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Research Article

Taxonomic studies of the genus *Tephrosia* Pers. (Papilionaceae) in Nigeria

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Abstract

The relationship between eleven *Tephrosia* species occurring in Nigeria was examined using a number of taxonomic tools. Fresh and herbarium specimens were used for this purpose and methods followed conventional taxonomic practice. Although the species occur in savanna ecosystems, herbarium collections revealed an abundant distribution in the southern part of Nigeria. Morphometric studies revealed that four quantitative characters viz: leaflet length, lamina length, fruit length and pedicel length can be used to delimit members of this genus. Based on the cluster analysis using average linkage within group, the closest species are *T. leptostachya* and *T. purpurea* with the shortest distance measure (0.713). *T. linearis* has the smallest leaflet, in length and width. Further morphological studies also showed that *T. vogelii* has the largest pod while *T. barbigera* has the highest number of seeds per pod. Generally, the species have very short petiolule ranging between 0.2cm and 0.3cm in length, on the average. Foliar micro-morphological studies also showed that the species generally possess polygonal cells with straight to curved anticlinal walls and anisocytic stomata types while pollen studies revealed tricolporate pollen grains to be predominant within the taxa. Although, the present study has added to the existing information regarding *Tephrosia* species, it also suggests further research to ascertain its taxonomic position within the Papilionaceae.

Keywords
Tephrosia; Papilionaceae; taxonomy; morphology; palynology; distribution

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1. Introduction

The genus *Tephrosia* Pers. belongs to tribe Millettieae of the family Papilionaceae which comprises trees, shrubs, climbers or herbs. Generally, the leaves or leaflets of members of this family are compound, imparipinnate or 3-foliate, or simple with stipules. Flowers are zygomorphic and hermaphroditic with 5 sepals, 5 petals, and 10 stamens with the anther opening lengthwise by slits. The family is also known to be cosmopolitan (Hutchinson & Dalziel 1958, Soladoye & Lewis 2003).

Tephrosia is a French word which means "staying green" (Phillips 1986). The genus comprises about 300-400 species found in the tropical and subtropical regions of the world and some of them have beneficial purposes (Barnes & Freyre 1967, Gaskins *et al.* 1972; Schrire 2005, Watson, 2008). There are about 15 species found in Nigeria (Hutchinson &

Dalziel 1958), although this does not include *T. candida* (Roxb.) DC. and *T. leptostachya* DC. as the latter was regarded as a subspecies under *T. purpurea* (Linn.) Pers. (Brummitt 1968). The genus has been known for its taxonomic complexity (Lewis *et al.* 2005), thus, this paper seeks to provide constant and reliable diagnostic characters that can be useful in the delimitation of the species even in fragmentary conditions.

Materials and methods

Fresh specimens collected from University of Ibadan premises, Ijaye, Deeper Life Camp Ground (Moniya) all in Oyo State, and Olokemeji in Ogun State as well as previously deposited specimens at Forest Herbarium Ibadan (FHI) and University of Ibadan Herbarium (UIH) were used for this work. The specimens were those of *T. bracteolata* Guill. & Perr., *T. linearis* (Willd.) Pers., *T. elegans* Schumach, *T. pedicellata* Bak. and *T. platycarpa* Guill. & Perr. Others include: *T. barbigera* Welw., *T. candida* (Roxb.) DC., *T. leptostachya* DC., *T. mossiensis* A. Chev., *T. purpurea* (Linn.) Pers., and *T. vogelii* Hook. f.

Species distributional studies

This was solely based on information obtained from herbarium collections deposited at the Forest Herbarium Ibadan (FHI) located at the Forestry Research Institute of Nigeria, Ibadan, and University of Ibadan Herbarium (UIH) at the Department of Botany, University of Ibadan, Nigeria. Relevant information such as the place of collection, name of collector, voucher number and date of collection were obtained from these herbarium specimens and a distributional map of the species showing their locations was generated at the Geography Department of the University of Ibadan using Arc GIS 9.3 software.

Macro-morphological Studies

For the morphometric studies, twenty representative specimens of each species were used and quantitative vegetative and reproductive characters were measured following conventional taxonomic practice (Olowokudejo 1999, Soladoye *et al.* 2010). Some of these characters include leaflet length, leaflet width, lamina length, petiolule length, amongst others. Qualitative characters were also taken into consideration and some of which include leaflet shape, apex, base, margin, etc. The mean and standard error were calculated for all the macro-characters and values were recorded on Microsoft excel spreadsheet. Raw data were coded and analysed using SPSS 20.0 statistical package.

Micro-morphological Studies

Foliar epidermal preparations

Pieces of each *Tephrosia* species were obtained from the standard median portion and soaked in separate Petri dishes containing concentrated solution of Nitric acid. For the freshly collected specimens, they were soaked almost immediately while herbarium specimens were first revived in boiling water for about 20 minutes. Lower and upper epidermal layers were separated carefully with the aid of forceps and camel hair brush, rinsed in water about three to five times, dehydrated in 50% alcohol, stained in safranin and left for about five minutes. Thereafter, they were thoroughly rinsed in distilled water and mounted in 25% glycerol on clear microscopic glass slides and carefully covered with cover slips with the edges of the cover slip well sealed with nail vanish in order to prevent dehydration. Slides were labeled properly and viewed under Fisher light microscope. Twenty random measurements of the epidermal cells and stomata were taken with the aid of micrometer eyepiece, and counting of cells, stomata and trichomes were also done for each species. The preparations however, followed the methods of Kadiri and Ayodele, (2003) and Ibrahim *et al.*, (2006). Photomicrograph of each slide was taken using an Olympus CX31 Microscope with a Hyper Crystal LCD camera attached. The mean, standard error and range were computed for each species while the Stomatal Index (SI) was calculated using the formula of Salisbury (1927).

$$SI = \frac{S \times 100\%}{E + S}$$

where S = number of Stomata per unit area, E= number of epidermal cells of the same area.

Micro-characters were studied under Fisher light microscope while photo-micrographic images were taken with an Olympus microscope with an attached camera at the department of botany, University of Ibadan. Measurements were taken with a micrometer eyepiece and carefully recorded.

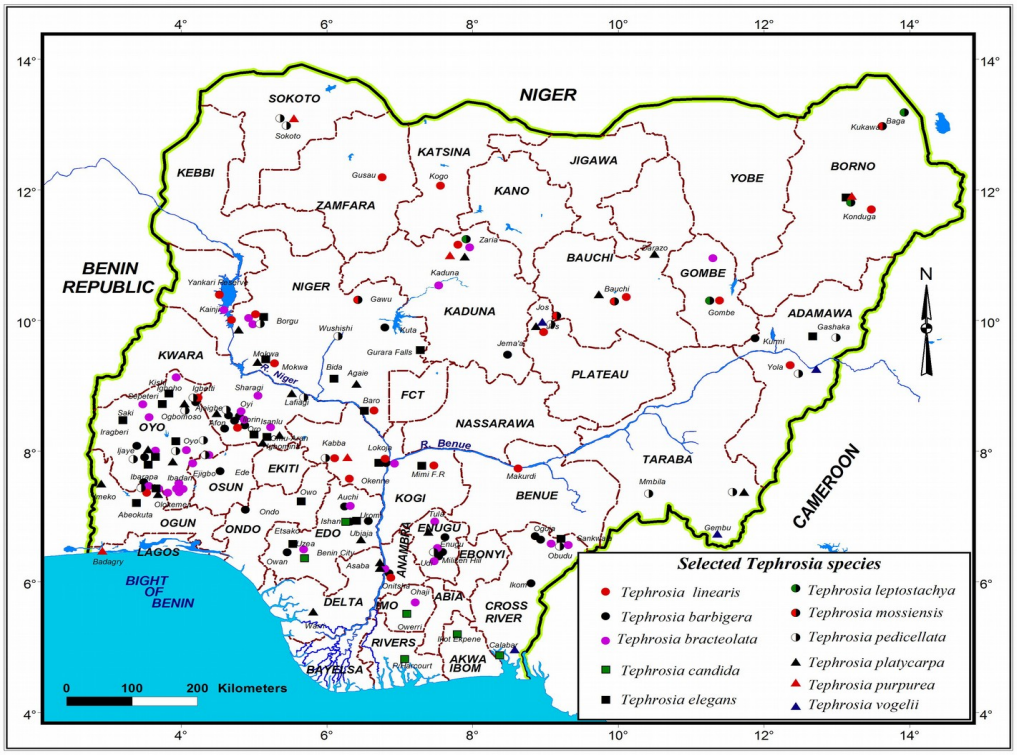

Figure 1. Map of Nigeria showing the distribution of *Tephrosia* species

Pollen preparation

Fresh flowers from collections as well as dried ones from herbarium samples were used for this purpose. The flowers of each *Tephrosia* examined species were crushed into fine powder inside a 50ml centrifuge bottle using a glass rod. These finely crushed powder were subjected to acetolysis method of pollen analysis following standard protocols as described by Erdtman (1960). The prepared specimens were individually mounted in 100% glycerol on clean microscopic glass slides, and each slide was carefully covered with cover slip. The slides were turned upside down so as to allow the pollen grains to settle on the cover slip for easy sighting when viewed under the light microscope. After about 10 minutes, the slides were turned, sealed with nail vanish and arranged vertically in slide box. The slides were carefully examined under fisher light microscope. Pollen descriptions followed Sowunmi (1973) while pollen shapes and class were also studied in accordance with Erdtman (1943).

Results and Discussion

Distributional information gathered from the herbaria consulted showed that most of the collections were those of *T. bracteolata*. Generally, the species were found in the Southern part of Nigeria, particularly Oyo and Kwara States. Other states include Edo, Enugu, Cross River, Anambra, Kogi and Kwara States (Figure 1). Although the family Papilionaceae generally thrives more in savanna regions and all the fresh collections used in his work were also obtained from derived savanna areas, from herbarium studies however, very few collections were made from the northern part of the country, despite being savanna region and none was made from some states like Kano, Jigawa, Yobe and Nassarawa.

Results obtained from the quantitative studies (Table 1) also showed that the leaflets of *T. linearis* are the smallest having the least value in length and width (2.5x0.2cm^2). Leaflets of *T. vogelii* are largest with a size of 4.9x1.2cm^2. However *T. bracteolata* has the highest mean leaflet length (6.0cm), while *T. purpurea* has the highest value for leaflet width, 3.3cm. All the species have an average petiolule length ranging between 0.2cm and 0.3cm. The shortest internode was observed in *T. mossiensis* (0.7cm) while the distance between nodes was highest in *T. purpurea* (4.5cm). *T. pedicellata* has the smallest pod (2.2x0.8cm^2) and *T. vogelii* has the largest pod (10.6x1.3cm^2) although *T. barbigera* has the highest number of seed in a pod (20).

Table 2 shows the result of the qualitative characters assessed. The leaflet shape of the 11 *Tephrosia* species studied range from linear in *T. bracteolata, T. linearis* and *T.*

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Table 1. Quantitative vegetative and reproductive characteristics of the *Tephrosia* species studied

Species	Leaflet Length	Leaflet width	Lamina Length	Petiolule Length	Distance between node	Fruit Length	Fruit width	Pedicel Length	No of seed/pod
T. barbigera	3.0(4.3±0.3)5.8	0.4(0.7±0.1)1.1	3.7(4.5±0.3)5.9	0.1(0.2±0.0)0.3	1.0(1.7±0.2)3.3	3.2(4.9±0.3)6.2	0.4(0.5±0.1)0.7	0.3(0.5±0.0)0.7	8.0(13.2±1.4)20.0
T. bracteolata	3.8(6.0±0.4)12.2	0.4(0.6±0.1)1.4	3.7(6.4±0.5)9.0	0.2(0.2±0.0)0.3	0.8(17±0.2)2.2	7.0(7.4±0.3)8.4	0.4(0.4±0.1)0.5	0.3(1.6±0.8)0.7	11.0(15.0±0.8)19.0
T. candida	3.9(4.9±0.2)5.6	0.8(1.0±0.0)1.3	4.1(5.3±0.2)6.0	0.2(0.3±0.0)0.4	1.8(3.6±0.7)4.8	6.6(9.0±0.4)10.6	0.6(0.7±0.1)0.8	1.1(1.6±0.1)2.0	6.0(10.5±0.7)12.0
T. elegans	3.0(4.6±0.3)6.7	0.6(0.8±0.0)0.9	3.5(4.9±0.3)6.8	0.1(0.3±0.0)0.5	1.9(2.7±0.2)3.6	3.7(4.5±0.3)6.3	0.4(0.4±0.1)0.5	0.1(0.1±0.0)0.2	6.0(7.2±0.4)10.0
T. leptostachya	1.0(1.7±0.2)2.5	0.3(0.6±0.0)0.8	0.7(1.8±0.2)2.5	0.2(0.3±0.1)0.5	3.0(4.1±0.4)6.5	3.3(4.1±0.1)4.9	0.3(0.4±0.0)0.4	0.3(0.5±0.0)0.7	6.0(7.8±0.3)9.0
T. linearis	1.2(2.5±0.2)3.5	0.1(0.2±0.0)0.3	1.5(2.8±0.2)3.9	0.1(0.2±0.0)0.4	2.5(3.4±0.2)5.1	3.4(4.4±0.3)5.6	0.2(0.3±0.1)0.4	0.1(0.2±0.0)0.3	8.0(10.0±0.6)13.0
T. mossiensis	1.5(2.2±0.1)3.2	0.5(0.8±0.1)1.4	1.7(2.5±0.2)3.8	0.1(0.2±0.0)0.3	0.4(0.7±0.1)1.4	4.4(5.4±0.3)6.8	0.4(0.5±0.1)0.8	0.2(0.4±0.1)0.7	3.0(8.9±1.5)14.0
T. pedicellata	1.3(2.4±0.2)2.9	0.5(0.7±0.1)1.0	2.0(2.8±0.2)4.0	0.1(0.2±0.0)0.3	1.0(2.4±0.2)3.5	1.7(2.2±0..2)3.5	0.3(0.8±1.1)0.5	0.2(0.4±0.0)0.5	5.0(7.2±0.6)12.0
T. platycarpa	2.2(3.3±0.3)4.7	0.4(0.8±0.1)1.3	2.8(3.8±0.3)5.2	0.1(0.3±0.0)0.3	2.1(3.5±0.4)6.2	3.1(3.9±0.2)5.0	0.2(0.5±0.2)0.8	0.2(0.5±0.1)0.7	6.0(8.1±0.8)11.0
T. purpurea	1.2(2.0±0.2)2.9	0.4(0.7±0.1)3.3	0.5(2.5±0.2)3.9	0.1(0.2±0.0)0.3	3.3(4.5±0.4)6.3	3.1(4.1±0.2)4.5	0.4(0.4±0.0)0.5	0.4(0.5±0.0)0.6	6.0(7.7±0.4)10.0
T. vogelii	2.0(4.9±0.4)6.3	0.7(1.2±0.1)1.9	2.5(5.3±0.4)6.9	0.1(0.3±0.0)0.4	3.1(4.1±0.2)5.3	5.5(10.6±0.8)13.3	0.4(1.2±0.5)1.8	1.1(2.4±0.3)3.6	10.0(14.3±1.0)17.0

All quantitative characters - min. (mean ± SEM) max.
All measurements are in cm

Table 2. Qualitative leaf characteristics of the *Tephrosia* species studied

Taxa	Leaflet apex	Leaflet base	Leaflet margin	Leaflet shape	Leaflet surface		Leaflet arrangement	Leaf arrangement
					Adaxial	Abaxial		
T. barbigera	Rounded to emarginate	Cuneate	Entire	Obovate to oblanceolate	Glabrous	Pubescent	Opposite	Alternate
T. bracteolata	Rounded	Cuneate	Entire	Linear	Glabrous	Pubescent	Opposite	Alternate
T. candida	Acute	Cuneate	Entire	Obovate to oblanceolate	Glabrous	Pubescent	Opposite	Alternate
T. elegans	Emarginate or mucronate	Cuneate	Entire	Linear oblanceolate	Glabrous	Pubescent	Opposite	Spiral
T. leptostachya	Emarginate	Cuneate	Entire	Obovate to Oblanceolate	Glabrous	Pubescent	Opposite	Alternate
T. linearis	Rounded	Cuneate	Entire	Linear	Silky pubescent	Silky pubescent	Opposite	Alternate
T. mossiensis	Obtuse	Cuneate	Entire	Obovate	Pubescent	Pubescent	Opposite	Alternate
T. pedicellata	Acute-rounded	Cuneate	Entire	Oblanceolate	Pubescent	Pubescent	Opposite	Alternate
T. platycarpa	Truncate or emarginated	Cuneate	Entire	Linear	Pubescent	Pubescent	Opposite	Alternate
T. purpurea	Rounded or emarginated	Cuneate	Entire	Obovate	Pubescent	Pubescent	Opposite	Alternate
T. vogelii	Rounded or truncate & mucronate	Cuneate	Entire	Oblanceolate	Pubescent	Pubescent	Opposite	Alternate

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Table 3. Similarity matrix based on Correlation coefficient of the species studied

		Leaflet Length	Leaflet Width	Lamina Length	Leaflet Petiolule Length	Distance Between Nodes	Fruit Length	Fruit Width	Pedicel Length	No of Seeds/ Pod
Correlation	Leaflet Length	**1.000**								
	Leaflet Width	.405	**1.000**							
	Lamina Length	.996	.431	**1.000**						
	Leaflet Petiolule Length	.301	.280	.267	**1.000**					
	Distance Between Nodes	-.226	.048	-.215	.569	**1.000**				
	Fruit Length	.682	.636	.689	.416	.093	**1.000**			
	Fruit Width	.330	.790	.368	.248	.146	.637	**1.000**		
	Pedicel Length	.660	.656	.682	.376	.153	.921	.737	**1.000**	
	No of Seeds/Pod	.722	.251	.718	.140	-.243	.728	.362	.746	**1.000**

Table 4. Cluster analysis using average linkage within groups

Stage	Cluster Combined		Coefficients
	Cluster 1	Cluster 2	Cluster 1
1	*T. leptostachya*	*T. purpurea*	0.713
2	*T. leptostachya*	*T. platycarpa*	4.528
3	*T. leptostachya*	*T. linearis*	5.960
4	*T. leptostachya*	*T. pedicellata*	7.598
5	*T. elegans*	*T. leptostachya*	10.115
6	*T. elegans*	*T. mossiensis*	11.813
7	*T. barbigera*	*T. bracteolata*	17.026
8	*T. candida*	*T. vogelii*	17.635
9	*T. barbigera*	*T. candida*	26.561
10	*T. barbigera*	*T. elegans*	42.466

Table 5. Principal component analysis of the macro-characters (Total Variance Explained)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.956	55.062	55.062	4.956	55.062	55.062
2	1.784	19.822	74.883	1.784	19.822	74.883
3	1.079	11.984	86.867	1.079	11.984	86.867
4	.557	6.184	93.051			
5	.281	3.124	96.175			
6	.199	2.208	98.383			
7	.098	1.094	99.477			
8	.046	.516	99.992			
9	.001	.008	100.000			

Extraction Method: Principal Component Analysis

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Table 6. Qualitative foliar characteristics of the *Tephrosia* species examined

Species	Cell shape		Anticlinal wall		Stomatal type		Trichome	
	Abaxial Surface	Adaxial Surface	Abaxial Surface	Adaxial Surface	Abaxial Surface	Adaxial Surface	Abaxial Surface	Adaxial Surface
T. barbigera	Polygonal	Polygonal	Straight	Straight	Anisocytic, paracytic	Anisocytic, paracytic	Present	Present
T. bracteolata	Polygonal	Polygonal	Straight/ curved	Straight/ curved	Anisocytic	Anisocytic	Absent	Absent
T. candida	Polygonal	Polygonal	Straight	Straight	Anisocytic, tetracytic	Absent	Absent	Absent
T. elegans	Polygonal	Polygonal	Straight	Straight	Anisocytic,	Paracytic	Present	Absent
T. leptostachya	Polygonal	Polygonal	Straight	Straight	Anisocytic, paracytic	Anisocytic	Present	Absent
T. mossiensis	Polygonal	Polygonal	Straight	Straight	Anisocytic	Anisocytic	Present	Present
T. pedicellata	Polygonal	Polygonal	Straight	Straight	Anisocytic, paracytic	Anisocytic, tetracytic	Present	Present
T. platycarpa	Polygonal	Polygonal	Straight	Straight	Paracytic	Anisocytic	Present	Present
T. purpurea	Polygonal	Polygonal	Straight	Straight	Anisocytic, paracytic	Anisocytic	Present	Absent
T. vogelii	Polygonal	Polygonal	Straight	Straight	Anisocytic, paracytic	Paracytic	Present	Present

Table 7. Quantitative foliar characteristics of the *Tephrosia* species examined

Species	Surface	Stomatal Index	Cell/view	Cell length (µm)	Cell width (µm)	Stomata/view	Stomata length (µm)	Stomata width (µm)	No. of trichomes
T. barbigera	Abaxial	21.7	118(143±6.04)184	27.0(35.1±1.4)43.2	13.5(21.8±1.4)27.0	33(40±1.0)46	16.2(20.0±0.6)24.3	8.1(11.1±0.6)16.2	1(4±0.4)7
	Adaxial	19.7	111(150±12.7)196	27.0(37.3±1.6)45.9	16.2(19.4±0.9)24.3	28(37±2.1)51	16.2(18.2±0.7)21.6	8.1(9.8±0.4)10.8	0(3±0.7)5
T. bracteolata	Abaxial	20.3	175(192±5.3)218	25.65(30.4±1.6)37.2	10.8(18.4±1.8)29.7	41(49±1.0)54	14.9(18.2±0.4)20.3	8.1(9.9±0.4)13.5	Absent
	Adaxial	14.0	142(173±7.8)200	21.6(35.6±1.9)43.2	18.9(21.6±1.1)29.7	21(28±1.3)35	18.9(20.6±0.5)24.3	8.1(11.5±0.5)13.5	Absent
T. candida	Abaxial	0.9	211(24±10.2)270	25.7(31.8±1.6)37.8	13.2(19.2±1.5)24.3	0(2±0.9)6	18.9(21.3±0.8)24.3	12.2(13.3±0.2)13.5	Absent
	Adaxial	0	269(371±16.1)429	18.9(32.4±1.5)43.2	10.8(16.7±0.7)21.6	Absent	Absent	Absent	Absent
T. elegans	Abaxial	27.4	175(182±3.3)191	18.9(30.7±2.4)40.5	13.5(17.4±1.1)25.7	54(67±3.0)80	16.2(19.5±0.6)24.3	9.45(11.3±0.4)13.5	Numerous
	Adaxial	25.4	185(199±4.4)212	27.0(34.1±1.9)43.2	10.8(19.3±1.1)24.3	53(68±3.1)79	13.5(16.1±0.6)18.9	8.1(11.3±0.5)12.2	Absent
T. leptostachya	Abaxial	27.3	195(224±7.1)260	27.0(34.7±1.5)45.9	16.2(17.3±0.6)21.6	63(85±2.7)96	16.2(19.6±0.6)21.6	10.8(13.3±0.4)16.2	0(1±0.3)3
	Adaxial	27.4	190(243±10.9)324	21.6(33.9±1.3)43.2	13.5(20.4±1.0)27.0	77(92±2.1)101	10.8(15.7±0.7)24.3	5.4(6.6±0.9)10.8	0(0±0.0)1
T mossiensis	Abaxial	19.8	90(120±4.5)157	43.2(48.8±2.9)67.5	18.9(28.0±1.5)37.8	20(30±0.8)36	21.6(28.5±0.7)35.1	10.8(14.6±0.8)16.2	6(7±0.2)8
	Adaxial	16.5	159(188±7.1)256	27.0(37.4±2.1)54	13.5(19.1±1.3)27.0	24(37±1.0)44	13.5(17.0±0.6)21.6	8.1(12.2±0.7)18.9	Absent
T pedicellata	Abaxial	28.9	44(97±6.6)122	27.0(39.3±2.5)51.3	13.5(20.9±2.0)32.4	28(40±1.5)52	18.9(24.3±0.6)24.3	10.8(13.6±0.4)18.9	2(5±0.4)7
	Adaxial	29.4	100(115±4.5)147	27.0(41.9±2.9)48.6	13.5(20.9±1.7)35.1	40(48±1.5)61	16.2(21.2±0.6)24.3	10.8(13.1±0.4)16.2	4(6±0.3)9
T platycarpa	Abaxial	17.4	126(178±10.5)215	31.1(38.9±2.4)51.3	13.5(18.6±1.4)24.3	30(38±1.1)42	16.2(22.6±1.7)37.8	8.1(10.0±0.4)13.5	6(9±0.8)14
	Adaxial	26.0	141(171±7.7)207	32.4(39.6±1.9)46.2	13.5(18.9±1.2)24.3	49(60±1.6)72	16.2(19.4±0.7)21.6	8.1(13.5±0.9)16.2	0(2±0.2)3
T purpurea	Abaxial	18.0	158(176±6.7)210	20.3(29.5±1.7)40.5	12.2(16.6±0.8)21.6	33(39±1.6)49	14.9(17.1±0.3)18.9	8.1(10.4±0.5)12.2	0(1±0.2)2
	Adaxial	24.4	120(147±6.0)171	27.0(32.9±2.1)48.6	17.6(20.6±0.8)27.0	36(47±2.4)58	14.9(17.3±0.4)18.9	8.1(11.6±0.5)12.2	Absent
T vogelii	Abaxial	19.4	188(213±6.9)241	21.6(30.2±1.5)37.8	13.5(18.4±1.0)24.3	41(51±3.0)66	14.9(18.9±0.7)21.6	10.8(12.0±0.4)13.5	8(10±0.7)14
	Adaxial	14.0	96(170±8.8)226	27.0(31.7±01.0)37.8	18.9(22.3±0.9)29.7	21(28±0.8)33	13.5(15.8±0.4)18.9	5.4(8.5±0.3)10.8	0(0±0.2)2

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platycarpa to obovate, oblanceolate or both in others. The leaflets all have entire margins, cuneate bases with pubescent abaxial surfaces and glabrous to pubescent adaxial surfaces. The leaflets are also oppositely arranged while the leaves are alternate except in *T. elegans* where they are spirally arranged.

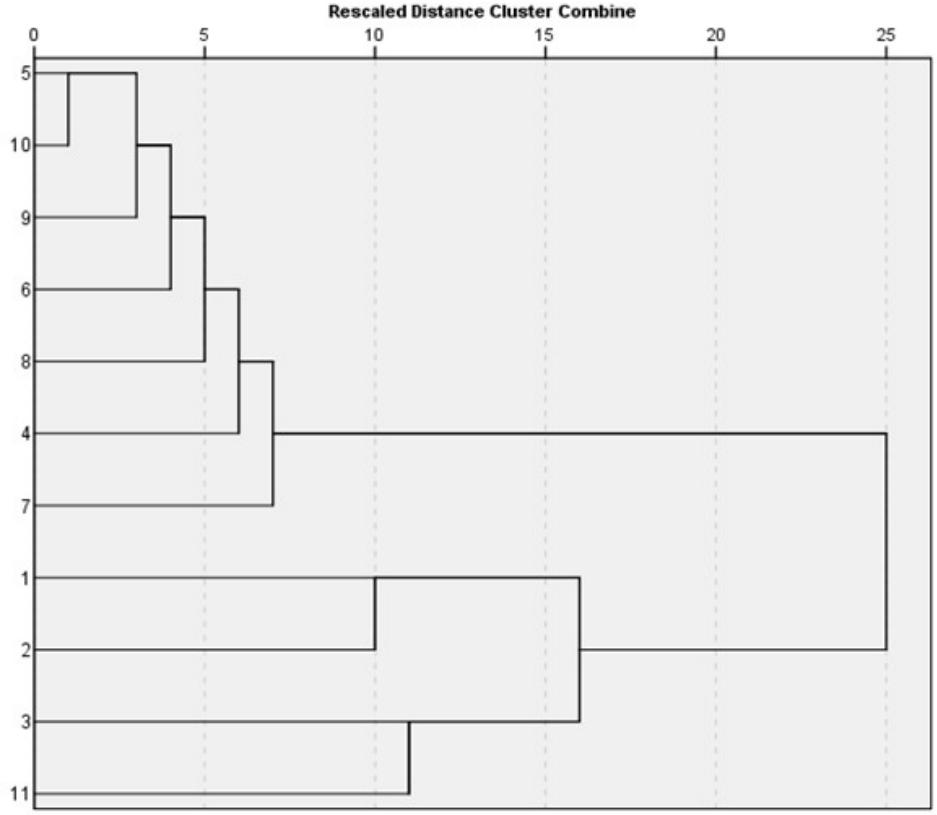
From the correlation coefficient carried out on the 11 species studied (Table 3), it was also observed that there is a highly positive correlation between leaflet length and lamina length with the highest value of 0.996, pedicel length and fruit length with a value of 0.921, leaflet width and fruit width with a value of 0.790. In consequence, these characters viz: leaflet length, lamina length, pedicel length and fruit length can be suggested to be more reliable in delimitation of the *Tephrosia* species studied. There is, in contrast, a highly negative correlation between number of seed/pod and distance between nodes (-2.43), which implies that they may not be useful in the taxonomic delimitation of the genus.

Results from the PCA analysis also revealed that several components were extracted. Of these components, only three contributed greatly to the delimitation of the taxa studied, while others were uninformative. These three components accounts for about 86.9% of the total variance (Table 5).

The remaining variance bits were distributed among other components, that they can hardly be retrieved and thus, using the first three components is an easy choice to obtaining best result.

As shown in Figure 2, two distinct groups were obtained based on the macro-morphological characters assessed. The first group comprises *T. leptostachya, T. purpurea, T. platycarpa, T. linearis, T. pedicellata, T. elegans* and *T. mossiensis.* The second group comprises *T. barbigera, T. bracteolata, T. candida* and *T. vogelii*.

Observations from the qualitative macro-morphological characters as shown in Table 6, also supports previous literatures, all the species have entire leaf margin with hairy abaxial surfaces. However, there were variations observed on their adaxial surfaces. Some were glabrous (*T. barbigera, T. bracteolata, T. candida, T. elegans* and *T. leptostachya*) and others pubescent (*T. linearis, T. mossiensis, T. pedicellata, T. platycarpa, T. purpurea* and *T. vogelii*). Their leaflets also range from linear to obovate to



Key: 1- *T. barbigera;* **2-** *T. bracteolata;* **3-** *T. candida*; **4-** *T. elegans;* **5-** *T. leptostachya;* **6-** *T. linearis,* **7-** *T. mossiensis;* **8-** *T. pedicellata;* **9-** *T. platycarpa*; **10-** *T. purpurea*; **11-** *T. vogelii*

Figure 2. Dendrogram using average linkage (within group)

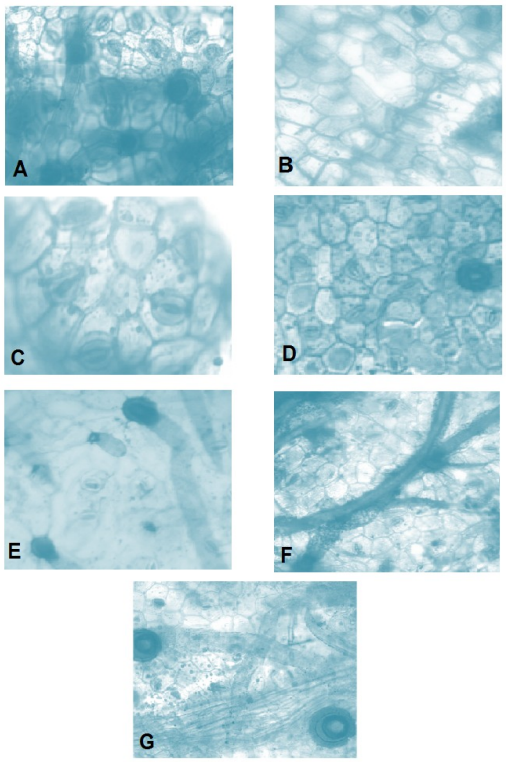


Plate 1. Photomicrographs of the abaxial surfaces of some of the *Tephrosia* species studied x400. **A**- *T. barbigera* showing straight anticlinal walls and simple trichome; **B&C**- *T. bracteolata* and *T. candida* respectively showing tetracytic stomata; **D**- *T. leptostachya* showing paracytic stomata; **E**- *T. pedicellata* showing glandular and simple trichomes; **F**- *T. platycarpa* showing simple trichome and polygonal cells; **G**- *T. vogelii* showing simple trichome and trichome bases.

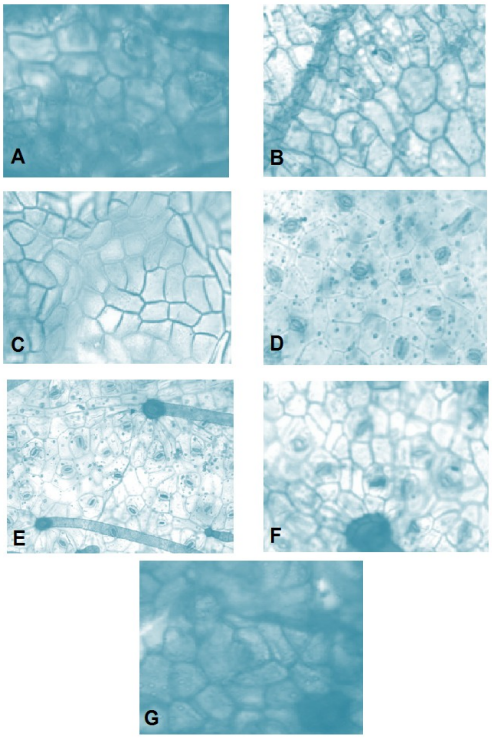


Plate 2. Photomicrographs of the adaxial surfaces of some of the *Tephrosia* species studied x400. **A,C&D**- *T. barbigera, T. candida* and *T. leptostachya* respectively showing polygonal cells; **B**- *T. bracteolata* straight to curved anticlinal wall; **E**- *T. pedicellata* showing glandular (uniseriate) and simple trichomes; **F**- *T. platycarpa* showing trichome base, polygonal cells and stomata; **G**- *T. vogelii* showing stomata, trichome and polygonal cells.

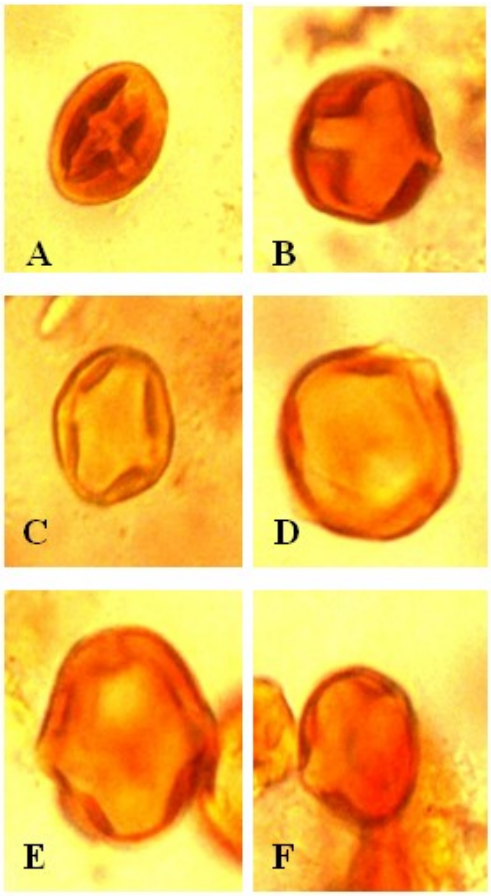


Plate 3. Pollen Photomicrographs of the polar view of some of the species studied. x400. **A-** *T. barbigera;* B- *T. bracteolata*; **C-** *T. candida;* **D-** *T. leptostachya;* **E-** *T. linearis;* **F-** *T. pedicellata.*

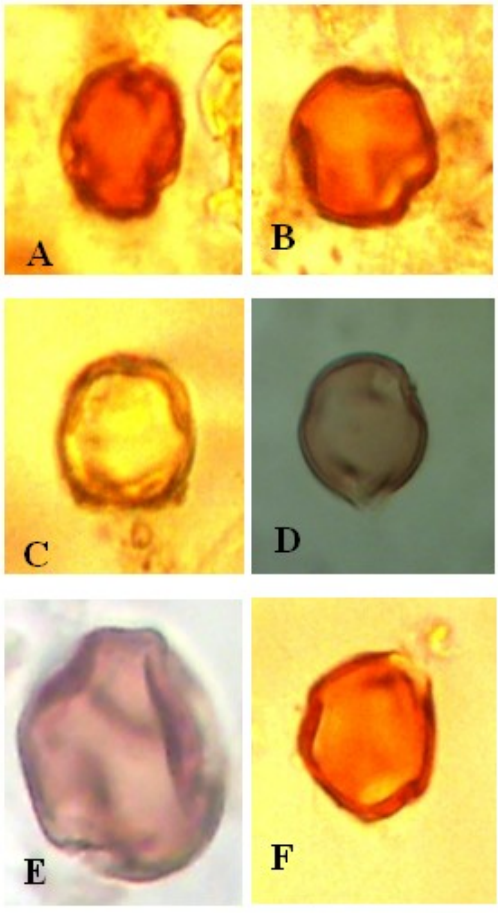


Plate 4. Pollen photomicrographs of the equatorial views of some of the species studied. x400. **A-** *T. barbigera;* **B-** *T. bracteolata*; **C-** *T. candida;* D- *T. leptostachya;* **E-** *T. linearis;* **F-** *T. pedicellata*

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oblanceolate or both (obovate and oblanceolate).

Stern (2000) opined that the importance of morphological features in taxonomic classification of plants cannot be overemphasized, and this is also supported by the present study. Based on macro-morphological characters employed and the dendrogram generated from these characters, there are two major groups. The first group comprises *T. leptostachya, T. purpurea, T. platycarpa, T. linearis, T. pedicellata, T. elegans* and *T. mossiensis*. The extent of similarity measured by the correlation coefficient may suggest a monophyletic origin of these species. The second group comprises *T. barbigera, T. bracteolata, T. candida* and *T. vogelii*. This group however appears paraphyletic. Further investigation may reveal the true phylogeny of members of both groups. The dendrogram also showed that *T. leptostachya* and *T. purpurea* are the closest and this may account for the reason why *T. leptostachya* was treated as a subspecies under *T. purpurea* as earlier reported by Brummitt (1968). These two species also have the lowest coefficient value of 0.713 (Table 4) and this corroborates the statistical rule that the smaller the coefficient value, the more the degree of affinity existing between the species, and hence they can be said to be the closest of all the species examined in this work.. It is also evident from the dendrogram, that the degree of affinity between *T. barbigera* and *T. bracteolata* is similarto that existing between *T. candida* and *T. vogelii*, as eviden by their coefficient values viz: 17.026 and 17.635 respectively.

Further findings from the foliar micro-morphological studies showed that all the species of *Tephrosia* examined have polygonal epidermal cells with straight to curved anticlinal walls on both adaxial and abaxial surfaces (Plates 1&2). This conforms to Stace (1965) who reported that plants that inhabit dry areas tend to have straight to curve anticlinal walls, and this genus comprises mainly savannah plants. Most of the species are epiamphistomatic except *T. vogelii, T. barbigera* and *T. elegans* which are hypoamphistomatic. Only *T. candida* is hypostomatic and has the least mean number of stomata on its abaxial surface. Generally, the species have paracytic and anisocytic stomata except two, *T. candida* and *T. pedicellata*, which have tetracytic stomata; a few of them have trichomes ranging from simple and long in *T. platycarpa* and *T. mossiensis* to glandular ones in *T. pedicellata* (Table 6). *T. candida* and *T. bracteolata* both lack trichomes on their two surfaces while *T. elegans* and *T. purpurea* only have on their abaxial surfaces. However for the quantitative foliar micro-characteristics (Table 7), the mean epidermal cell number varies from 97 in *T. pedicellata* to 239 in *T. candida* and 114 in *T. pedicellata* to 370 in *T. candida* on abaxial and adaxial surfaces respectively. The smallest cell was observed in *T. purpurea* (29.5x16.6 μm^2) and the largest ones were observed in *T. mossiensis* (48.8x28.0μm^2) on their abaxial surfaces. On the adaxial surface however, *T. bracteolata* has the smallest cell (30.4x18.4μm^2) while *T. pedicellata* has the largest cell (41.9x20.9μm^2). Stomata number range from 2 in *T. candida* to 28 in *T. bracteolata* and 0 in *T. candida* to 92 in *T. leptostachya* on the abaxial and adaxial surfaces respectively. Trichomes, where present, and ranged from 1 to 14.

As observed from the pollen analysis, all the species have similar pollen type (tricolporate), with psilate exine and pollen shapes that range from prolate, subprolate to prolate spheroidal. According to Erdtman (1952), pollen of same genus show more or less similar type with few exceptions. Species with the thinnest exine are *T. bracteolata* and *T. candida* (1.2µm) while *T. elegans* has the thickest exine (2.6µm). The smallest grain was observed in *T. candida* (24.6x20.3) μm^2 and the largest grains in *T. vogelii* (36.0x30.9) μm^2. Values of P/E range between 108 in *T. mossiensis* to 139 in *T. barbigera*.

Conclusion

Although, the macro-morphological characters can be used to delimit the studied taxa to some extent, it is also important to compliment the obtained results with other taxonomic tools. Macro-morphologically, the results obtained separate the eleven species of *Tephrosia* into two main groups, and none of these species was distantly related from others except when comparing a member of one group with a member from another group. Micro-morphologically, epidermal preparations unite the species together as well as the pollen studies. Herbarium collections however do not reflect the true habitat of this genus as there could have been some limitations on the part of collectors. It is

therefore suggested that further systematic studies be conducted in the genus to ascertain its taxonomic position.

Competing Interests:

The authors do not have any competing interests.

Author's contribution:

DMC and AEA initiated the research and participated in collection of the plant materials. DMC performed the laboratory works and data analysis. Both authors prepared the manuscript. AEA proofread the final manuscript.

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References

Barnes, D.K. & R.H. Freyre 1967. Recovery of natural insecticides from *Tephrosia vogelii*. III. An improved procedure for sampling and assaying rotenoid content in leaves. *Economic Bot* 21:93-8. http://dx.doi.org/10.1007/BF02897179

Brummitt, R.K. 1968. New and little known species from the Flora Zambesiaca area. XX. *Tephrosia*. *Bol Soc Brot ser* 2(41):24–351

Erdtman, G. 1943. An Introduction to Pollen Analysis. Waltham Mass. 239 pp.

Erdtman, G. 1952. Pollen Morphology and Plant Taxonomy. Angiosperms. Almqvist and Wiksell, Stockholm. 539 pp.

Erdtman, G. 1960. The acetolysis method; a revised description. *Svensk Bot. Tidskr*, 54:561-564.

Gaskins, M.H., G.A. White, and F.W. Martin 1972. *Tephrosia vogelii*; A Source of Rotenoids for Insecticidal and Piscicidal Use. US Dept Agricultural Technol Bull 1445.

Hutchinson, J. and J.M. Dalziel 1958. Flora of West Tropical Africa (Vol. 1 Pt. 2). Crown Agents for Oversea Governments and Administrations, Millbank, London. pp505-587.

Ibrahim, A.J., A.E. Ayodele., A.I. Jegede and Y.F. Kunle 2006. Comparative Studies of *Khaya* A. Juss. (Meliaceae) in Nigeria. *African Journal of Biotechnology.* 5(11):1154-1160.

Kadiri, A.B. and A.E. Ayodele 2003. Comparative Leaf micro-morphological Characters of the Nigerian Species of *Rauvolfia* Linn. (Apocynaceae). *Bioscience Research Communications.* 15(6):35-41.

Lewis, G., B. Schrire., B. Mackinder. and M. Lock 2005. *Legumes of the world*. p. 386. Royal Botanic Gardens, Kew:London.

Olowokudejo, J.D. 1999. Comparative Morphology of leaf epidermis in the genus Annona (Annonaceae) in West Africa. *Phytomorphol*., 40: 407 – 422.

Phillips, B.G. 1986. *Webster Third New International Dictionary of the English language.* Merrian-Webster Incorporation U. S. pp 1, 044

Salisbury, E.J. 1927. On the causes of and ecological significance of stomatal frequency, with special reference to the woodland flora. *Phil. Trans. R. Soc.* 216B, 1-65.

Schrire, B.D. 2005. Tribe Millettieae. In: Lewis, G., Schrire, B., Mackinder, B. and Lock, M. (eds) Legumes of the world. Royal Botanic Gardens, Kew, 367–387.

Soladoye, M.O. and G.P. Lewis 2003. A Checklist of Nigerian Legumes. Center for Environmental Research and Development (CENRAD), Ibadan. 141pp.

Soladoye, M.O., M.A. Onakoya., E.C. Chukwuma and M.A. Sonibare 2010. Morphometric study of the genus Senna Mill. in South-western Nigeria. *African Journal of Plant Science*. 4 (3): 044-052.

Sowunmi, M.A. 1973. Pollen grains of Nigerian plants. *Grana* 13:145–186. http://dx.doi.org/10.1080/00173137309429891

Stace C.A. 1965. Cuticular studies as an aid to plant taxonomy. *Bulletin of the museum* (*National History*). Botany 4:1-78.

Stern, K. R. 2000. Introductory plant biology, MacGraw-Hill Company Inc. United States of America, pp: 630.

Watson, L. 2008. *Tephrosia* Pers. Syn. Pl. 1:328 (1806) Current Scientific Description.