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Macro and Micro Morphological Evaluation of Loganiaceae Liana as Medicinal Plants in South-Western Nigeria

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----------***ABSTRACT***----------
Seven Strychnos species (Loganiaceae family) that are constantly being explored for their medicinal values throughout the world were collected from four major extant forests in South-Western part of Nigeria. They are all giant lianas that grow in thick rain forests. Forty two micro and macro morphological characters were assessed for delimitation of these economically important species. Their leaf shapes range from Elliptic, oblong, obovate to oblanceolate. The leaves lengths are between 3.5cm and 11.7cm; widths are between 2.5cm and 5.2cm. They generally possess single or paired hooks as modification for climbing and inflorescence are axillary cymose type. They possess straight to sinuous anticlinal wall pattern and their cell shapes are polygonal to irregular. They are all hypostomatic with paracytic or anomocytic stomata types and two species have non-glandular unicellular trichomes. Principal component analysis (PCA) and unweighted pair group method with arithmetic average (UPGMA) revealed that their leaf shapes, sizes, hook types, epidermal cell dimension, stomata indices, cuticular foldings among other characters are sufficient to delimit these economically useful species in their populations both for their medicinal importance and conservation purposes. Taxonomic key for the species of Strychnos identification was also prepared to facilitate these activities.

KEYWORDS: *Strychnos, Liana, micro-morphology, medicinal, conservation.*

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I. INTRODUCTION AND LITERATURE REVIEW

Lianas are woody climbing plants that rely on other plants for mechanical support. Their axes have reduced amount of supporting tissue and they are highly light demanding (Campbell and McNewbery, 1993; Padaki and Parthasarathy, 2000). Lianas are a conspicuous and characteristic life-form in tropical rain forests and their high abundance is an important physiognomic feature differentiating tropical from temperate forests (Gentry 1991; Baars, *et al*., 1998). In Nigeria, it is heartrending to hear that majority of the forests environment have been removed as a result of human activities and the usual dense forest canopies have disappeared from many forested zones. Important plant species have been eroded from our lofty vegetations; some are extinct, while many others are referred to as endangered species. What do we expect when the cover has been removed from Faunas? They become more vulnerable as they wander with fear in their natural environment and eventually, they are gone (Bongers *et al*., 2002). Lianas primarily utilise the crown of forest shrubs and trees as they extend themselves from one plant to the other and above them (Ewers and Fisher, 1991; Caballe, 1998; Campbell and Mc Newbery, 1993). Hence, they are the backbones of networks and connections of forest crowns which uniquely typify the tropical rain forests. Forests play important roles as carbon sink in alleviating global climate change and as a reservoir of vegetal and animal biodiversity. Forests are also important for inhabitants (it is habitable) and a source of income via wood and non-wood forest products (Ros-Tonen, 2000). Owing to the problem of deforestation, tropical rain forest biodiversity gives a great concern and its conservation has become an issue of increasing priority (Bongers *et al.,* 2002). Climbers generally have modifications for climbing, hence they are found as: (a) Twiners (b) Hook climbers and stragglers (c) Rooting climbers (d) Tendril climbers (e) Rattans - climbing palms (Padaki and Parthasarathy, 2000). *Strychnos* Linn. are the main woody climbers and largest genus in Loganiaceae family with approximately 200 species that grow in tropical rain forests and savannas as lianas, shrubs, or small trees. In the neotropics *Strychnos* is distributed from Mexico down through Bolivia (Frasier, 2008). In the paleotropics it is found throughout tropical Africa and Madagascar, in India, Sri Lanka, Southeast Asia and the northern tropical part of Australia (Bisset *et al*., 1973; Leeuwenberg and Leenhouts, 1980). Hutchinson and Dalziel (1972), in the Flora of West Tropical Africa recognised 35 species of *Strychnos.* However, they have greatly depleted from Nigerian forests over the last decades as a result of overexploitation due to their ethno-botanical values.

These have not helped their conservation, not in any of the West African countries within the tropical rain forests. Some of *Strychnos* ethno-botanical values are here highlighted: Their leaves, roots, bark, fruit and seeds are used for medicine throughout Nigeria; as analgesic, a purgative, for uterine problems, to treat sore eyes, for parasitic infections like guinea worm, to treat malaria fever (in combination with other plants), rheumatism, ulcers, leprosy, snake-bites etc (Burkill *et al*., 1995; Neuwinger, 1996; Rafatro *et al*., 2000). Some *Strychnos* species have indole alkaloids and cytotoxic actions; they are used for arrow and ordeal poisons. Strychnine and Brucine (active poisons) are produced by some *Strychnos* species - stored in Barks and seeds (Bisset and Phillipson, 1971a; Bisset *et al*., 1973; Samuelsson, 1992; Yan *et al*., 2006).

II. MATERIALS AND METHODS

Strychnos samples from four major extant forests were collected in South-Western part of Nigeria. They were all authenticated and herbarium samples deposited in University of Lagos herbarium (LUH) and Forestry Herbarium Ibadan (FHI). Samples were assessed on the field and in the laboratory for their morphological and anatomical characters. The anatomical slides preparation followed the procedures used by Olowokudejo (1993). In each case, anatomical slide replicates were prepared from plant samples collected from the field. The Anatomical characters; both 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 XSZ-N107 Model light microscope and Motic Camera 'Moticam 2300, 3.0 M.Pixel, USB 2.0 model. Counting of cellular structures was done at x620-Magnification, Measurement at x400-Magnification and image taken at x620-Magnification. Descriptive statistics of mean, standard deviation, standard error and Principal Component Analysis (PCA); extraction method and rotation Method was Varimax with Kaiser Normalization carried used. Pair wise distance (similarity) matrices were computed using sequential, hierarchical and nested (SAHN) clustering option of the NTSYS-pc version 2.02j software package (Rohlf, 1993). The program generated dendrograms which grouped the *Strychnos* species according to their morpho-anatomical characters using unweighted pair group method with arithmetic average (UPGMA) cluster analysis (Sneath and Sokal, 1973). Taxonomic key for the species of *Strychnos* identification was also prepared to facilitate the conservation and their medicinal uses.

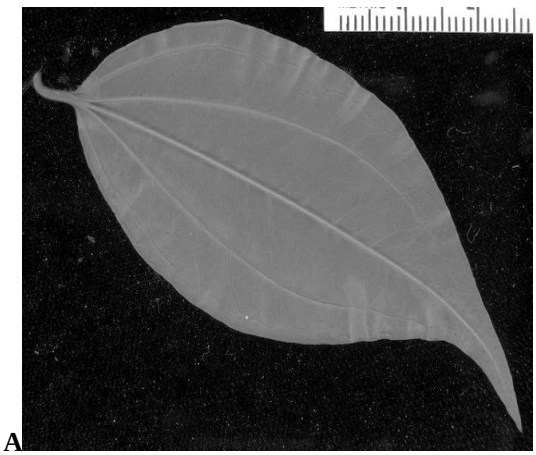
III. RESULTS

Seven *Strychnos* species from four major extant forests were giant lianas that grow in thick rain forests.

Table 1: Locations of representative species collected

S/N	Name of plant specimens	Place of collection	Frequency encountered	Coordinates of collection (GPS)
1	*Strychnos barteri*	J_4 Omo Forest Reserve, Ijebu	4	N 06°50′17.4″ E004°21′52.6″
2	*Strychnos boonei*	Onigambari Forest Reserve, Ibadan	5	N 07°11′.01″ E003°52′42.6″
3	*Strychnos asterantha*	Akure Forest Reserve, Owena	6	N 07°12′07.1″ E005°01′43.9″
4	*Strychnos congolana*	Akure Forest Reserve, Owena	8	N 07°12′16.25″ E005°01′10.29″
5	*Strychnos longicaudata*	Akure Forest Reserve, Owena	3	N 07°12′12.4″ E005°01′.20.28″
6	*Strychnos nigritana*	Aponmu Forest Reserve, Ondo	10	N07° 14' 14.0'' E005° 03' 21.8''
7	*Strychnos urceolata*	Aponmu Forest Reserve, Ondo	4	N 07°13′17.4″ E005° 03' 19.8''

A

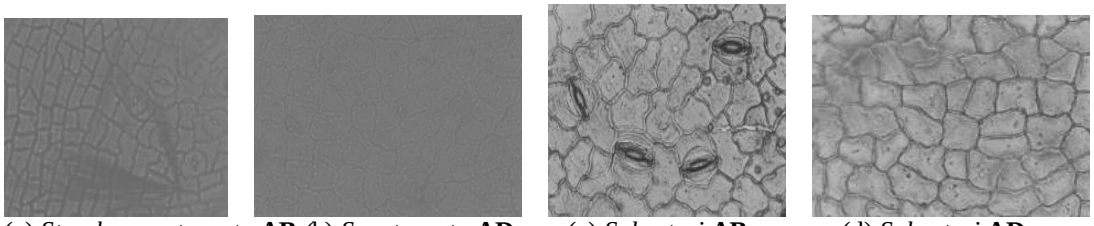


B

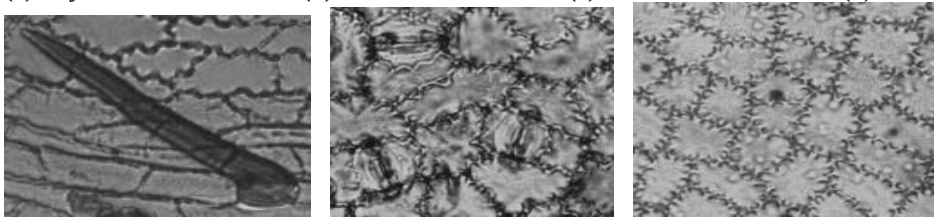
C **D**

E **F**

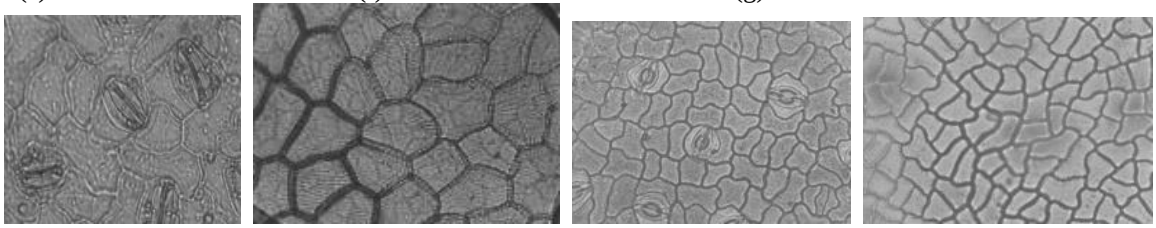
Figure 1: Vegetative structure of *Strychnos* species (a) Basal acrodromous venation of *S. dinklagei* (a) *S. usambarensis* (c) paired hook of *S. nigritana* (d*)* two pairs hook of *S. aculeata* (e) *S. icaja* (f) *S. talbotie*



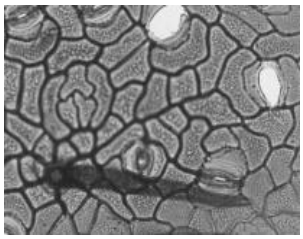
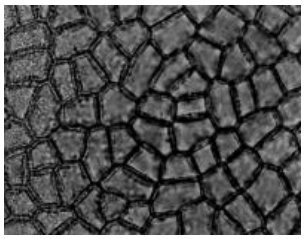
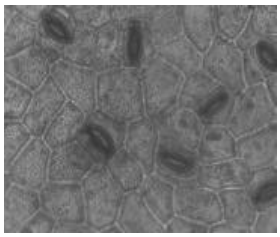
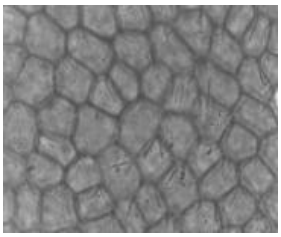
(a) *Strychnos asteranta* **AB** *(*b) *S. asteranta* **AD** (c) *S. barteri* **AB** (d) *S. barteri* **AD**



(e) *S. boonei* **AB** (f) *S. boonei* **AB** (g) *S. boonei* **AD**



(h) *S. congolana* **AB** (i) *S. congolana* **AD** (j) *S. longicaudata* **AB** (k) *S. longicaudata* **AD**

(l) *S. nigritana* **AB** (m) *S. nigritana* **AD** (n) *S. urceolata* **AB** (o) *S. urceolata* **AD**

Plates (a – o): Adaxial and abaxial surfaces of *Strychnos* species, Magnification: x 640. AB and AD stand for Abaxial and Adaxial surfaces respectively.

Table 2a: Leaf epidermal features of *Strychnos* liana

Strychnos species	Leaf surface	Anticlinal wall pattern	Cell wall thickness (µm). min.(mean ± s.e.) max	Epidermal Cell shape	Epidermal Cell number *min. (mean ± s.e.) max*	Epidermal Cell length min. (mean ± s.e.) max	Epidermal cell width min. (mean ± s.e.) max
S. asteranta	Abaxial	straight	1(1.13 ± 0.0497)1.5	polygonal to irregular	380(394.90 ± 1.471)405	5(9.4 ± 0.455)13	4(6.150 ± 0.293)8
S. barteri	Abaxial	undulate	2(2.45 ± 0.114)3	irregular	96(97.70 ± 0.512)100	11(23.75 ± 0.369)46	8(9.15 ± 0.933)11
S. boonei	Abaxial	Barbed/ straight	2(2.45 ± 0.114)3	penta to polygonal	120(126.8 ± 0.618)130	13(17.550 ± 0.667)25	4(5.45 ± 0.211)7
S. congolana	Abaxial	curved	2(3.05 ± 0.170)4	irregular	69(76.4 ± 0.796)80	15(21.4 ± 0.860)27	5(9.10 ± 0.518)15
S. longicaudata	Abaxial	curved	1.2(1.89 ± 0.054)2	irregular	160(163.75 ± 0.542)168	10(16.15 ± 0.930)23	5(6.60 ± 0.245)8
S. nigritana	Abaxial	curved	1(1.23 ± 0.057)1.5	irregular	104(108.80 ± 0.647)115	10(23.60 ± 1.346)30	8(9.90 ± 0.355)13
S. urceolata	Abaxial	straight	1(1.23 ± 0.057)1.5	polygonal	108(110.05 ± 0.320)112	15(23.20 ± 1.065)30	8(11.20 ± 0.501)15

Table 2b: Leaf epidermal features of *Strychnos* liana contn'd

Strychnos species	Stomata length (µm) min (mean ± s.e) max	Stomata width (µm) min (mean ± s.e) max	Stomata number	Stomata type	Stomata index %	Trachoma type	Trichome length	cuticular ornamentation
S. asteranta	2(2.350 ± 0.109)3	1(1± 0.0)1	37(39.7 ± 0.300)42	paracytic	9.14	-	0	-
S. barteri	6(7.65 ± 1.039)9	2(2.40 ± 0.503)3	4(5.35 ± 0.6708)6	paracytic	5.19	-	0	-
S. boonei	5(5.35 ± 0.109)6	1.5(1.78 ± 0.057)2	11(12.35 ± 0.150)13	paracytic	8.88	simple unicellular	43(58.5 ± 2.29)80	-
S. congolana	7(8.90 ± 0.204)10	1.5(1.825 ± 0.055)2	14(15.1 ± 0.161)16	paracytic	16.50	-	0	radiating striae
S. longicaudata	3(3.55 ± 0.114)4	1.5(2.00 ± 0.063)2.5	10(12.15 ± 0.357)15	anomocytic	6.91	-	0	g/c striation
S. nigritana	4(5.00 ± 0.162)6	1(1.48 ± 0.099)2	12(13.10 ± 0.191)14	paracytic	10.75	simple unicellular	30(82.00 ± 10.655)240	g/c striation
S. urceolata	7(9.60 ± 0.328)12	3(4.40 ± 0.169)5	7(14.65 ± 5.505)119	anomocytic	11.75	-	0	-

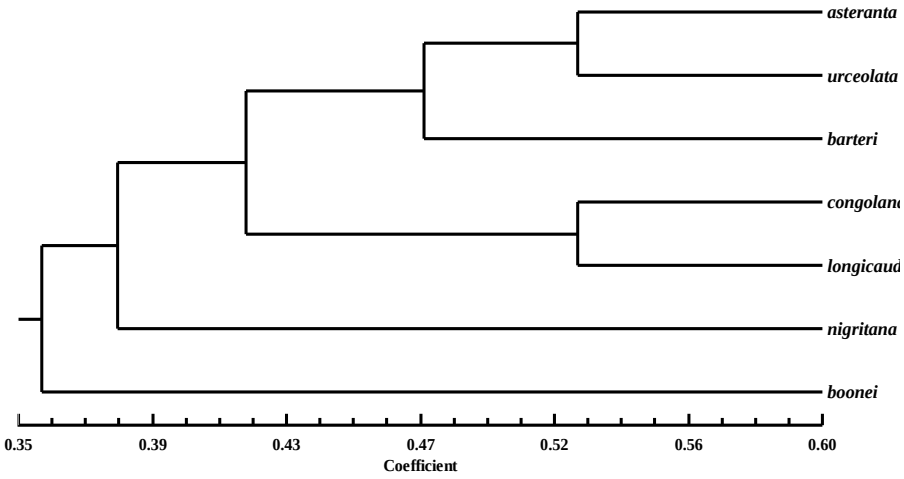


Figure 2: Dendrogram from clustering analysis showing
The relationship of *Strychnos* species in West Africa using 42 characters

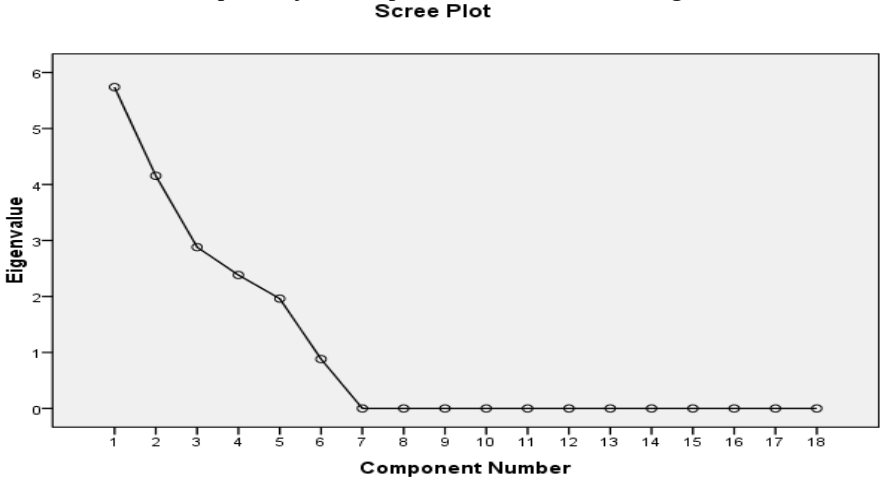


Figure 3: Scree plot of Eigenvalue against 18 components.

Table 3: PCA with three components contributing up to 70% from 42 characters

Total Variance Explained									
	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	5.739	31.885	31.885	5.739	31.885	31.885	4.757	26.426	26.426
2	4.155	23.083	54.969	4.155	23.083	54.969	3.930	21.833	48.259
3	2.881	16.004	70.972	2.881	16.004	70.972	3.203	17.796	66.055
4	2.383	13.237	84.209	2.383	13.237	84.209	2.615	14.527	80.582
5	1.961	10.894	95.103	1.961	10.894	95.103	2.614	14.521	95.103

Taxonomic Key preparation for *Strychnos* species from South-West Nigeria

1a. Hook single, Bark smooth ---------- 2
2a. Leaf apex acuminate, Flower fresh color white ---------- 3
3a. Leaf surface pubescent, Cuticular striations absent ---------- *Strychnos boonei*
3b. Leaf surface glabrous, Cuticular striations present ---------- *Strychnos congolana*
2b. Leaf apex Caudate, Flower fresh colour Yellow ---------- *Strychnos longicaudata*
1b. Hook paired, Bark rough ---------- 4
4a. Anticlinal wall pattern Straight, Epidermal cell shape Polygonal ---------- 5

5a. Mean epidermal cell number 395, Stomata type Paracytic ------ *Strychnos asteranta*
5b. Mean epidermal cell number 110, Stomata type Anomocytic -- *Strychnos urceolata*
4b. Anticlinal wall pattern Curved, Epidermal cell shape Irregular ---------- 6
6a. Stomata Index 5.19, Trichome absent ---------- *Strychnos barteri*
6b. Stomata Index 10.75, Trichome simple unicellular ---------- *Strychnos nigritana*

IV. DISCUSSIONS

During the collection of these *Strychnos* in the forests, we observed that they are abundant in virgin forest vegetation or forests that are above 40 years from the last date of cultivation. Otherwise, they are found as strangling shrubs which may lack some essential diagnostic features. Our lofty forested vegetation are gone, these we observed during the collection of the samples. Active lumbering was in progress as we progressed in our collection activity in the 'extant' Nigeria forest environments, very incredible. Morphological and anatomical characters, forty two of them revealed the following: The leaves have basal and suprabasal acrodromous primary venation pattern and their leaf shape range from Elliptic, oblong, obovate to oblanceolate. The leaves lengths are between 3.5 cm and 11.7 cm; widths are between 2.5 cm and 5.2 cm (Table 2 a and b; Figure 1). Their inflorescence are axillary cymose type, flower fresh colour is either white or yellow with long corolla tube which are proportional to their width, diagnostic for different species (Leeuwenberg, 1980). Three species possess single hook while four species possess paired hooks as modification for climbing (Figure 1). The dendrogram generated (Figure 2) revealed that *S. boonei* is basal in their evolutionary relationship while *S. asteranta* and *S. urceolata* are the most recent diverging clades. *Strychnos* species have completely separated out at about 53 % similarity (Figure 2). Principal component analysis (PCA, Table 3) revealed that their leaf shapes, sizes, hook types, epidermal cell dimension, stomata indices, cuticular foldings among other characters are sufficient to delimit these economically useful species in their populations both for their medicinal importance and conservation purposes. Taxonomic key for the species of *Strychnos* identification also revealed the grouping of the species based on the proximity of their traits exhibited.

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