (abaxial) surfaces were treated separately for each plant specimen. Photomicrograph of the leaf epidermis was obtained with Olympus CH 30. Measurement and counting of cellular structures were done at x1000-Magnification.

STATISTICAL ANALYSES

Descriptive statistics of mean, standard deviation and Principal Component Analysis (PCA) were used. The program generated dendrograms which grouped the *Millettia* species according to their morpho-anatomical characters using cluster analysis (Sneath and Sokal, 1973).

RESULTS

The following account is based on the epidermal characters of ten species of *Millettia.* Qualitative and quantitative epidermal features of *Millettia* species were employed for the delimitation of the taxa with their means and standard deviations shown in Table 1. Mean and standard errors of the abaxial foliar characters are contained in Table 2. Correlation coefficient of *Millettia* species are presented as Table 3 which shows that close resemblance of species could be observed when certain characters are employed. It is indicated that there is high significant ($P \leq 0.01$) correlation between: epidermal cell length and epidermal cell width, epidermal cell width and subsidiary cell

length, guard cell length and subsidiary cell length, guard cell length and subsidiary cell width, stomatal index and stomatal frequency, stomatal index and trichome frequency, trichome frequency and stomatal frequency, trichome index and stomatal frequency, and trichome index and stomatal index.

From the Principal Component Analysis (PCA) carried out on the data set, four out of the eleven characters examined accounted for about 93.3% importance in the delimitation of the taxa (Table 4). Fig. 1 which shows the component plot in rotated space revealed that characters weighing above 0.5 could also help largely in the delimitation of the group while the remaining characters indicate the similarities existing between the species. It is indicated that subsidiary cell width, epidermal cell length and width, and guard cell length have higher values above 0.5 than the remaining seven characters. It is therefore affirmed that these characters contribute heavily to the delimitation.

Furthermore, the result shown in Table 5 gives the average linkage between groups based on agglomeration schedule. The coefficient of cluster existing between *M. barteri* (2) and *M. griffoniana* (6) is 51.529 while that existing between *M. aboensis* (1) and *M. chrysophylla* (3) is 1040.054 indicating great degree of variation in their morphometry. Cluster analysis produced the dendrogram illustrated in Figure 3 from which two main clusters are produced. In the first cluster, specimens of *M. barteri, M. griffoniana, M. aboensis, M. dinklagei, M. thonningii, M. pilosa, M. zechiana* and *M. macrophylla* occur together. The second cluster separate out at the lowest level of similarity, and it comprises *M. chrysophylla* and *M. drastica.* In the same vein, the dendrogram revealed that greater affinity exists between *M. barteri* and *M. griffoniana, M. barteri* and *M. aboensis* than the one existing between *M. barteri* and *M. chrysophylla*.

The results of the scatter plot of epidermal cell length and epidermal cell width (Fig. 3) revealed that *M. pilosa* has the highest value of epidermal cell length, *M. thonningii* has the lowest epidermal cell length and width, and *M. griffoniana* has the highest value of epidermal cell width. Scatter plot of guard cell length and width (Fig. 4) showed that *M. griffoniana* has the highest guard cell length and lowest guard cell width, *M. pilosa* has highest guard cell width and *M. chrysophylla* has the lowest guard cell length.

Main Description of Epidermal Characters in the Species

Great range of variation was observed in the epidermal cells. The shape of epidermal cells varied from polygonal to irregular with straight and undulating wall patterns. In *Millettia aboensis,* the epidermal cells have polygonal and straight walls with paracytic stomata; *M. barteri* has polygonal, straight or curve walls with paracytic and anomocytic stomata; *M. chrysophylla* has irregular and undulating walls with anomocytic stomata; in *M. dinklagei,* polygonal and straight epidermal walls are found with anomocytic stomata; *M. drastica* has epidermal cells that are polygonal, wavy, straight and undulate with paracytic stomata; *M. griffoniana* has irregular, polygonal epidermal cell shape with undulating wall pattern and anomocytic stomata; the epidermal cells of *M. macrophylla* are polygonal in shape and paracytic stoma type; in *M. pilosa* irregular, polygonal, straight and undulating epidermal walls are found with paracytic stomata type; *M. thonningii* has straight walls, polygonal epidermal shape and paracytic stomata; and in *M. zechiana,* polygonal, irregular, straight and undulating walls are found with anomocytic and paracytic walls (Plate 1; Table 6).

M. chrysophylla differs from other species by its irregularly shaped epidermal cells with undulating walls. Irregular epidermal cell with undulating walls are found only on adaxial surfaces of *M. drastica* and *M. griffoniana,* and on abaxial surface of *M. pilosa.* The epidermal cell length and width also varied among species, smallest average epidermal cell length and breadth was recorded in *M. aboensis* abaxial surface (12.62±3.86 µmand 6.7±2.60 µm respectively) and largest epidermal cell length and width in *M. drastica* adaxial surface (26.5±4.46 µm and 14.53±4.56 µm). Variation in epidermal frequency was also observed with the highest (86.2±9.46mm^2) in *M. dinklagei* adaxial surface and the lowest (36.95±7.47mm^2) in *M. drastica* adaxial surface.

Generally, all the ten species are hypostomatic except *M. barteri* and *M. zechiana* which are amphistomatic. All species have paracytic and anomocytic stomata. Stomata frequency values were calculated for all the ten species and were found variable in all the species, for which was highest in *M. pilosa* abaxial surface (8.25±2.36mm^2) and lowest in *M. dinklagei* abaxial surface (1.40±0.50mm^2). Like the stomatal frequencies, the stomatal indices of the species are different. Of the ten taxa, *M. dinklagei* abaxial surface, recorded a minimum value of 1.86±0.78 % and *M. chrysophylla,* a maximum value of 15.27±7.32 % for the lower epidermis. Variations in length and width subsidiary cell and guard cells were also recorded, with the minimum subsidiary length appearing in *M. chrysophylla* abaxial surface (10.08±2.53µm) and minimum width in *M. zechiana* adaxial surface (2.43±0.65µm) and maximum subsidiary length and width appearing in *M. macrophylla* abaxial surface (19.78±1.58µm and 6.83±1.43µm). The highest guard cell length was observed in *M. griffoniana* abaxial surface (13.63±1.64µm) and the lowest in *M. chrysophylla* abaxial surface (7.85±1.87µm). Guard cell width was highest in *M. pilosa* abaxial surface (3.95±1.35µm) and lowest in *M. griffoniana* abaxial surface (1.70±0.41µm).

Trichomes are widespread in *Millettia.* Observed foliar trichomes were non-glandular, unicellular with swollen base in all *Millettia* species. Trichomes were observed on both surfaces of the leaves except on the upper surface (adaxial) of *M. chrysophylla* and *M. drastica.* The highest trichome frequency (2.25±0.97mm^2) appeared in *M. pilosa* abaxial and lowest (1.35±0.49mm^2) was observed in both *M. barteri* abaxial and *M. macrophylla* adaxial surface. The trichome index was also calculated for all species and variation was observed among species, with minimum value in *M. barteri* abaxial surface (1.84±0.75 %) and maximum value in *M. dinklagei* adaxial surface (5.59±2.75 %)

Table 1: *Mean and standard deviation of the foliar epidermal characters of Milletia species*

Species	Epiderm-al Layer	Ep length (µm)	Ep width (µm)	Ep freq (mm^2)	G length (µm)	G width (µm)	Sub length (µm)	Sub width (µm)	Stomfreq (mm^2)	Stom index %	Tri freq (mm^2)	Tri index %
M. aboensis	Abaxial	12.63±3.86	6.70±2.60	69.65±8.0	9.70±1.25	3.15±0.95	11.85±1.34	4.73±0.68	1.45±0.51	2.10±0.73	1.40±0.50	2.04±0.81
	Adaxial	13.13±3.41	8.25±2.38	61.95±5.74							1.42±0.61	2.32±1.01
M. barteri	Abaxial	14.4±3.14	8.73±1.91	74.6±9.23	11.1±2.04	3.18±0.83	17.95±2.28	5.30±1.40	2.25±1.16	3.07±1.66	1.35±0.49	1.84±0.75
	Adaxial	15.0±2.80	7.70±7.47	78.2±1.72	8.48±1.72	2.25±0.47	16.55±3.44	5.13±1.65	6.55±1.67	8.56±2.77	1.45±0.61	1.88±0.84
M. chrysophylla	Abaxial	15.23±3.21	8.85±2.23	45.65±9.79	7.35±1.87	2.13±0.79	10.08±2.53	2.95±1.03	6.70±3.08	15.27±7.32	2.00±0.73	4.53±1.78
	Adaxial	21.03±3.50	10.35±3.79	53.05±6.38								
M. dinklagei	Abaxial	13.43±2.24	9.13±2.99	77.55±9.15	8.58±3.27	3.10±1.20	10.55±2.06	4.55±1.88	1.40±0.50	1.86±0.78	1.45±0.5	1.89±0.70
	Adaxial	15.0±2.98	8.13±2.36	86.2±9.46							4.75±2.34	5.59±2.75
M. drastica	Abaxial	14.15±2.71	7.55±1.92	36.95±7.47	11.45±1.01	2.03±0.50	14.7±2.02	5.53±0.97	5.40±1.81	14.82±5.37	15±0.51	4.27±1.77
	Adaxial	26.5±4.46	14.53±4.56	39.2±4.01								
M. griffoniana	Abaxial	17.83±3.34	10.45±2.22	70.3±5.70	13.63±1.64	1.70±0.41	15.43±1.73	6.7±2.08	1.65±0.81	2.39±1.28	1.75±0.79	2.52±1.22
	Adaxial	20.4±3.89	10.3±3.54	60.65±4.58							1.75±0.85	2.87±1.33
M. macrophylla	Abaxial	14.58±2.09	9.25±2.00	62.7±5.50	11.15±1.30	2.78±0.84	19.78±1.58	6.83±1.43	1.85±0.67	3.02±1.23	1.50±0.52	2.44±0.93
	Adaxial	19.38±2.72	13.25±2.21	53.8±6.33							1.35±0.49	2.53±0.93
M. pilosa	Abaxial	17.93±3.59	9.53±2.78	65.8±9.6	11.05±2.61	3.95±1.35	12.9±2.76	4.75±0.94	8.25±2.36	12.84±4.39	2.25±0.97	3.57±1.80
	Adaxial	17.78±2.02	12.75±1.86	59.0±3.81							1.45±0.51	2.50±0.97
M. thonningii	Abaxial	12.23±2.06	5.43±0.90	77.85±5.62	9.93±0.88	3.9±0.62	14.3±2.94	5.65±0.78	6.6±1.6	8.51±2.12	2.05±0.76	2.65±1.02
	Adaxial	14.1±3.37	7.3±1.64	75.15±5.01							2.1±0.64	2.81±0.90
M. zechiana	Abaxial	14.93±4.48	8.95±3.53	59.85±7.5	7.73±0.5	1.83±0.52	10.98±0.90	2.43±1.65	3.85±1.81	6.56±3.24	1.9±0.72	3.21±1.30
	Adaxial	16.63±2.65	10.3±1.98	63.35±7.22	7.6±0.64	1.8±0.64	10.8±0.64	2.4±0.72	4.15±1.81	6.53±2.78	2.4±0.90	3.88±1.56

Ep length- Epidermal length; Ep width - Epidermal width; Ep freq - Epidermal frequency; G length - Guard cell length; G width - Guard cell width; Sub length – Subsidiary cell length; Sub width – Subsidiary cell width; Stomfreq - Stomata frequency; St index - Stomata index; Tri freq- Trichome frequency; Tri ind - Trichome index

Table 2: *Mean and standard error of the abaxial foliar micro-characters of Milletia species*

Species	Ep length (µm)	Ep width (µm)	Ep freq (mm^2)	G length (µm)	G width (µm)	Sub length (µm)	Sub width (µm)	Stomfreq (mm^2)	Stom index %	Trifreq (mm^2)	Tri index %
M. aboensis	12.63±0.86	6.70±0.58	69.65±1.79	9.70±0.28	3.15±0.21	11.85±0.30	4.73±0.15	1.45±0.11	2.09±0.16	1.40±0.11	2.04±0.18
M.barteri	14.40±0.70	8.73±0.43	74.60±2.06	11.10±0.46	3.18±0.19	17.95±0.51	5.30±0.31	2.25±0.26	3.07±0.37	1.35±0.11	1.84±0.17
M. chrysophylla	15.23±0.72	8.85±0.50	45.65±2.19	7.35±0.42	2.13±0.18	10.08±0.56	2.95±0.23	6.70±0.69	15.27±1.64	2.00±0.16	4.53±0.40
M. dinklagei	13.43±0.50	9.13±0.67	77.55±2.05	8.58±0.73	3.10±0.27	10.55±0.46	4.55±0.42	1.40±0.11	1.86±0.17	1.45±0.11	1.89±0.16
M. drastica	14.15±0.60	7.55±0.43	36.95±1.67	11.45±0.23	2.03±0.11	14.70±0.45	5.53±0.22	5.40±0.41	14.82±1.20	1.50±0.11	4.27±0.40
M. griffoniana	17.83±0.75	10.45±0.50	70.30±1.28	13.63±0.37	1.70±0.09	15.43±0.39	6.70±0.47	1.65±0.18	2.39±0.29	1.75±0.18	2.52±0.27
M. macrophylla	14.58±0.47	9.25±0.45	62.70±1.23	11.15±0.29	2.78±0.19	19.78±0.35	6.83±0.32	1.85±0.15	3.02±0.27	1.50±0.11	2.44±0.21
M.pilosa	12.23±0.46	5.43±0.20	77.85±1.26	9.93±0.20	3.90±0.14	14.30±0.21	5.65±0.17	6.60±0.36	8.51±0.47	2.05±0.17	2.65±0.23
M. thonningii	17.93±0.80	9.53±0.62	65.80±2.15	11.05±0.58	3.95±0.30	12.90±0.62	4.75±0.21	8.25±0.53	12.84±0.98	2.25±0.22	3.57±0.40
M. zechiana	14.93±1.00	8.95±0.79	59.85±1.68	7.73±0.11	1.83±0.12	10.98±0.20	2.43±0.15	3.85±0.41	6.55±0.72	1.90±0.16	3.21±0.29

Ep length- Epidermal length; Ep width - Epidermal width; Ep freq - Epidermal frequency; G length - Guard cell length; G width - Guard cell width; Sub length – Subsidiary cell length; Sub width – Subsidiary cell width; Stomfreq - Stomata frequency; St index - Stomata index; Tri freq- Trichome frequency; Tri ind - Trichome index

Table 3: Correlation coefficient of the abaxial foliar micro characters employed

Correlation		Epidermal length	Epidermal width	Epidermal frequency	Guard cell length	Guard cell width	Subsidiary cell length	Subsidiary cell width	Stomata frequency	Stomata index	Trichome frequency	Trichome index
	Epidermal length	1.000	.808	-.157	.429	-.269	.073	.074	.223	.202	.446	.314
	Epidermal width		1.000	-.063	.231	-.426	.098	.016	-.224	-.146	.031	.035
	Epidermal frequency			1.000	.135	.577	.133	.269	-.390	-.740	-.086	-.868
	Guard cell length				1.000	-.006	.704	.865	-.199	-.217	-.183	-.223
	Guard cell width					1.000	.086	.177	.275	-.059	.177	-.357
	Sub length						1.000	.798	-.284	-.304	-.364	-.332
	Sub width							1.000	-.314	-.354	-.326	-.396
	Stomata frequency								1.000	.895	.821	.756
	Stomata index									1.000	.592	.942
	Trichome frequency										1.000	.549
	Trichome index											1.000

Highly significant at P≤0.01

Table 4:*Principal component analysis based on abaxial foliar micro-morphological characters*

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.422	40.203	40.203	4.422	40.203	40.203
2	2.539	23.078	63.281	2.539	23.078	63.281
3	1.859	16.902	80.182	1.859	16.902	80.182
4	1.446	13.143	93.325	1.446	13.143	93.325
5	.343	3.116	96.441			
6	.249	2.265	98.706			
7	.101	.920	99.626			
8	.037	.340	99.966			
9	.004	.034	100.000			
10	-1.27E-016	-1.16E-015	100.000			
11	-1.13E-015	-1.03E-014	100.000			

Extraction Method: Principal Component Analysis.

Note: 1- Epidermal length; 2- Epidermal width; 3 - Epidermal frequency; 4 - Guard cell length; 5 - Guard cell width; 6 – Subsidiary cell length; 7 – Subsidiary cell width; 8 - Stomata frequency; 9 - Stomata index; 10- Trichome frequency; 11 - Trichome index

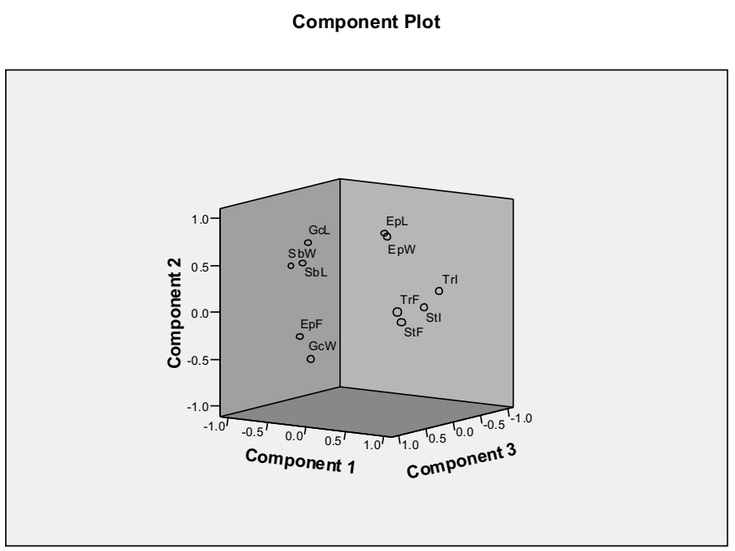

Fig. 1:Component plot in rotated space of the abaxial foliar micro morphological characters

Ep L- Epidermal length; EpW - Epidermal width; EpF - Epidermal frequency; GcL - Guard cell length; GcW - Guard cell width; SbL – Subsidiary cell length; SbW – Subsidiary cell width; StF - Stomata frequency; StI - Stomata Index; Tr - Trichome frequency; TrI - Trichome index

T

able 5:*Cluster analysis from the abaxial foliar micro characters based on average linkage within species of Milletia*

	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
Stage	Cluster 1	Cluster 2	Cluster 1	Cluster 2	Cluster 1	Cluster 2
1	2	6	51.529	0	0	3
2	1	4	72.003	0	0	3
3	1	2	88.297	2	1	6
4	3	5	125.591	0	0	9
5	8	10	128.523	0	0	7
6	1	9	134.528	3	0	8
7	7	8	174.111	0	5	8
8	1	7	244.493	6	7	9
9	1	3	1040.054	8	4	0

Note: 1 – *M.aboensis*, 2 – *M. barteri*, 3 – *M. chrysophylla*, 4 – *M. dinklagei*, 5– *M. drastica*, 6 – *M. griffoniana*, 7 – *M. macrophylla*, 8 – *M .pilosa*, 9 – *M. thoningii*, 10 – *M. zechiana*

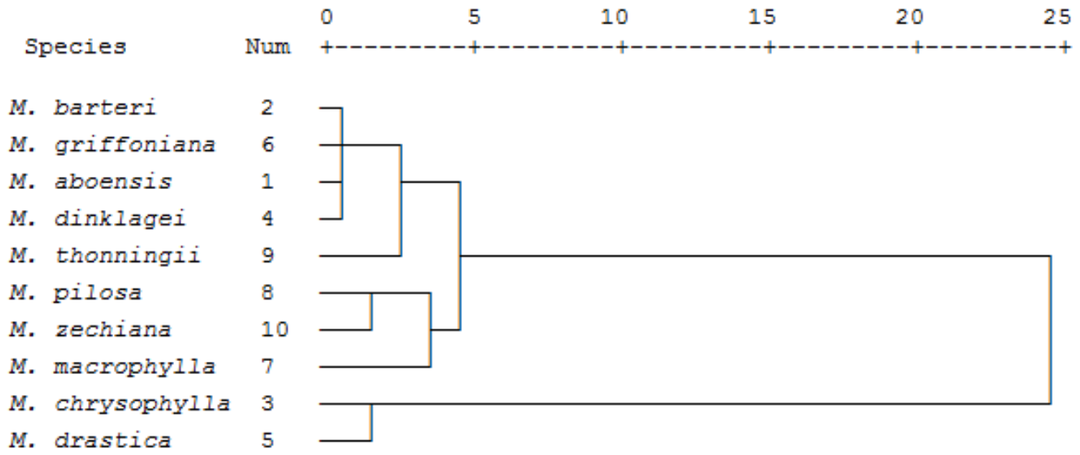

Fig.2: *Dendrogram using Average Linkage (Within Group) based on the abaxial foliar micro-morphological characters*

Table 6: *Qualitative features of the epidermal morphology of ten species of genus Millettia*

Taxa	Leaf surface	Stomatal types	Epidermal cell shape	Epidermal wall pattern	Trichome type
M. aboensis	Adaxial	Absent	Polygonal	Straight	Unicelluar
	Abaxial	Paracytic	Polygonal	Straight	Unicelluar
M. barteri	Adaxial	Paracytic and anomocytic	Polygonal	Straight	Unicelluar
	Abaxial	Anomocytic	Polygonal to irregular	Straight to undulating	Unicelluar
M. chrysophylla	Adaxial	Absent	Irregular	Undulate	Absent
	Abaxial	Anomocytic	Irregular	Undulate	Unicelluar
M. dinklagei	Adaxial	Anomocytic	Polygonal	Straight	Unicelluar
	Abaxial	Absent	Irregular	Undulate	Unicelluar
M. drastic	Adaxial	Absent	Irregular	Undulate	Absent
	Abaxial	Anomocytic	Polygonal	Straight	Unicelluar
M. griffoniana	Adaxial	Absent	Irregular	Undulate	Unicelluar
	Abaxial	Anomocytic	Polygonal	Straight	Unicelluar
M. thonningii	Adaxial	Absent	Polygonal	Straight	Unicelluar
	Abaxial	Paracytic	Polygonal	Straight	Unicelluar
M. pilosa	Adaxial	Absent	Polygonal	Straight	Unicelluar
	Abaxial	Paracytic	Irregular	Undulate	Unicelluar
M. macropylla	Adaxial	Absent	Polygonal	Straight	Unicelluar
	Abaxial	Paracytic	Polygonal	Straight	Unicelluar
M. zechiana	Adaxial	Anomocytic and paracytic	Polygonal	Straight Undulate	Unicelluar
	Abaxial	Anomocytic	Polygonal to irregular	Straight	Unicelluar

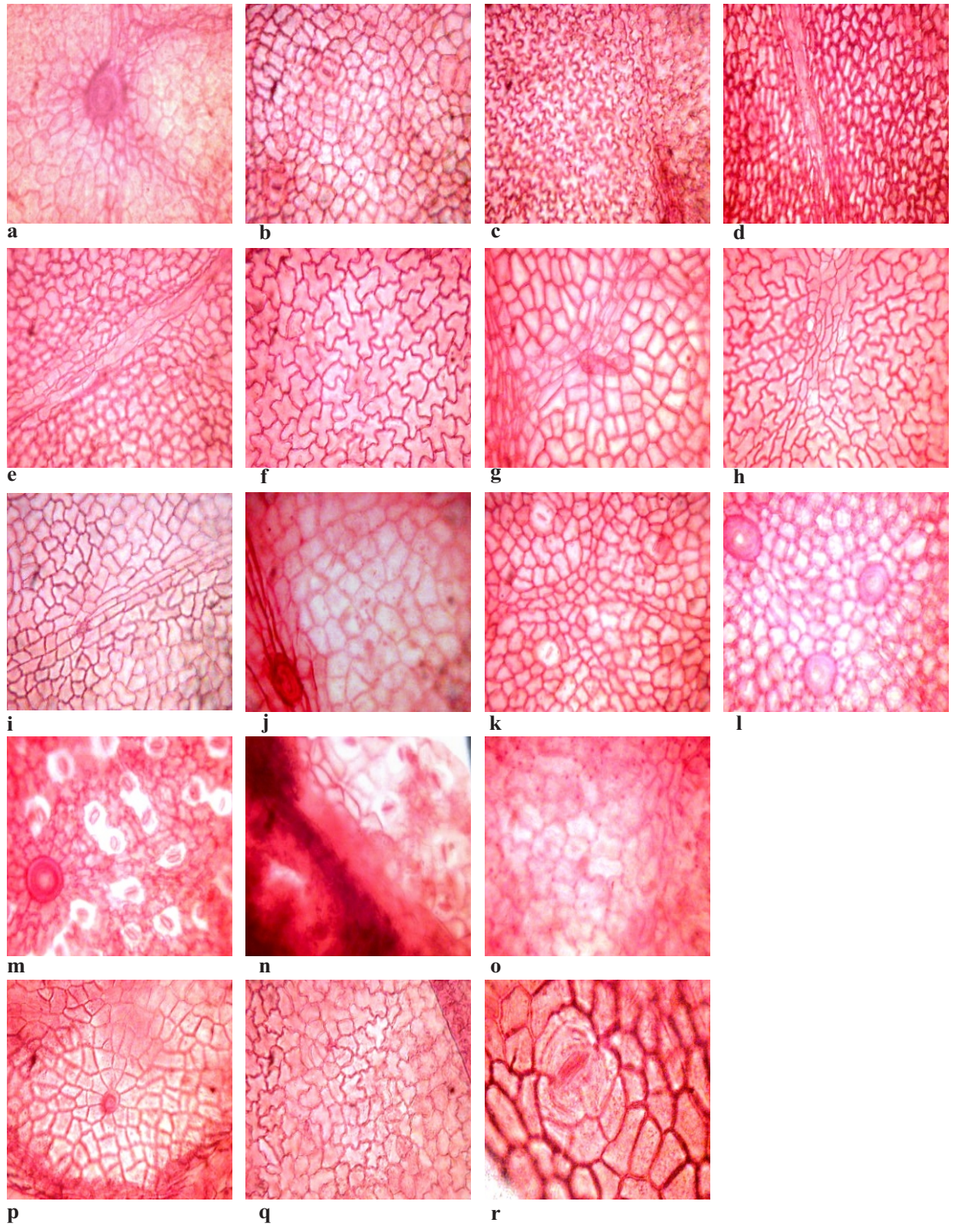

***Plate 1**: Light Microscopy of Leaf Epidermis of the Taxa ofMillettiaStudied, Magnification: x 400 and 1000*

a-*M. aboensis*$_{(adaxial)}$,**b** – *M. barteri*$_{(adaxial)}$, **c** – *M. chrysophylla*$_{(adaxial)}$, **d** – *M. dinklagei* $_{(adaxial)}$, **e** – *M. dinklagei*$_{(abaxial)}$, **f** – *M. drastica*$_{(adaxial)}$, **g** – *M. drastica*$_{(abaxial)}$,**h** – *M. griffoniana*$_{(adaxial),}$ **i** – *M. griffoniana*$_{(abaxial),}$, **j** - *M. macrophylla*$_{(adaxial),}$ **k** - *M. macrophylla*$_{(abaxial),}$ **l** - *M. pilosa*$_{(abaxial)}$, **m** - *M. pilosa*$_{(abaxial)}$**n** - *M. thonningii*$_{(abaxial),}$**o** - *M. thonningii*$_{(adaxial),}$ **p**- *M. zechiana*$_{(abaxial),}$**q**- *M. zechiana*$_{(abaxial)}$ and**r**- *M. zechiana*$_{(adaxial)}$ (Magnification x1000)

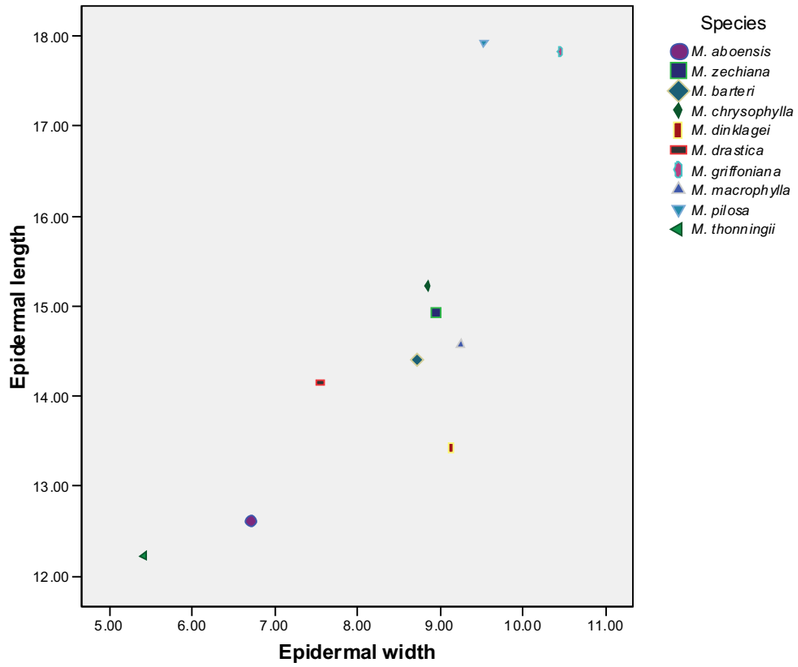

***Fig. 3 :** Scatter plot of epidermal length andepidermal width of Milletia species*

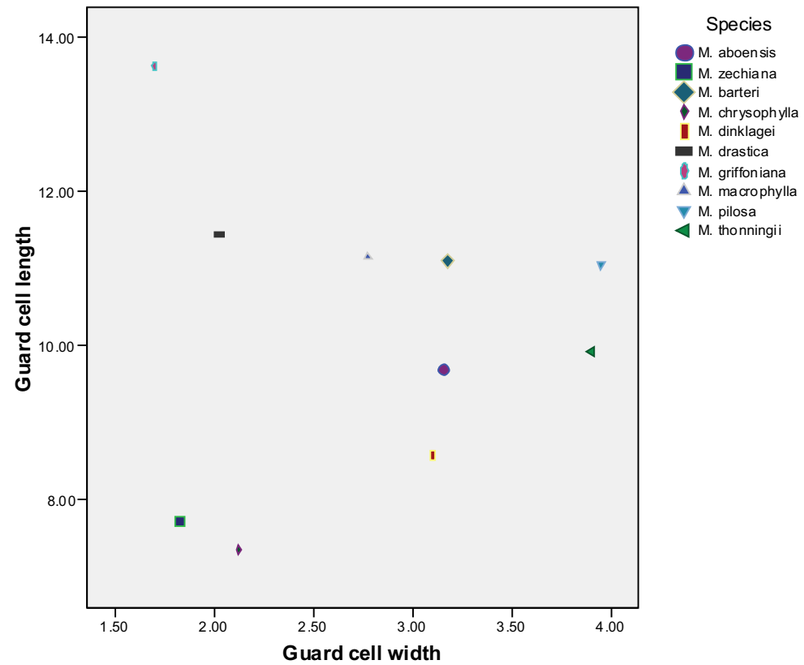

***Fig. 4:** Scatter plot of guard cell length and guard cell width of Milletia species*

DISCUSSION AND CONCLUSION

The comprehensive work done by Roy (2008) on plant anatomy shows that angiosperms exhibit a great variation in the arrangement, structure, shape, size, frequency of their leaf anatomical features. However, the investigated taxa revealed a number of characters in the epidermal cells, stomatal and trichome types on adaxial and abaxial surface both quantitatively and qualitatively. These morpho-anatomical features have provided noteworthy supplementary evidence and are of taxonomic value (Stace, 1965; Soladoye, 1985). The leaf anatomical characters studied in this work correspond largely to those of Albert and Sharma (2013); Taia (2003); Hűsenyïn (2011). They used foliar epidermal features in characterizing some species in the family Leguminosae. However, few salient epidermal characters that were not recorded by these authors and which may enhance the taxonomy of this genus and species in Nigeria have been established in this study

The species analysed in the genus *Millettia* showed variation in shape and size of epidermal cells. Polygonal, irregular shaped epidermal cells with straight and undulate walls were observed in the species. It is clear from the present findings that epidermal frequency is high when epidermal cells are small and frequency is low when the epidermal cells are large. The diagnostic importance of the morphology of guard cells and subsidiary cells was strongly emphasized by Albert and Sharma (2013). Leaves are hypostomatic in all species except *M. barteri* and *M. zechiana* whose leaves are amphistomatic. Anomocytic and paracytic types of stomata were noted in the present study of which anomocytic stomata is more frequent than paracytic. Paracytic stomata characterizes five species such as *M. aboensis, M. barteri, M. thonningii, M. pilosa, M. macrophylla* and *M. zechiana*

In the family Leguminosae, the earlier relevant works included the structure of trichome, stomatal structure and developmental, stomatal frequency (Huseyin, 2011). According to Stace (1965), the distribution and frequency of stomata are useful in in solving several problems of plant systematics. This study revealed that the stomatal frequencies are more or less constant for a particular species in spite of ecological variations. Moreover, no two species show identical values and this specificity contributed to the fact that stomatal studies can be a basis for taxonomic revision in combination with other characters.

It has been proved that during this study that most of epidermal characters are extremely peculiar and stable and not decisive enough either by their presence or absence in most of the species of the genus. In addition, it would appear that although there is considerable diversity in foliar anatomy but insufficient enough to recognize different taxa at generic level. These characters could not be solely used as the base for the taxonomic classification of the genus *Millettia*. There are some differences at specific level that permit the delimitation of the taxa and can serve as good taxonomic tools.

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Artificial Key Based on Abaxial Foliar Micromorphology for the Taxonomic Identification of *Millettia* species

1a. Epidermal cell wall straight; epidermal cell shape polygon……...2

1b. Epidermal cell wall undulating; epidermal cell shape irregular……..……....3

2a. Stomata amphistomatic……..……4

2b. Stomata hypostomatic……..……..… 5

3a. Stomatal index 15.27%; trichome frequency 2 mm^2……..*M. chrysophylla*

3a. Stomatal index 1.86%; trichome frequency 1.45 mm^2……....*M. dinklagei*

4b. Epidermal frequency 82 mm^2 ……..….*M. barteri*

4b. Epidermal frequency 72 mm^2 ……..……..…*M. zechiana*

Fig. 4: *Scatter plot of guard cell length and guard cell width of Milletia species*

5a. Mean epidermal cell length 17.93 µm ……...*M. thonningii*

5b. Mean epidermal cell length 12.23µm …….....*M.pilosa*

6a. Stomatal paracytic; stomatal frequency 1.85 mm^2……..*M. macrophylla*

6b. Stomatal anomocytic; stomatal frequency 1.45 mm^2……...…*M. aboensis*

7a. Subsidiary cell length 14.7 µm; guard cell length 11.45 µm..…….....*M.drastica*

7a. Subsidiary cell length 15.43 µm; guard cell length 13.63 µm..…….....…*M.griffoniana*

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ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ETHANOL EXTRACT OF TEN DIFFERENT COMMERCIAL SPICES AND HERBS AGAINST THREE CLINICAL BACTERIAL ISOLATES

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ABSTRACT

The use of spices in the treatment of diseases has not been fully exploited. Ethanol and aqueous extract of the following spices: Garlic (*Alium sativum*), Onion (*Allium cepa*), Cinnamon (*Cinnamomum verum*), Thyme (*Thymus vulgaries*), Ginger (*Zingiber officinale*), Lime leaf (*Citrus aurantifolia*), Curry (*Murraya koenigii*), Bay leaf (*Laurus nobilis*), Red pepper (*Capsicum annum* L.) and Tumeric (*Curcuma longa*) were tested for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae,* using agar well diffusion method at concentrations of 100, 75, 50 and 25mg/mL. The Minimum Inhibitory Concentration (MIC), synergistic effect of two spices in the same family and antimicrobial sensitivity were also determined. Aqueous and ethanol extract of cinnamon were the most effective against the test organism with zones of inhibition between 9mm to 25mm; closely followed by tumeric which also showed good inhibitory effect on the test organisms except that the aqueous and ethanol extract did not have any effect on *E. coli* and *S. aureus* respectively. Garlic–onion aqueous extract inhibited the test organisms with zones of inhibition ranging between 9mm to 24mm. Ginger - tumeric aqueous extract did not have any effect on *E. coli* and *S. aureus*. The MIC for aqueous and ethanol extract of cinnamon was 3.125mg/ml and 12.5mg/ml respectively. *E. coli* was highly susceptible to gentamicin than the remaining two organisms. This work has shown that both cinnamon and turmeric can be used as antimicrobial agents in the treatment of some diseases.

Keywords: ethanol, aqueous, spices, *K. pneumoniae, E. coli, S. aureus*

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INTRODUCTION

Spices can be defined as 'vegetable products used for flavouring, seasoning and imparting aroma in foods'. Herbs are leafy spices, and some, can provide both spice seeds and leafy herbs (Douglas *et al.*, 2005). Herbs and spices are essential part of man's nutrition. They have been used to enrich the taste, colour and smell of food (Chandarana *et al.*, 2005); as traditional medicine for thousands of years (Babuskin *et al*., 2014); and also as food preservatives (Nakatani, 1994). They are used to a large extent in many Asian, African and other countries. Currently, in regard of their useful effects, the consumption of spices/herbs has been steadily improving in developed countries too (Indu *et al.,* 2006).

Phytochemicals are compounds responsible for the exceptional taste and smell in spices and herbs (Avato *et al.,* 2000). Some spices are known to kill (bactericidal) or stop (bacteriostatic) microbial growth (Ağaoğlu *et al.,* 2007). There has been an incredible upsurge in resistance to antibiotics by various bacterial pathogens (Wood *et al.,* 1996), which has led to the search by scientists for alternative antimicrobials like spices instead of other chemotherapeutic agents. *S. aureus*, *E. coli* and *K. pneumoniae* are all important human pathogens and are known to be causative agent of different diseases ranging from boil, pneumonia, meningitis to UTI (Prescott *et al*., 2008). These pathogens have been found by different investigators to be inhibited by at least one of the following: cinnamon, ginger, garlic, curry and lime leaf (Ağaoğlu *et al*., 2007; Ojo *et al*., 2007; Akintobi *et al*., 2013; Khan Pathan *et al.,* 2012; Malwal and Sarin, 2011).