The Significance of Leaf Morphological Characters in the Identification of Some Species of Apocynaceae and Asclepiadaceae

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Abstract: The relationships among seven species of Apocynaceae and seven species of Asclepiadaceae have been concentrated on the basis of information derived from macro- and micro-morphological characters of leaves. The macro-morphological characters of the leaves recorded important feature in the leaf arrangement, blade outline, blade apex and blade texture. Also the present study recorded one species leafless and one sessile. The outline of the petiole demonstrates that the studied species of Apocynaceae and Asclepiadaceae can be distinguished into four patterns (Half circle, half circle with 2 ridges, terete, terete with two wings). Also the vascular structure of petiole revealed four main types: **A.** One main bundle only without subsidiary bundles recorded in five species, **B.** One main bundle accompanied by one subsidiary bundle in one species, **C**. One main bundle accompanied by two (1+1) subsidiary bundles in five species, **D.** One main bundle accompanied by six (3+3) subsidiary bundles in one species. The study of leaf surface of studied species of Apocynaceae and Asclepiadaceae by using light microscope presented 15 different types of trichomes. Some species were free from glandular trichomes and others were free from non-glandular trichome beside the taxa which have the two types of the trichomes. The data recorded from the morphological and anatomical characters are used in numerical analysis and showed that species were grouped into two major clusters, the first one (**I**) consisted of one species; while the second cluster (**II**) comprises the remainder. The second cluster divided into two groups: group **"G1"** incorporated one species; and group **"G2"** divided into two sub group: sub group **"Sb.G1"** included seven species; while sub group **"Sb.G2"** have five species. This study support consider the two family as large family: Apocynaceae. The diagnostic morphological and anatomical characters of leaf appeared to be significant in differentiation between the species of Apocynaceae and Asclepiadaceae. A dichotomous indented key to the species is constructed.
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Key words: Leaf morphology, Anatomy, Apocynaceae, Asclepiadaceae.

1. Introduction

Apocynaceae and Asclepiadaceae are relatively large families (in total approximately 5000 species) consist of mainly tropical and subtropical plants with abundant latex and usually with simple opposite leaves.

The Apocynaceae was first described by **(Jussieh, 1789)** as *"Apocinae"*. After short time later a part of the family was separated into a new family, the *Asclepiadaceae,* by **(Brown, 1810 a& b)** as *"Asclepia/deae"*. Ever since the *Asclepiadaceae* were separated from the Apocynaceae by **(Brown, 1810 b).** The close affinities of the two families and the fact that Asclepiadaceae exhibits the culmination of evolutionary trends apparent in Apocynaceae have been recognized by early taxonomists **(Brown, 1810; Endlicher, 1838; Don, 1838; Decaisne, 1844; Bentham and Hooker, 1862; Baillonv 1891 and Schumann, 1895)** as well as modern **(Stebbins, 1974; Cronquist, 1981; Dahlgren, 1980; Takhtajan, 1987; Rosatti, 1989 and Thorne, 1992)**. The circumscription of this close set of relationships for these families has been expressed differently by taxonomists. Some authors still consider them one large family: Apocynaceae *s.l.* **(Demeter, 1922; Safwat, 1962; Stebbins, 1974; Thorne, 1992; Judd *et al.*, 1994; Struwe *et al.*, 1994; Sennblad and Bremer 1996 and Endress and Bruyns 2000)**; others as two distinct families: Apocynaceae *s.str.* and Asclepiadaceae *s.l.* **(Cronquist, 1981; Dahlgren, 1980; Takhtajan, 1987; Rosatti, 1989).** Alternative treatments have included placing them as separate families in a separate order, the Apocynales **(Hutchinson 1973)** also including Plocospermataceae or in a suborder of the Gentianales, the Apocynineae **(Rosatti, 1989 and Nicholas and Baijnath, 1994)**.

In Egypt the two families are separated from each other's; Apocynaceae are represented by two wild genera both are very rare and 17 cultivated genera including 21 species**.** While Asclepiadaceae represented by 12 wild genera, 18 species and 6 cultivated genera including 6 species **(Täckholm, 1974 and Boulos, 2000).**

Our knowledge about the anatomy of these

plants is only measly. So far only a few species have been worked out **(Omino, 1996; Sidiyasa, 1998; Abdel Kawy, 2003; Radwan, 2007; Middleton, 2007; Abdul Jaleel *et al.*, 2009** and **Pinto *et al.*, 2012).** Therefore the purpose of this study is to investigate the leaf (petiole and blade) morphology and anatomy of some species of Apocynaceae and Asclepiadaceae and to evaluate their taxonomic value.

Material & Methods

The present study is based on 14 species of Apocynaceae and Asclepiadaceae collected fresh from different localities in Egypt **(Table I)**. In this work the materials studied were identified by means of comparison with specimens kept in the herbarium of the Agricultural Museum (CAIM). In addition, keys of **Bailey (1916), Lindley (1932), Hutchinson and Dalziel (1963), Tãckholm (1974**), **Davis (1975)**, **Pandey (1997), Endress and Bruyns (2000) and Boulos (2000).** Reference herbarium specimens of studied species were prepared and kept in the herbarium of Botany and Microbiology Department, Faculty of Science (Girls Branch) Al-Azhar University. For anatomical investigation each specimen was killed and then fixed according to **Nassar and El-Sahhar, (1998)** in F.A.A. (formalin - glacial acetic acid - 70% alcohol) with the ratio of 5: 5: 90 by volume. The leaves (petiole & blade) were hand sectioned at 20-30 mμ in thickness in the concerned organs. The sections were stained according to **Dilcher (1974)** in safranin (1% solution in 50% ethanol) and light green (1% solution in 96% ethanol) and photographs.

The stomata and trichomes types were determined by stripping and fixing the lower leaf epidermis in 70% ethanol and cleared in 1% warm lactic acid before examination by light microscope **Nassar and El-Sahhar, (1998)**.

The terminology concerning the mesophyll types and trichomes is given according to **Fahn (1974)**, **Metcalfe and Chalk (1979)**, **Garces (2013) and Gilberto and Alexander (2014)**.

A total of 45 comparative morphological and anatomical characters for the leaves studied species were scored and coded for creating data matrix used for numerical analysis. The relationships between the studied species have been demonstrated as dendrograms **(Plates 4 & 5)** by using the statistical program PRIMER software, version 5.0.

Table 1: List of the collected species for the present study

Family	Species	Locality and date
Apocynaceae	***Acokanthera spectabilis* Hook.**	El- Orman Garden, 3/ 2009
	***Carissa spinarum* L.**	The Zoo, 3/2009
	***Thevetia peruviana* (Pers.) K. Schum.**	Agriculture Museum Garden,3/ 2009
	***Catharanthus roseus* (L.) G. Don.**	The Zoo, 3/2009
	***Alstonia scholaris* (L.) R. Br.**	The Zoo, 3/2009
	***Nerium oleander* L.**	El- Kobba Palace, 3/ 2009
	***Mascarenhasia elastica* k.Schum.**	Asswan Botanical Garden, 4/2009
Asclepiadaceae	***Leptadenia arborea* (Forssk.) Schweinf.**	Asswan, 4/2010
	***Leptadenia pyrotechnica* (Forssk.) Decne.**	Wadi Hagol, 3/2010
	***Calotropis procera* (Aiton) W. T. Aiton.**	Al–Azhar University,6/2009
	***Cynanchum acutum* L.**	El- Fayoum,3/2009
	***Solenostemma argel* (Delile) Hayne.**	Asswan, 4/2010
	***Asclepias curassavica* L.**	Asswan Botanical Garden, 4/2009
	***Cryptostegia grandiflora* R.Br.**	Agriculture Museum Garden,3/ 2009

3. Results and Discussion

The different macro- and micro-morphological features of leaves (petioles, blades and trichomes) of species were extensively studied to indicate the importance of these characters. Variation in these aspects among the species is listed in Table 2 and recorded comparatively for individual species in Table 3 and illustrated in Plates 1 & 2. The study of leaf surface of studied species of Apocynaceae and Asclepiadaceae by using light microscope presented 15 different types of trichomes showed in Plate 3. The results were used to build a dichotomous indented key to the investigated species.

The outline of the petiole demonstrates that the studied species of Apocynaceae and Asclepiadaceae can be distinguished into four patterns as follows: Pattern (i): Half circle in *Acokanthera spectabilis*, *Carissa spinarum*, *Thevetia peruviana, Catharanthus roseus, Nerium oleander, Solenostemma argel,* and *Asclepias curassavica*. Pattern (ii): Half circle with 2 ridges in *Cryptostegia grandiflora*. Pattern (iii): Terete in *Mascarenhasia elastic, Leptadenia arborea and Cynanchum acutum* Pattern (iv): Terete with two wing in *Alstonia scholaris*.

A- Plant is leafless……..……..…… ***Leptadenia pyrotechnica***
AA- Plants are leafy
B- Leaves whorled
C- Blade outline ovate with obtuse apex & asymmetric base and surface cavity absent…….... ***Alstonia scholaris***
CC- Blade outline elliptic with acute apex & symmetric base and surface cavity present……..……. ***Nerium oleander***
BB- Leaves not whorled
D- Leaves alternate and linear…….. ***Thevetia peruviana***
DD-Leaves not so
E- Leaves opposite
F- Blade outline cordate and glandular trichome with bicellular-biseriate stalk & unicellular head present……....***Cynanchum acutum***
FF- Blade outline not so
G-Blade broad ovate with apiculate apex and glandular trichome with unicellular stalk & bicellular head with cylindrical apex present……..… ***Carissa spinarum***
GG- Blade outline elliptic and glandular trichome absent
H- Blade with acuminate apex, asymmetric base and petiole outline terete ……..….. ***Mascarenhasia elastic***
HH- Blade with acute apex, symmetric base and petiole outline half circle
I-Vascular tissue one main bundle with 1+1 subsidiary in petiole, stone cell & druses are present and non-glandular trichome only present…….……..…***Acokanthera spectabilis***
II- Vascular tissue one main bundle with 1+0 subsidiary in petiole, stone cell & druses are absent and glandular & non-glandular trichome present……..***Asclepias curassavica***
EE- Leaves opposite decussate
J- Leaf obovate with apiculate apex
K- Leaf tomentose, symmetric, sessile and glandular trichome with bicellular-uniseriate stalk & unicellular head present…….…. ***Calotropis procera***
KK- Leaf glabrous, asymmetric, petiolate and glandular trichome with bicellular-uniseriate stalk & unicellular head absent……..…. ***Catharanthus roseus***
JJ- Leaf elliptic with acute apex
L- Petiole outline terete with 1+1 subsidiary vascular bundle and druses absent……..…. ***Leptadenia arborea***
LL- Petiole outline half circle, subsidiary vascular bundle absent and druses present
M- Petiole outline half circle with two ridges, ground tissue parenchyma & sclerenchyma, mesophyll dorsiventral and nonglandular trichome only present……. ***Cryptostegia grandiflora***
MM- Petiole outline half circle, ground tissue parenchyma & collenchyma, mesophyll isobilateral and glandular trichome present…….. ***Solenostemma argel***

The vascular structure of petiole revealed four main types: **A.** One main bundle only without subsidiary bundles recorded in five species, **B.** One main bundle accompanied by one subsidiary bundle in one species, **C**. One main bundle accompanied by two (1+1) subsidiary bundles in five species, **D.** One main bundle accompanied by six (3+3) subsidiary bundles in one species.

Stomata are anomocytic in most studied species, paracytic only in (*Acokanthera spectabilis*), anomocytic with paracytic in (*Cryptostegia grandiflora*), anomocytic with anisocytic in (*Catharanthus roseus*), anomocytic with paracytic and actinocytic in (*Mascarenhasia elastica*) and anomocytic with actinocytic in (*Calotropis procera*).

Mesophyll; is isobilateral in (*Nerium oleander*, *Leptadenia arborea*, *Calotropis procera* and *Solenostemma argel*), while dorsiventral in (the remainders). Schizogenous canals are recorded in all studied species, while surface cavities are recorded in *(Nerium oleander) only*.

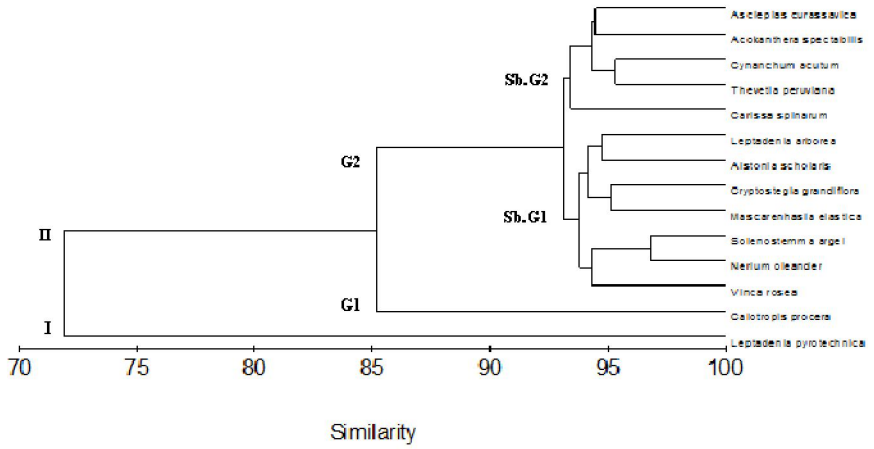

Plate 4: Dendrogram showing the interrelationships between 14 species of Apocynaceae and Asclepiadaceae based on 45 characters of morphological and anatomical features by using PRIMER Program.

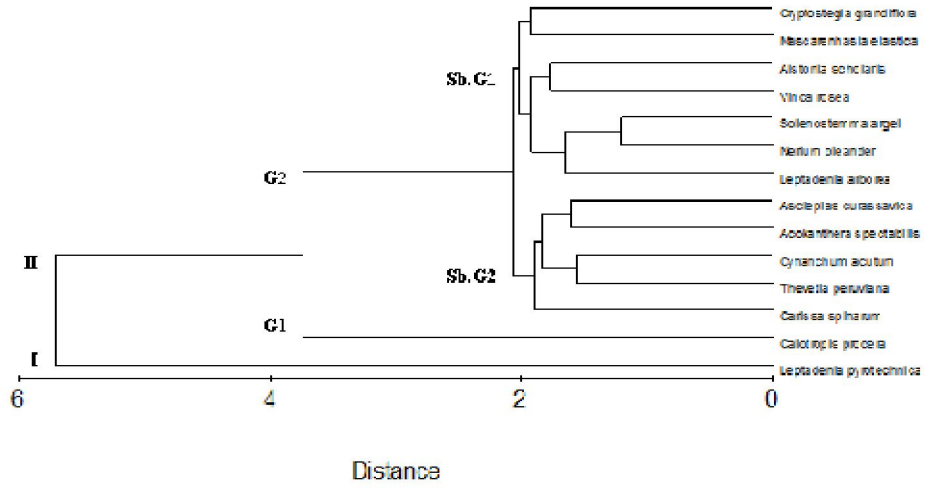

Plate 5: Dendrogram showing the interrelationships between 14 species of Apocynaceae and Asclepiadaceae based on 45 characters of morphological and anatomical features by using PRIMER Program.

The trichomes used to differentiate between the species. Some of these trichomes are specific for some species for example: Glandular hairs (unicellular stalk and unicellular cylindrical head) is present in *Asclepias curassavica*; Glandular with unicellular stalk and bicellular head in *Carissa spinarum*; Glandular with bicellular –biseriate stalk and unicellular head in *Cynanchum acutum*; Glandular with bicellular – uniseriate stalk and unicellular head in *Calotropis procera;* Glandular with multicellular stalk and unicellular head acute apex in *Asclepias curassavica*; Non glandular, simple with long, acute & apical cell in *Carissa spinarum* and Non glandular with two short basal cell and long acute apical cell in *Catharanthus roseus*. The non-glandular with two long, acute apical cells are specific for two species (*Carissa spinarum* and *Solenostemma argel*). Also two species have glandular trichomes only; *Alstonia scholaris* and *Cynanchum acutum*. Another has non-glandular trichomes as *Acokanthera spectabilis* and *Cryptostegia grandiflora*.

Numerical analysis: Tables (2 and 3)

All characters from morphological and anatomical leaf structure for 14 species of Apocynaceae and Asclepiadaceae were used for numerical analysis by using the method of clustering as a tool in the identification of the studied species and in taxonomic relationships among Apocynaceae and Asclepiadaceae.

The results of clustering particularly analysed by the agglomeration of Schedule measure distance and similarity, using average linkage between groups **(Plates 4 & 5)** showed that species were grouped into two major clusters, the first one (**I**) consisted of one species; *Leptadenia pyrotechnica;* while the second cluster (**II**) comprises the remainder. The second cluster divided into two groups: group **"G1"** incorporated one

species; *Calotropis procera*. The group **"G2"** incorporated the remainder. The group **"G2"** divided into two sub group: sub group **"Sb.G1"** included seven species; *Catharanthus roseus*, *Nerium oleander*, *Solenostemma argel*, *Mascarenhasia elastica*, *Cryptostegia grandiflora, Alstonia scholaris* and *Leptadenia arborea*; while sub group **"Sb.G2"** have five species; *Carissa spinarum, Thevetia peruviana*, *Cynanchum acutum*, *Acokanthera spectabilis* and *Asclepias curassavica*.

Table 2: Characters list for the numerical analysis of the studied species of Apocynaceae and Asclepiadaceae.

Leaf	1- Present [1]/ absent [2].
	2- Arrangement: Opposite [1] / Alternate [2] / Opposite Decussate [3]/ Whorled [4].
	3- Petiole length: Sessile [1]/ Very short [2]/ Short [3]/ Long [4].
	4- Blade outline: Elliptic [1]/ Ovate [2]/ Linear [3]/ Obovate [4]/ Cordate [5].
	5- Blade apex: Acute [1]/ Apiculate [2]/ Obtuse [3]/ Acuminate [4].
	6- Blade base: Symmetrical [1]/ Asymmetrical [2].
	7- Blade texture: Glabrous [1]/ Tomentose [2].
	8- Blade length: Very short [1]/ Short [2]/ Long [3]/ Very long [4].
Petiole	9- Present [1]/ Absent [2].
	10-Outline: Half circle [1]/ Terete [2]/ Terete with two wings [3]/ Half circle with 2 ridges [4].
	11- Cuticle: Thick [1]/ Very thick [2].
	12- Cuticle: Smooth [1]/ Warty [2].
	13- Epidermis: Radial [1] / Tangential [2]/ Mixed [3].
	14- Ground tissue: One type [1]/ Two types [2].
	15- Subsidary vascular bundles: 1+ 0 [1]/ 1+ 1 [2]/ 3+ 3 [3]/ Absent [4].
	16- Tissue associated with bundles: Stone cells [1]/ None [2].
	17- Schizogenous canals: Present [1]/ Absent [2].
	18- Laticeferous canal: Present [1]/ Absent [2].
	19- Druses: Present [1]/ Absent [2].
Blade	20- Cuticle: Thick [1]/ Very thick [2].
	21- Cuticle: Smooth [1]/ Warty [2]/ Striated [3].
	22- Epidermis: Radial [1] / Radial with tangential [2].
	23- Surface cavities: Present [1]/ Absent [2].
	24- Stomata: One type [1]/ Two types [2]/ Three types [3].
	25- Midrib: One type [1]/ Two types [2].
	26- Stone cell: Present [1]/ Absent [2].
	27- Mesophyll: Dorsiventral [1]/ Isobilateral [2].
	28- Schizogenous canals: Present [1]/ Absent [2].
	29- Laticeferous canals: Present [1]/ Absent [2].
	30- Druses: Present [1]/ Absent [2].
Trichomes	31- Unicellular stalk and unicellular head: Present [1]/ Absent [2].
	32- Unicellular stalk and unicellular head with cylindrical apex: Present [1]/ Absent [2].
	33- Unicellular stalk and bicellular head: Present [1]/ Absent [2].
	34- Long unicellular stalk and multicellular head: Present [1]/ Absent [2].
	35- Bicellular –biseriate stalk and unicellular head: Present [1]/ Absent [2].
	36- Bicellular –uniseriate stalk and unicellular head: Present [1]/ Absent [2].
	37- Multicellular- uniseriate stalk and unicellular head with acute Aapex: Present [1]/ absent [2].
	38- Unicellular papillose: Present [1]/ Absent [2].
	39- Simple with long broad, blunt & smooth apical cell: Present [1]/ Absent [2].
	40- Simple with long broad, acute & smooth apical cell: Present [1]/ Absent [2].
	41- Two long broad and acute apical cell: Present [1]/ Absent [2].
	42- Multicellular with long acute apical cell: Present [1]/ Absent [2].
	43- Short basal cell and long curved apical cell: Present [1]/ Absent [2].
	44- Short basal cell and two long acute apical cells: Present [1]/ Absent [2].
	45- Two short basal cells and long acute apical cell: Present [1]/ Absent [2].

Table 3: Data matrix of morphological and anatomical characters listed in table 2.														
Species / Characters	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1	1	1	1	1	1	1	1	1	2	1	1	1	1	1
2	1	1	2	3	4	4	1	3	0	3	1	3	1	3
3	2	3	2	3	4	3	3	4	0	1	4	2	4	4
4	1	2	3	4	4	1	1	1	0	4	5	1	1	1
5	1	2	`1	2	3	1	4	1	0	2	1	1	1	1
6	1	1	1	2	2	1	2	2	0	1	1	1	1	2
7	1	1	1	1	1	1	1	1	0	2	1	1	1	1
8	3	1	4	2	4	4	4	2	0	4	2	2	3	3
9	1	1	1	1	1	1	1	1	2	2	1	1	1	1
10	1	1	1	1	3	1	2	2	0	0	2	1	1	4
11	1	1	2	1	1	1	1	1	0	0	1	1	1	1
12	1	1	1	2	1	2	2	2	0	0	1	2	1	1
13	1	1	3	1	1	1	1	1	0	0	2	1	1	3
14	1	2	1	2	2	2	2	2	0	0	2	2	2	2
15	2	4	2	4	2	3	4	2	4	4	2	4	1	4
16	1	2	2	2	2	2	2	2	0	0	2	2	2	2
17	1	2	2	2	2	2	2	2	2	2	1	2	2	2
18	1	1	1	1	1	1	1	1	2	2	1	1	1	1
19	1	2	1	1	1	1	1	2	2	2	1	1	1	1
20	1	1	1	1	1	1	1	2	0	1	1	1	1	1
21	1	1	1	2	3	2	3	3	0	2	1	2	1	1
22	1	1	2	2	2	1	2	2	0	2	2	1	2	2
23	2	2	2	2	2	1	2	2	2	2	2	2	2	2
24	1	1	1	2	1	1	3	1	1	2	1	1	1	2
25	2	2	2	2	1	2	2	2	0	1	2	2	2	2
26	1	2	2	2	2	1	1	2	2	2	2	2	2	2
27	1	1	1	1	1	2	1	2	0	2	1	2	1	1
28	2	2	2	2	2	2	2	2	2	1	2	2	2	2
29	1	1	1	1	1	1	1	1	2	1	1	1	1	1
30	1	1	2	2	1	1	1	1	2	2	1	1	2	1
31	2	1	1	1	1	1	2	1	2	2	1	1	1	2
32	2	2	2	2	2	2	2	2	2	2	2	2	1	2
33	2	1	2	2	2	2	2	2	2	2	2	2	2	2
34	2	2	1	1	1	1	2	1	2	2	1	2	2	2
35	2	2	2	2	2	2	2	2	2	2	1	2	2	2
36	2	2	2	2	2	2	2	2	2	1	2	2	2	2
37	2	2	2	2	2	2	2	2	2	2	2	2	1	2
38	2	1	1	1	2	2	2	1	2	2	2	2	2	2
39	1	2	2	1	2	1	2	2	2	2	2	1	2	1
40	2	1	2	2	2	2	2	2	2	2	2	2	2	2
41	2	1	2	2	2	2	2	2	2	2	2	1	2	2
42	2	2	2	2	2	2	2	1	2	1	2	1	1	2
43	2	2	2	1	2	1	2	2	2	2	2	1	2	2
44	2	2	2	1	2	2	2	1	2	2	2	2	1	2
45	2	2	2	1	2	2	2	2	2	2	2	2	2	2

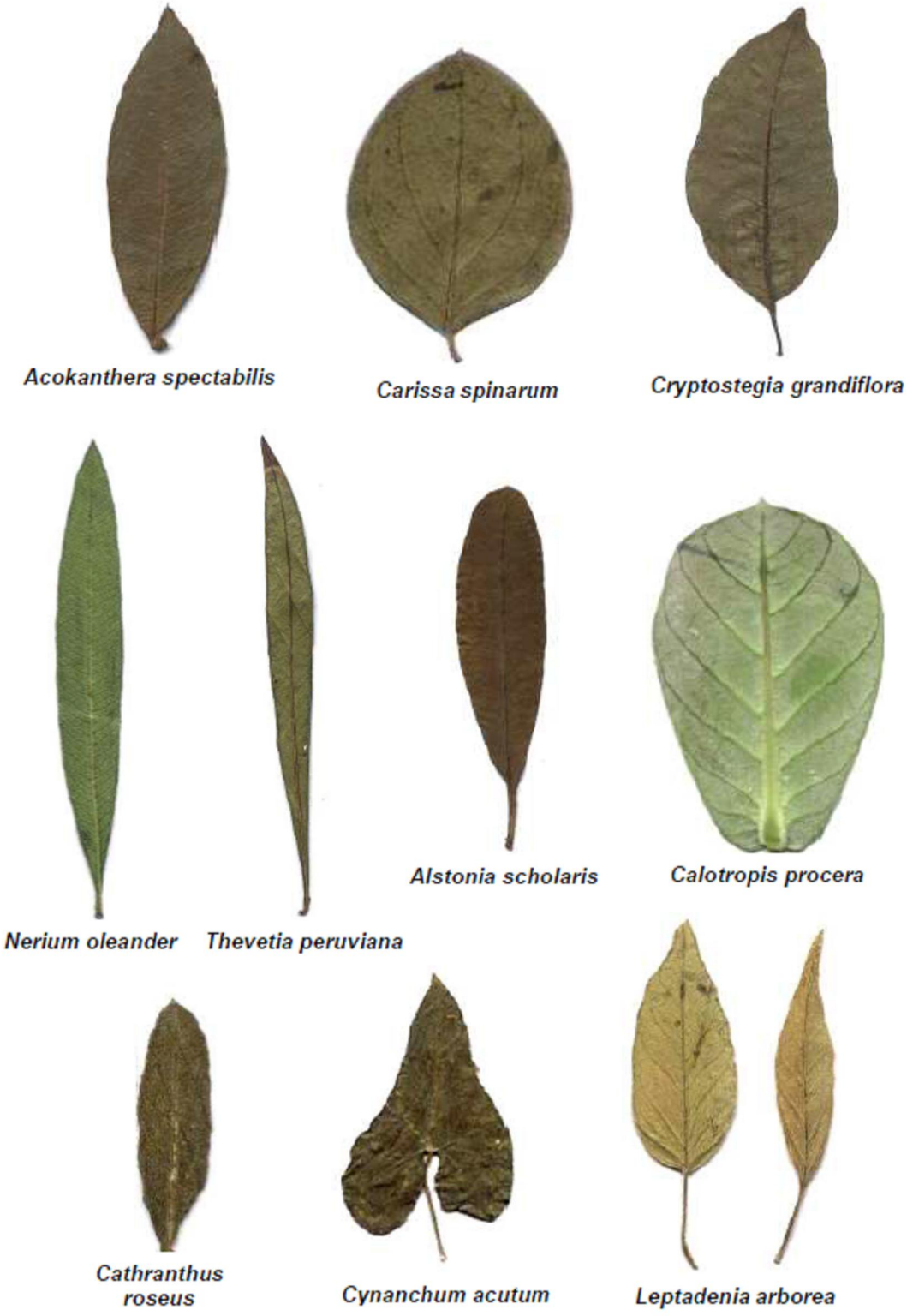

Plate 1: The main morphological types of leaves in studied species.

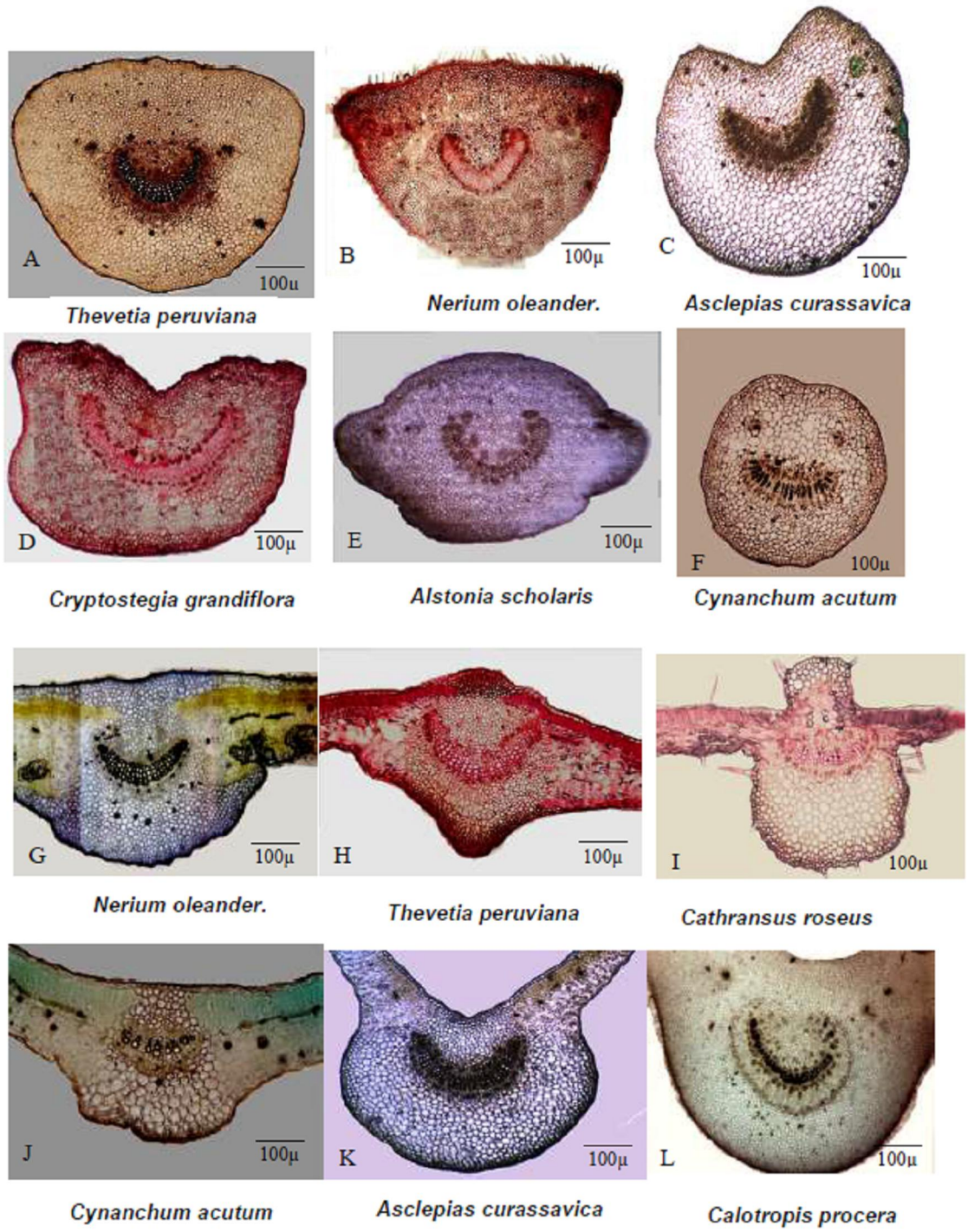

Plate 2: Micromorphological characters of the studied species of Apocynaceae and Asclepiadaceae

Fig. A-F: Petiole cross section.
Fig. G-L: Blade cross section.

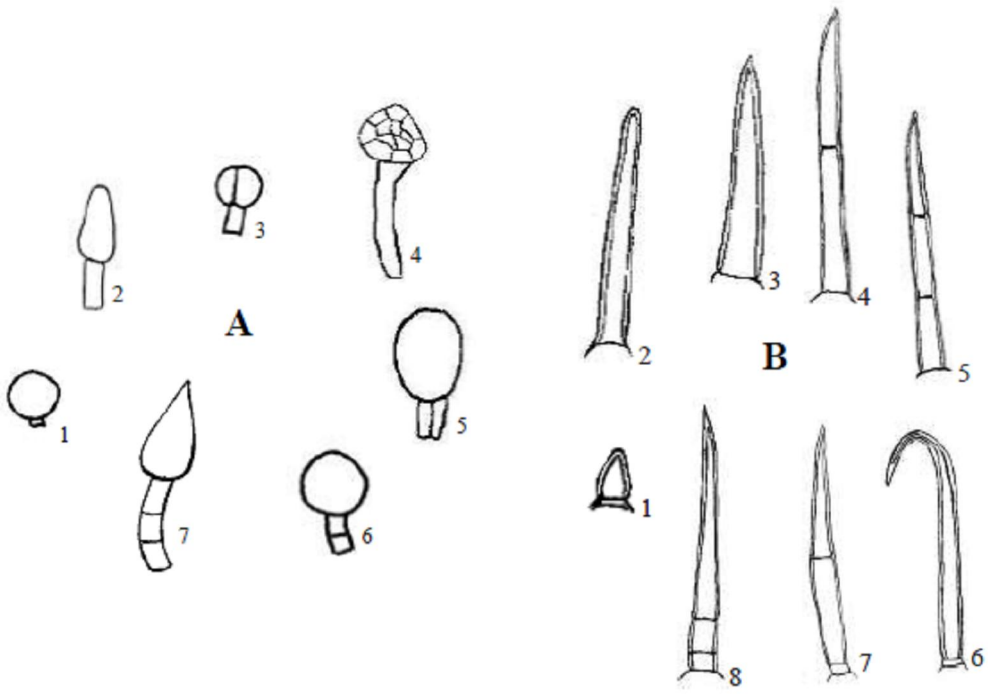

Plate 4: Foliar trichome types within the studied species
A- Glandular trichomes B- Non-glandular trichomes

A- Glandular

1- Glandular with unicellular stalk and unicellular head (*e.g. Thevetia peruviana*).
2- Glandular with unicellular stalk and unicellular cylindrical head (*e.g. Asclepias curassavica*).
3- Glandular with unicellular stalk and bicellular head (*e.g. Carissa spinarum*).
4- Glandular with long unicellular stalk and multicellular head (*e.g.Cathransus roseus*).
5- Glandular with bicellular –biseriate stalk and unicellular head (*e.g. Cynanchum acutum*).
6- Glandular with bicellular –uniseriate stalk and unicellular head (*e.g. Calotropis procera*).
7- Glandular with multicellular stalk and unicellular head acute apex (*e.g. Asclepias curassavica*).

B-Non-glandular

1- Non glandular, unicellular papillose (*e.g. Leptadenia arborea*).
2- Non glandular, simple with long broad, blunt & apical cell (*e.g. Acokanthera spectabilis*).
3- Non glandular, simple with long, acute & apical cell (*e.g. Carissa spinarum*).
4- Non glandular with two long, acute apical cell (*e.g. Solenostemma argel*).
5- Non glandular with multicellular long broad and acute apical cell (*e.g. Calotropis procera*).
6- Non glandular with short basal cell and long curved apical cell (*e.g.Nerium oleander*).
7- Non glandular with short basal cell and two long acute apical cells (*e.g. Leptadenia arborea*).
8- Non glandular with two short basal cell and long acute apical cell (*e.g. Cathransus roseus*).

Conclusion

From the all characters of morphology, anatomy and numerical analysis of the leaves for the studied species, this study support consider the two family as large family: Apocynaceae *s.l.* and this agree with **(Demeter, 1922; Safwat, 1962; Stebbins, 1974; Thorne, 1992; Judd *et al.*, 1994; Struwe *et al.*, 1994; Sennblad and Bremer 1996 and Endress and Bruyns 2000)**

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