

Leaf Morphology and Venation Patterns of *Euphorbia* L. (Euphorbiaceae) in Egypt with Special Notes on Their Taxonomic Implications

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Abstract

Euphorbia L. (Euphorbiaceae) is the largest genus of flowering plants in the flora of Egypt. The present paper deals with the study of leaf architecture including venation patterns, marginal configuration and leaf shape characters in the *Euphorbia* species in Egypt. A classical clustering analysis (UPGMA) and principle component analysis (PCA) by PAST 2.17c software are conducted based on 27 architectural leaf characters to discriminate the investigated taxa. Plates of light microscope for cleared leaf, marginal ultimate veins details as well as tooth shape for studied taxa were provided. Results from multivariate analysis are kept in line with the traditional taxonomic sections of the genus in Egypt. The obtained phenogram is slightly matched with the tradition and modern classification of genus *Euphorbia*. The arrangement and attachment of leaves, laminar size, apex and base leaf features, symmetry of base and medial of blade, primary vein framework, major secondary veins course, minor secondary veins, tertiary veins course and areolation development have been considered to be the most important distinguishable characters in *Euphorbia*. Leaf morphology and venation characters can be considered as good taxonomic indicators in segregating *Euphorbia heterophylla* in a distinct section (*Poinsettia*) within subgenus *Chamaesyce*, in addition they can discriminate the closely related species of *Euphorbia* as shown in the constructed key.

Keywords: Areolation, *Euphorbia*, Idioblasts, PCA, Venation, Architecture, UPGMA.

1. Introduction

Euphorbiaceae *sensu lato* is one of the six largest plant families after Orchidaceae, Asteraceae, Fabaceae, Rubiaceae and Poaceae (Christenhusz, and Byng, 2016). It includes around 8000–9000 species in 340 genera, and strongly represented in the tropical regions of the world (Radcliffe-Smith, 1980; Govaerts *et al.*, 2002; Secco *et al.*, 2012). According to Webster (1994), Euphorbiaceae *s.l.* comprises 52 tribes and 5 subfamilies: Phyllanthoideae, Oldfieldioideae, Acalyphoideae, Crotonoideae and Euphorbioideae. Recently, the Angiosperm Phylogeny Group (APG, 2016) recognized five lineages of Euphorbiaceae *s.l.* at family level: Phyllanthaceae, Putranjivaceae, Pandaceae, Picrodendraceae and Euphorbiaceae *sensu stricto*.

Euphorbia L. is one of the largest genera of angiosperms and the largest genus of Euphorbiaceae; it has a cosmopolitan distribution with about 2150 species (Govaerts *et al.*, 2000; Bruyns, 2006). Despite its great vegetative diversity, the genus is morphologically characterized by having a cyathiate inflorescence and a highly reduced inflorescence that resembles a single flower (Steinmann and Porter, 2002). Based on

geographical distribution, habit, leaves and stipules characters, branching of inflorescence, number and morphology of involucre glands and seed characters, the genus has been divided into four subgenera: *Esula* Pers., *Athymalus* Neck., *Chamaesyce* Raf., and *Euphorbia*.

Regionally, *Euphorbia* is considered as the largest genus in the flora of Egypt, represented by 41 species, distributed in all phyto-geographical regions of the country with different habits and habitats (Boulos, 2000).

El-Hadidi (1973), critically revised sect. *Anisophyllum* (Haw.) Roesler, while Fayed (1973) made a taxonomic revision of 20 species represented in different sections namely: *Anisophyllum*, *Lyciopsis* Boiss., *Poinsettia* (Graham) Boiss., *Pseudoacalypha* Boiss., *Tirucalli* Boiss., and *Tithymalus* Boiss. Accordingly, Fayed (1973) indicated the importance of some morphological characters, such as habit, leaves, cyathia, capsules, and seed features in distinguishing the Egyptian taxa of *Euphorbia*.

According to Laraño and Buot (2010), the leaf architecture and other vegetative characters are often ignored by some taxonomist in identification and classification of plant taxa due to their belief that these characters have high grade of phenotypic plasticity; however, it can be pointed out that leaf characters, particularly venation patterns are, in general, genetically

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fixed and can be used as a taxonomic tool. Moreover, foliar micromorphology and architecture can be used as a valuable aid to taxonomy in various groups (e.g. Abd El-Ghani *et al.*, 2007; Laraño and Buot Jr, 2010; Salvaña and Buot Jr, 2013; Thepsithar and Thongpukdee, 2013)

The plant leaves are commonly used in taxonomic analyses, particularly in fitting with morphometric analysis (Viscosi and Cardini, 2011). Leaf characters may stand as appropriate taxonomic characters mainly in plant fossils in which the flowering organs are degenerated or absent (Hickey, 1973; Dilcher, 1974; Hickey and Taylor, 1991). Many authors discriminated and identified different taxa based only on morphological characters of leaves (Levin, 1986 a, b; Todzia and Keating, 1991; Hershkovitz, 1992; Christophel *et al.*, 1996; Roth-Nebelsick *et al.*, 2001; Wang *et al.* 2001; Luo and Zhou, 2002; Fuller and Hickey, 2005; Loutfy *et al.*, 2005; Martínez-Millán and Cevallos-Ferriz, 2005; Cervantes *et al.*, 2009; Pacheco-Trejo *et al.*, 2009).

Recently, Sarala and Vijay (2014) studied the foliar micromorphology and architecture of 44 species belonging

Table 1: Tribal and sectional classification as well as source of taxa under investigation. Herbarium acronym is following Thiers (2017).

Subgenus	Section	Taxa	Collection	
<i>Euphorbia</i> subgenus <i>Chamaesyce</i> Raf.	<i>Anisophyllum</i> (Haw.) Roeper	<i>E. hirta</i> L.	Nile valley, Assiut university ground, 5-5-2018, Mona Hassan (ASTU)	
		<i>E. indica</i> Lam.	Nile valley, Assiut university ground, 5-5-2018, Mona Hassan (ASTU)	
		<i>E. peplis</i> L.	Mediterranean region, Alexandria: Baltim, 31-7-1990, Fayed & El- Garf (ASTU)	
		<i>E. lasiocarpa</i> Klotzsch	Nile valley, Cairo: Maadi garden, 27-9-2011, Fayed (ASTU)	
		<i>E. hyssopifolia</i> L.	Nile valley, Giza, no date, Abdel Salam Galaly (ASTU)	
		<i>E. forsskaolii</i> J. Gay	Nile valley, Assiut El Jadida, 9-4-2018, Mona Hassan (ASTU)	
		<i>E. scordifolia</i> Jacq.	Gebel Elba, Haliab Triangle Area: Wadi Umm Shleem, 13-1-2005, Kadry Abdel Khalik (ASTU)	
		<i>E. granulata</i> Forssk. var. <i>granulata</i>	Gebel Elba, Haliab Triangle Area: Wadi Umm Sheeb, 13-5-2013, Kadry Abdel Khalik (ASTU)	
		<i>E. serpens</i> Kunth	Nile valley, Assiut university ground, 5-5-2018, Mona Hassan (ASTU)	
		<i>E. prostrata</i> Ait.	Nile valley, Assiut university ground, 5-5-2018, Mona Hassan (ASTU)	
<i>Poinsettia</i> (Graham) Boiss.	<i>E. heterophylla</i> L.	Nile valley, Assiut university ground, 5-5-2018, Mona Hassan (ASTU)		
<i>Euphorbia</i> subgenus <i>Athymalus</i> Neck.	<i>Lyciopsis</i> Boiss.	<i>E. cuneata</i> Vahl	Gebel Elba, Haliab Triangle Area: Sambeek Embeek, no date, Usama Abdel Rady (ASTU)	
<i>Euphorbia</i> subgenus <i>Esula</i> Pers.	<i>Pachycladae</i> (Boiss.) Tutin	<i>E. dendroides</i> L.	Gebel Elba, Sollum plateau, 15-4-2016, Faried and Banhaway (ASTU)	
		<i>E. terracina</i> L.	Mediterranean region, Alexandria-Burg-El-Arab, 6-4-2015, Faried <i>et al.</i> (ASTU)	
	<i>Chylogala</i> (Fourr.) Prokh.	<i>E. retusa</i> Forssk.	Mediterranean region, wadi Hagol, 13-4-2010, Zareh and Aboul-Ela (ASTU)	
		<i>Helioscopia</i> Dumort.	<i>E. arguta</i> Banks & Sol.	Assiut University ground, 1-1961, Badari (ASTU)
	<i>E. helioscopia</i> L.		Nile valley, Assiut university ground, 12-1-2018, Mona Hassan (ASTU)	
	<i>Exiguae</i> (Geltman) Riina & Molero	<i>Tithymalus</i> Boiss.	<i>E. dracunculoides</i> Lam.	Gebel Elba, Haliab Triangle Area: Wadi Maarafawy, 4-2-2005, Kadry Abdel Khalik (ASTU)
			<i>E. peplis</i> L.	Nile valley, Assiut university ground, 17-4-2018, Mona Hassan (ASTU)
<i>E. chamaepeplis</i> Boiss.			Gebel Elba, Gebel Serbal region, wadi Rem, 23-4-2004, Fayed <i>et al.</i> (ASTU)	
<i>Pithyusa</i> (Raf.) Lázaro		<i>E. obovata</i> Decne	Southern Sinai: Wadi Gebal region, wadi Gebal, 13-5-2004, Fayed <i>et al.</i> (ASTU)	

2.2. Leaf clearing

For leaf venation study, method of Yu and Chen (1986) was followed with some modifications. Leaves were boiled in water for 10-20 minutes, then placed in 1-5%

to 20 genera in Euphorbiaceae, and showed that these characters can be used for differentiating taxa. Kakkar and Paliwal (1972) made detailed studies on the leaf anatomy of the genus *Euphorbia* with regard to tracheoid idioblasts and vein endings. Sehgal and Paliwal (1974) studied the leaf venation patterns of 150 species of *Euphorbia* and they divided the genus into three major groups (uni-, bi- and tri-veined).

The present investigation was conducted to evaluate the importance of leaf morphological characters as well as patterns of venation in studying the diversity and patterns of variation of 21 taxa of *Euphorbia* in Egypt.

2. Materials and Methods

2.1. Sampling

The present study was based mainly on specimens preserved in ASTU Herbarium (Table 1) as well as fresh materials of the most species that collected from their appropriate localities.

NaOH, the strength depending on the thickness of the material. NaOH solution was changed every 1-2 days during the clearing process, which generally took 2-10 days. Cleared leaves were then rinsed in running water thoroughly, dried, stained in 1% safranin, and mounted on

slides with Glycerin. Leaves were examined and photographed by Olympus SZ61 stereomicroscope provided with a digital Olympus camera SC100. Characters and characters states of leaf morphology were described based on terminologies of Melville (1976), Hickey (1973), Pole (1991), Ash *et al.* (1999) and Ellis *et al.* (2009).

2.3. Numerical analysis

For the numerical analysis, PAST version 2.17 c program of Hammer *et al.* (2001) was used. Hamming/P-distance clustering algorithms test was used to assess the degree of similarity inside data matrix by un-weighted pair-group method with arithmetic means (UPGMA) to generate the current phenograms (Figure 1).

3. Results

Samples of 21 species of *Euphorbia* were undergone for analysis. The morphological characters and character states were evaluated and recorded (Table 2). Data were analyzed by the software PAST version 2.17c (Hammer *et al.*, 2001) using the data matrix organized for 21 OUTS x 57 binary and multistate traits (Table 3). Within the current study, the phenogram (Figure 1) was performed by UPGMA; it achieved the highest co-phenetic correlation coefficient (0.8745) which showed a good fit between the phenogram and the distance matrix. PCA was performed to check the uniformity of the grouping achieved with cluster analysis by using combined data in which the cumulative variance for PC1 and PC2 increased 52% of the variation for the two first principal components and accounted 100% for the first 20 principal components (Table 4). The arrangement and attachment of leaves, laminar size, symmetry of leaves base, medial symmetry of blade and primary vein framework, major secondary veins course have been considered to be important distinguishable characters in *Euphorbia* at cluster level. The numerical analysis separated the 21 taxa into three major clusters (Figure 1).

The constructed phenogram showed that *E. heterophylla* in section *Poinsettia* was separated from the rest of the examined taxa and form the most basal cluster (C1) at the similarity level of 0.7; the second cluster (C2) includes ten taxa which was separated into three main groups (A, B and C) at similarity level 0.39: the basal group (A) comprises *E. hirta*, *E. lasiocarpa*, *E. hyssopifolia* and *E. indica*, the second group (B) included *E. peplis* and *E. scordifolia*, *E. forsskaolii*, *E. prostrata*, the last group (C) included two species, *E. serpens* and *E.*

granulata var. *granulata*. Group A was further divided into two subgroups at the similarity level 0.29, *E. hirta* in the first subgroup, *E. indica*, *E. lasiocarpa* and *E. hyssopifolia* were included in second subgroup. Group B was classified into two subgroups at the same last similarity level includes *E. prostrata* in third subgroup and *E. forsskaolii*, *E. peplis* and *E. scordifolia* in fourth subgroup. *Euphorbia peplis* was separated in a single clade in the same last subgroup (Figure 1). The third cluster (C3) includes ten species: *E. helioscopia*, *E. arguta*, *E. terracina*, *E. retusa*, *E. cuneata*, *E. dracunculoides*, *E. dendroides*, *E. peplus*, *E. chamaepeplus* and *E. obovata*. These species were separated in two major groups (D and E). Group D included four species segregated into two sub-groups at similarity level of 0.3; the basal sub-groups (subgroup 5) included three species: *E. terracina*, *E. helioscopia* and *E. arguta*, *E. terracina* was separated. The second subgroup (subgroup 6) was represented by *E. retusa*. Group E included six species which can be separated in four sub-groups at similarity level of 0.22. The most basal subgroups (subgroup 7) consists of only *E. dendroides*, the second one (subgroup 8) consists of *E. dracunculoides*, the third subgroup (subgroup 9) consists of *E. peplus*, *E. chamaepeplus*, and the last one (subgroup 10) consists of *E. obovata* and *E. cuneata*.

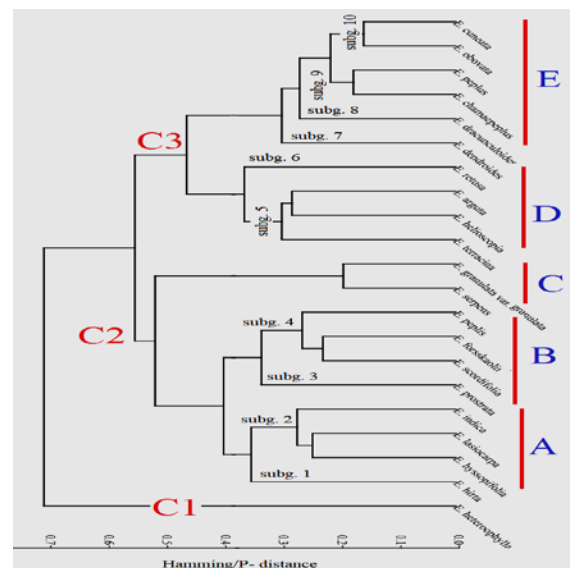


Figure 1. UPGMA dendrogram of the 21 taxa of *Euphorbia* using Hamming/ P- distance measure (0.8684) based on 57 architectural characters, the letters refer to divided groups, (C1, C2, C3) main clusters; (A-E) different groups.

Table 2. Characters and character states and their codes; NA= Non Applicable.

Code	Morphological characters	Characters state	Code	Morphological characters	Characters state
1	Leaf attachment	Petiolate (1); Sessile (2); Subsessile (3)	6	Laminar ratio	Less than 1.5 cm (1); 2–3 cm (2); 4–7 cm (3); > 8 cm (4)
2	Leaf arrangement	Alternate (1); Opposite (2); Sub-opposite (3)	7	Laminar size	Leptophyll(1); Nanophyll (2); Microphyll (3); Notophyll (4)
3	Leaf organization	Simple (1); Compound (2)	8	Laminar shape	Elliptic (1); Obovate (2); Ovate (3); Oblong (1); Linear (5); Oblong-lanceolate (6); Oblong-ovate (7); Linear-lanceolate (8); Spathulate (9); Oblong-obovate (10); Oblong-elliptic (11)
4	Petiole features	Terete (1); NA (2)	9	Medial symmetry	Symmetry (1); Asymmetry (2)
5	Position of lamina attachment	Marginal (1); NA (2)	10	Base symmetry	Symmetry (1); Asymmetry with basal width (2); Asymmetry with basal extension (3); Asymmetry with basal insertion (4)

Code	Morphological characters	Characters state	Code	Morphological characters	Characters state
11	Lobation	Unlobed (1); Lobed (2)	34	Intersecondary frequency	Less than one per intercostal area (1); usually one per intercostal area (2); more than one per intercostal area (3); NA (4)
12	Margin type	Untoothed (1); Toothed (2)	35	Intercostal tertiary vein	Percurrent (1); Reticulate (2)
13	Type of teeth	Dentate (1); Serrate (2); Serrate-crenate (3); NA (4)	36	Course of percurrent tertiary	Mixed (opposite-alternate) (1); NA (2)
14	Special margin features	Involute (1); Papillate (2); Erode (3); NA (4)	39	Epimedial tertiary	Alternate percurrent (1); Reticulate (2); Ramified (3); Mixed (4)
15	Apex angle	Acute (1); Obtuse (2); Reflex (3)	40	Proximal course of epimedial tertiary	Parallel to percurrent (1); NA (2)
16	Apex shape	Straight (1); Convex-rounded (2); Acuminate (3); Emarginate (4); Convex-truncate (5); Convex rounded or convex truncate (6)	41	Distal course of epimedial tertiaries course	Parallel to percurrent (1); NA (2)
17	Base angle	Acute (1); Obtuse (2); Reflex (3); Oblique-reflex (4)	42	Exterior tertiaries course	Absent (1); Looped (2); Terminating at margin (3)
18	Base shape	Cuneate (1); Convex rounded (2); Convex truncate (3); Convex with basal extension (4); Decurrent (5); Cordate (6); Concave-convex (7)	43	Quaternary vein fabric	Percurrent (1); Irregular reticulate (2); Absent (3)
19	Terminal apex features	Mucronate (1); Retuse (2); NA (3)	44	Quinternary vein fabric	Irregular reticulate (1); NA (2)
20	Surface texture	Glabrous (1); Pubescent on both surface (2); sparse pubescent on one side (3)	45	Areolation	Poor development (1); Moderate development (2); Good development (3)
21	Surficial glands	Marginal (1); NA (2)	46	Freely Ending Veinlets (FEVs)	Absent (1); Mostly unbranched (2); mostly one branch (3); Dichotomous branching (4); Dendritic (5)
22	Primary vein framework	Pinnate (1); Palmate basal acrodromous (2); Palmate basal acrodromous (3)	47	FEVs terminal	Simple (1); Tracheoid idioblasts (2)
23	Number of basal veins	One (1); 3-4 (2); 5-6 (3)	48	Marginal ultimate venation	Absent (1); Incomplete loops (2)
24	Major secondary vein framework	Semicraspedodromous (1); Fестоoned semicraspedodromous (2); Cladodromous (3); brochidodromous (4); Fестоoned brochidodromous (5)	49	Tooth spacing	Regular (1); Irregular (2); NA (3)
25	Minor secondary	Craspedodromous (1); Semicraspedodromous (2); Brochidodromous (3); NA (4)	50	Order of teeth	One (1); Two (2); NA (3)
26	Perimarginal veins	Intramarginal secondary (1); Fimbrial vein (2); Absent (3)	51	Number of teeth per 0.5 cm	4-7 (1); 8-12 (2); NA (3)
27	Major secondary spacing	Regular (1); Irregular (2); Gradually increasing proximally (3); Abruptly increasing proximally (4)	52	Sinus shapes	Angular (1); Rounded (2); NA (3)
28	Variation of major secondary angle to midvein	Uniform (1); Inconsistent (2); Smoothly decreasing proximally (3)	53	Tooth shapes	CC/ ST – CC/ CC (1); ST/ RT (2); ST/ St (3); RT/ RT- RT/ CC (4); CC/ CC- CC/ FL (5); RT /ST (6); CC/ RT (7); CV/ CC (8); ST/ CC (9); CC/ CC (10); NA (11)
29	Major secondary attachment to midvein	Decurrent (1); Basally Decurrent (2); Deflected (3); Excurent (4)	54	Principle vein	Present (1); Absent (2)
30	Intersecondary veins	Present (1); Absent (2)	55	Principle vein terminating	Submarginal (1); At apex of tooth (2); On proximal flank (3); On distal flank (4); At nadir of superjacent sinus (5); NA (6)
31	Intersecondary proximal course	Parallel to major secondary (1); Perpendicular (2); NA (3)	56	Course of accessory veins	Looped (1); Straight to concave (2); NA (3)
32	Intersecondary length	Less than 50% (1); More than 50% (2); NA (3)	57	Special features of tooth apex	Simple (1); Glandular (2); Cassidate (3); NA (4)
33	Intersecondary distal course	Reticulate or ramifying (1); Parallel (2); Perpendicular (3); Basiflexed (4); NA (5)			

Table 3: Matrix obtained from scoring (21 OTUs x 57 characters)

Table with 57 columns (Characters 1-57) and 21 rows (OTUs: E. hirta, E. indica, E. lasiocarpa, E. hyssopifolia, E. pepilis, E. granulata var. granulata, E. caryoc, E. forsskaolii, E. scordifolia, E. prostrata, E. heterophylla, E. cinerea, E. androidea, E. rufica, E. arguta, E. heliopsis, E. thymoides, E. pepilis, E. rhomboides, E. glabra, E. terracina). Each cell contains a numerical score from 1 to 7.

Table 4. Eigenvalue, percentage of variance and cumulative percentage of variance of the first 20 principal components

Table with 4 columns: PC, Eigenvalue, % variance, % Cumulative variance. Rows 1-20 showing principal component data.

4. Key to the taxa

Leaves notophyll, intercostal tertiary veins fabric mixed percurrent.....E.heterophylla
Leaves microphyll, nanophyll or leptophyll, intercostal tertiary veins fabric reticulate or absent2
2- Leaf base asymmetrical; primary vein palmately basal actinodromous3
Leaf base symmetrical; primary vein pinnate or palmately basal acrodromous.....12
3- Leaves margin toothed.....4
- Leaves margin entire.....11
4- Leaves leptophyll E. prostrata

Leaves nanophyll or microphyll5
Major secondary veins and minor secondary veins semicraspedodromous6
Major secondary veins cladodromous; minor secondary veins craspedodromous or absent9
6- Leaves microphyll, pubescent; major secondary veins attachment excurrent to the midvein, tooth apex glandular E. hirta
- Leaves nanophyll, sparsely hairy or entirely glabrous, major secondary veins attachment decurrent to the midvein, tooth apex eglandular 7
7- Leaves oblong, base convex with oblique reflex extension; major secondary veins abruptly increasing proximally, uniformly angledE. indica
Leaves oblong-lanceolate, base cordate with reflex width; major secondary veins irregular, inconsistently angled.....8
8- Leaves sparsely hairy; tooth spacing regular, tooth number up to 7 per 5 mm, sinus shapes angular, principle vein terminating at nadir of superjacent sinus, tooth apex simple E. lasiocarpa
Leaves entirely glabrous; tooth spacing irregular, tooth number less than 5 per 5 mm, sinus shapes rounded, principle vein terminating at submarginal, tooth apex cassidate E. hyssopifolia
9- Leaves entirely glabrous, apex reflex-emarginated, asymmetrical base insertion, margin dentate E. pepilis
- Leaves pubescent, apex convex; asymmetrical base extension; margin serrate 10
10- Leaves sparsely pubescent on one surface, apex rounded-truncate; craspedodromous minor secondary veins; perimarginal secondary veins present; secondary veins angle uniform, attachment to midvein basely decurrent; tooth spacing irregular E. forsskaolii
- Leaves pubescent on both surfaces, apex convex rounded; minor secondary veins absent; perimarginal

secondary veins absent; secondary veins angle inconsistent, attachment to midvein deflected; tooth spacing regular, **E. scordifolia**

Table 5: Correlation between the morphological characters and the first two principal components PC1, PC2; ⁽¹⁾ indicates traits with high scores in PC1, while ⁽²⁾ indicates traits with high scores in PC2

Code	Morphological characters	PC 1	PC 2
1	Leaf attachment	0.022	-0.038
2	Leaf arrangement	-0.068	0.037
3	Leaf organization	0.000	0.000
4	Petiole features	0.051	-0.049
5	Position of lamina attachment	0.000	0.000
6	Laminar ratio	0.028	-0.179
7	Laminar size	-0.006	-0.176
8	Laminar shape ²	0.150	0.558
9	Medial symmetry ²	-0.071	0.107
10	Base symmetry ²	-0.071	0.107
11	lobation	0.000	0.000
12	Margin type	-0.088	-0.05
13	Type of tooth	0.102	0.046
14	Special margin features	-0.007	0.052
15	Apex angle	-0.008	0.071
16	Apex shape ²	-0.002	0.268
17	Base angle ²	-0.139	0.295
18	Base shape ¹	-0.141	0.079
19	Terminal apex features	-0.136	-0.072
20	surface texture	-0.074	0.012
21	Surficial glands	-0.000	0.036
22	Primary vein framework	-0.052	0.086
23	Number of basal veins	-0.058	0.076
24	Major secondary framework	0.085	-0.088
25	Minor secondary course ¹	0.176	-0.047
26	Perimarginal veins ²	0.055	-0.169
27	Major secondary spacing	-0.027	-0.080
28	Variation of major secondary angle	0.033	0.084
29	Major secondary attachment to midvein	0.018	0.027
30	Inter secondary	0.017	0.055
31	Intersecondary proximal course	0.004	0.121
32	Intersecondary length	0.029	0.066
33	Intersecondary distal course ²	0.006	0.216
34	Intersecondary frequency	0.026	0.112
35	Intercostal tertiary vein fabric	-0.001	0.035
36	Course of Percurrent tertiary	-0.001	0.035
37	Angle of percurrent tertiary	-0.001	0.035
38	Intercostal tertiary vein angle variability	-0.001	0.035
39	Epimedial tertiaries ²	-0.068	0.182
40	Proximal course of epimedial tertiaries	-0.001	0.035
41	Distal course of epimedial tertiaries	-0.001	0.035
42	Exterior tertiary course ¹	-0.103	-0.022
43	Quaternary vein fabric	-0.026	0.127
44	Quinternary vein fabric	-0.031	0.059
45	Areolation	-0.044	-0.038
46	Freely Ending Veinlets branching	0.056	0.003
47	FEVs terminal	-0.025	0.047
48	Marginal ultimate venation	0.053	-0.058
49	Tooth spacing	0.126	0.104
50	Order of teeth	0.126	0.104
51	Number of teeth per 0.5 cm ¹	0.153	0.091
52	Sinus shape	0.108	0.112
53	Tooth shapes ^{1,2}	0.735	-0.233
54	Principle vein	0.014	0.049
55	Principle vein terminating ^{1,2}	0.384	0.265
56	Course of accessory veins	0.002	0.063
57	Specific tissue on teeth apex ^{1,2}	0.208	0.157

11- Leaves glabrous; apex reflex-emarginated, base cordate, reflexed angle; major secondary veins angle variation uniform; decurrent attachment to midvein; epimedial third veins ramified; areolation poor **E. serpens**

- Leaves pubescent; apex obtuse; base convex with basal extension, oblique reflexed angle; major secondary veins angle variation inconsistent, basally decurrent attachment to midvein; epimedial third veins mixed; areolation moderate...**E. granulata var. granulata**

12- Leaves margin toothed **13**

- Leaves margin entire..... **16**

13- Leaves linear, margin involute; major secondary veins semicraspedodromous; inter secondary veins absent..... **E. retusa**

- Leaves oblong-lanceolate or obovate, margin features papillate or absent; major secondary veins festooned semicraspedodromous or simple brochidodromous; inter secondary veins present **14**

14- Leaves apex mucronate; major secondary veins simple brochidodromous, angle variation smoothly decreasing proximally; marginal ultimate venation incomplete loops.....

E. terracina

Leaves apex obtuse or acute; major secondary veins festooned craspedodromous, angle variation uniform; marginal ultimate venation looped **15**

15- Leaves obovate, glabrous, apex convex rounded, margin features not papillate; major secondary veins spacing abruptly increasing proximally; tooth spacing regular, sinus shape angular
.....

... **E. helioscopia**

Leaves oblong-lanceolate, pubescent, apex acute, margin features papillate; major secondary veins spacing irregular; tooth spacing irregular, sinus shape rounded **E. arguta**

16-Leaves linear-lanceolate..... **17**

- Leaves ovate or obovate **18**

17- Leaves microphyll, margin entire; major secondary veins simple brochidodromous, spacing irregular; freely ending veins termination simple; marginal ultimate veins incomplete looped.....**E. dendroides**

Leaves nanophyll, margin erose; major secondary veins semicraspedodromous, spacing smoothly increasing proximally; freely ending veins termination freely tracheoid idioblasts; marginal ultimate veins absent **E. dracunculoides**

18- Leaves petiolate; major secondary veins angle variation uniform **19**

Leaves sessile; major secondary veins angle variation inconsistent or smoothly decreasing proximally **20**

19- Leaves obovate, apex not mucronate, base decurrent; major secondary veins festooned brochidodromous with decurrent attachment to the midvein; quaternary veins present; freely ending veins termination simple **E. peplus**

Leaves ovate, apex mucronate, base obtuse; major secondary veins semicraspedodromous with deflected attachment to the midvein; quaternary vein absent, freely

ending veins termination tracheoid idioblasts **E. chamaepeplus**

20- Leaves obovate; primary vein palmately basal acrodromous; major secondary angle variation inconsistent, spacing irregular, deflected attachment to the midvein.....**E. obovata**

- Leaves spatulate; primary vein pinnate; major secondary angle variation smoothly decreasing proximally, spacing regular, decurrent attachment to the midvein**E. cuneata**

5. Discussion

This study emphasizes the significance of leaf characters for recognizing most of the taxa under investigation. Leaf shapes and venation patterns are considered as essential traits that generally play a significant role in discriminate *Euphorbia* members at either subgeneric or sectional levels. The PCA is providing the numerical values correlative to the morphological traits used in this taxonomic analysis. PCA is considered as a standard multivariate statistical method that aims to make analysis to obtained numerical data (Mardia *et al.*, 1979). In this study, PCA results are slightly consistent with the latest taxonomic studies using the leaf morphology as the most important factor for classification of angiosperms.

Our primary phenetic data cannot yet reflect the true evolutionary history and the phylogeny among *Euphorbia* species in Egypt; however our findings are somewhat consistent with the previous traditional sectional classification of Pax and Hoffmann (1931) and recently with the molecular circumscriptions of the some studies such as Yang *et al.* (2012), Peirson *et al.* (2013), Riina *et al.* (2013). Leaf shapes, venation patterns and tooth characters were considered as essential traits that generally play a significant role in delimiting *Euphorbia* members both subgeneric and sectional

levels and can be considered as good taxonomic indicators in segregating closely related species in *Euphorbia*. In this study, only three subgenera covering all members of *Euphorbia* are selecting (Table 1). The first one is *E.* subgen. *Chamaesyce* which includes 11 taxa, the second is *E.* subgen. *Esula* with nine taxa, while the last one is *E.* subgen. *Athymalus* which is representing here by only one taxon (Table 1).

Euphorbia subgen. *Chamaesyce* is represented in our study by two sections, namely: *Anisophyllum* and *Poinsettia*. Section *Poinsettia* is represented by only one species, *E. heterophylla*. Morphologically, this species is unique in having some synapomorphic characters such as opposite-alternate leaves, glandular stipules, peltate glands which are often reduced to one gland, and seed caruncle reduced or minute (Boulos, 2000). Our results showed that *E. heterophylla* differs from all investigated taxa mainly by having notophyll leaves, mixed percurrent intercostal tertiary veins, alternate percurrent epidermal tertiary and percurrent quaternary veins (Figures 3A and 5A). Based on these characters, it was solitary placed in cluster C1 (Figure 1). All taxa of section *Anisophyllum* are characterized by a number of unique characters, such as opposite leaves, asymmetrical base, stipulate, clustered cyathium, axillary or terminal cythial, glands often with membranous appendages and ecarunculate seeds (Radcliff-Smith, 1980). Hooker (1885) stated that “sect.

Anisophyllum is forever multiplied”; in addition, El-Ghazaly & Chaudhary (1993) showed that this section is heterogamous in response to the shape of aperture and sexine pattern of its pollen grains. Based on the current results, the architectural characters of species belonging to sect. *Anisophyllum* are rather variable. Furthermore, the phylogenetic results obtained by Yang *et al.* (2012) using the ITS and chloroplast *ndhF* sequence proved that *Anisophyllum* is monophyletic group. In our study, the represented taxa of this section share numerous characters (e.g. opposite leaves, asymmetrical base, asymmetrical medial and palmately basal actiondromous veins), (Figure 2). In this study, ten species of sect. *Anisophyllum* are grouped together in cluster C2 (Table 1 and Figure 1), which is divided into three groups: A, B, and C. Within the species of sect. *Anisophyllum*, leaf characters and venation patterns provide a significant value to distinguish the studied taxa. *Euphorbia hirta*, *E. lasiocarpa*, *E. hyssopifolia*, and *E. indica* were placed together in group A (Figure 1), and share some macro-morphologically characters, such as: erect to ascending habit and cyathia clustered into capitates inflorescences (Boulos, 2000; Zohary, 1972).

Boulos (2000) and Zohary (1972) recognized *E. hirta* (sect. *Anisophyllum*) by its densely pubescent leaves and leaves length reaching to 4–4.5 cm in length. Nevertheless, leaf architectural characters of *E. hirta* (e.g. microphyll leaves with densely serrate margin, glandular tooth) were considered as good diagnostic characters and can be used to circumscribe the distinct Subgroup 1 (Figures 1, 2A and 4A).

On the other hand, *E. lasiocarpa*, *E. hyssopifolia* and *E. indica* (sect. *Anisophyllum*) share some morphological characters such as oblong to oblong-lanceolate leaves, loose clustered cyathia terminated at lateral or axillary shoots (Boulos, 2000). The current results show that these taxa are clustered together in the distinct Subgroup 2 (Figure 1) by having a similar architectural characters such as nanophyll leaves; major and minor secondary veins are semicraspedodromous, fimbrial perimarginal veins terminating at margin, major secondary veins decurrently attached to midvein and exterior tertiary terminating at leaf margin (Figures 2B, 2C, 2D, 4B, 4C and 4D). Radcliffe-Smith (1980) remarked that there is morphological ambiguity among *E. hyssopifolia* and *E. indica*; however, based on its narrow leaves as well as black seeds, El-Hadidi (1973) considered that *E. hyssopifolia* is a distinct species and differs from *E. indica*. El-Hadidi's (1973) aspect has been approved by the current results that *E. hyssopifolia* exhibits glabrous lanceolate leaves, with asymmetrical basal width, acute apex, cordate base with reflex angle, angles of secondary veins are inconsistent, proximal course of intersecondary veins is parallel, reticulate intersecondary veins course, quaternary veins fabric absent and freely ending veins (FEVs) with one branch (Figures 2D and 4D). Moreover, *E. indica* have hairy oblong leaves, with asymmetrical basal extension, obtuse apex, cordate base with oblique reflex angle, secondary veins angles uniform, intersecondary veins proximal course perpendicular, intersecondary veins course basiflexed, quaternary veins fabric presented and freely ending veins with dichotomous branching (Figures 2B and 4B). According to Boulos (2000), *E. lasiocarpa* seems to be more closely related to *E. indica* than other

taxa; they share some morphological characters such as sparsely hairy leaves with obtuse apex; however, the present results offer distinct architectural characters between the both species with a low similarity index (25%); *Euphorbia lasiocarpa* can be recognized by presence of sparse hairs towards the margin of leaf base (while being spread out on whole leaf in *E. indica*); in addition, *E. lasiocarpa* is characterized by cordate leaf base with reflex angle, inconsistent secondary veins angles are, regular tooth spacing, angular sinus shape and the principle vein is terminating at the nadir of superjacent sinus (Figures 2C and 4C).

Morphologically, *E. forsskaolii*, *E. scordifolia*, *E. prostrata* and *E. peplis* have small leaves (c. 1.5 x 0.5 cm) and a solitary cyathium (El-Hadidi, 1973; Fayed, 1973; Boulos, 2000). The dendrogram (Figure 1) reveals the location of the four species together in Cluster C2, group B by sharing architectural leaf features such as: oblique reflex base with convex basal extension, irregular spacing of secondary veins, mostly unbranched FEVs, round sinus, simple submarginal principle veins in the tooth apex.

The dendrogram (Figure 1) shows that group B is subdivided into two subgroups: subgroup 3 and subgroup 4. Based on floral and seeds characters documented by Fayed (1973), Boulos (2000) and Fayed and Hassan (2007), *E. prostrata* is easily distinguished from the related taxa by having minute appendage glands and transversely wrinkled seeds. Our results revealed that *E. prostrata* can be separated from all involved taxa by having elliptic leptophyll leaves with unequal basal width, minor semicraspedodromous secondary veins and the fimbrial perimarginal veins (Figures 2J and 4J). These differentiated characters confirm the placement of *E. prostrata* in a separate subgroup (Subgroup 3) away from *E. forsskaolii*, *E. peplis* and *E. scordifolia* (Figure 1).

Within subgroup 4, *E. forsskaolii*, *E. peplis* and *E. scordifolia* share architectural characters, such as nanophyll leaves and cladodromous secondary veins course. *E. peplis* is morphologically different from other taxa within this subgroup by having glabrous leaves, and seed length over 1.5 mm with 4-angles in transverse section (El-Hadidi, 1973; Fayed, 1973; Fayed and Hassan, 2007); these characters are in agreement with architectural results showing in figures 2E and 4E, in which *E. peplis* have entirely glabrous leaves, reflex-emarginated apex, asymmetrical insertion base, dentate margin, and fimbrial perimarginal veins.

Euphorbia scordifolia and *E. forsskaolii* are distinguishable on macro-morphological characters (Fayed, 1973) and seed characters (Fayed and Hassan, 2007); in addition, our results show a similarity value reaching to 21% due to the discriminating characters between those two taxa, in which the major secondaries veins are deflected in attachment to midvein, exterior tertiary veins terminating at margin, areolation is well developed and tooth spacing is regular in *E. scordifolia* (Figures 2I and 4I), while *E. forsskaolii* is characterized by decurrent major secondaries veins, absence of exterior tertiary veins, tooth spacing being irregular and areolation is poorly developed (Figures 2H and 4H).

The group C in cluster C2 is represented by two taxa, *E. serpens* and *E. granulata* var. *granulata*, they share the leptophyll leaves with untoothed margin, absence of inter

secondary veins (Figures 2F, 4F, 2G and 4G), but can easily be discriminated in morphology.

The second subgenus involved in this study is *Athymalus* which is representing by one section, *Lyciopsis*, with only one species *Euphorbia cuneata*. The dendrogram (Figure 1) shows that, *E. cuneata* placed together with *E. obovata* (subgenus *Esula*, section *Pithyusa*) with a similarity value reaching to 18%. According to Fayed and Hassan (2007), *E. cuneata* shares smooth seeds as character with some members of subgenus *Esula*. Our results cannot be used to place *E. cuneata* in a separate cluster; it will be useful to discriminate this species from all other involved specie.

The third subgenus involved in present study is *Euphorbia* subgen. *Esula*. It is represented in this study by nine species within six sections (Table 1). Most members of *E. subgen. Esula* are characterized by exstipulate leaves, absence of petaloid appendages, dichasial cyathia, and carunculate seeds (Zohary, 1972; Fayed, 1973; Boulos, 2000). They distribute mainly in temperate region particularly in the Mediterranean regions. Figure 1 shows the placement of all taxa belonging to *E. subgen. Esula* in a separate cluster (C3). Only one species, *Euphorbia retusa*, of the first section, *Chylogala*, was sampled in this study. According to Riina *et al.* (2013) and Boulos (2000), *E. retusa* can be easily separated from related taxa by having caruncle (about half as long as the seeds). Based on our results, *E. retusa* is placed solitarily as subgroup 6 (Figure 1), with involute margin and without intersecondary veins (Figures 3D and 5D).

Two species: *Euphorbia helioscopia* and *E. arguta*, of (the second section, *Helioscopia*) were sampled in this study. They were grouped together with *E. terracina* (section *Pachycladae*) in the same subgroup 5 (Figure 1) with a similarity index 31%. However, *E. helioscopia* and *E. arguta* are closer with each other than to *E. terracina* because they share absence of terminal mucronate apex and festooned semicraspedodromous secondary veins (Figures 3E, 5E, 3F and 5F).

According to Riina *et al.* (2013), *E. terracina* is placed in *Pachycladae* (the third sampled section) with *E. dendroides* by sharing some seed characters and their geographical distribution. Our result is not agreement with this view, whereas *E. terracina* and *E. dendroides* were placed in subgroups 5 and 7 respectively (Figure 1). In this study, the analysis of leaf architectural characters confirms the placements of *E. terracina* and *E. dendroides* in different sections as reported by Pax and Hoffmann (1931). *Euphorbia terracina* differs from *E. dendroides* mainly in having oblong-lanceolate leaves, toothed margin, major secondary spacing abruptly increasing proximally (linear lanceolate, untoothed margin, irregular spacing in *E. dendroides*), Figures (3C, 5C, 3K and 5K).

The fourth sampled section within subgenus *Esula* is *Exiguae*, which is represented here by *E. dracunculoides*. Morphological characters of leaves can be helpful to distinguish *E. dracunculoides* from all other involved taxa, it is unique in having linear-lanceolate leaves with mucronate apex, semicraspedodromous major secondary veins, well developed areolation as well as dendritic freely ending veins (Figures 3G, 5G). The placement of *E. dracunculoides* in subgroup 8 (Figure 1) is in agreement with the morphological and molecular results of Riina *et*

al. (2013) in which, *E. dracunculoides* was located in a separate clade within section *Exiguae*.

According to Boulos (2000) and Riina *et al.* (2013), *E. peplus* and *E. chamaepeplus* are included in section *Tithymalus* (the fifth sampled section). They are closely related by having some morphological characters. Figure 1 shows the clustering of both species together within subgroup 9 based on leaf morphology and venation patterns. Although, *E. peplus* and *E. chamaepeplus* shared characters such as petiolate leaves, uniform secondary veins variation, irregular spacing, poorly developed areolation, unbranched freely ending veins, *E. peplus* can be easily distinguished from *E. chamaepeplus* by some leaf characters, *E. peplus* is characterized by obovate leaves, apex features absence festooned brochidodromous

major secondary veins with decurrent attachment to midvein, irregular reticulate quaternary veins and simple freely ending veins (Figures 3H and 5H), while *E. chamaepeplus* can easily be distinguished by ovate leaves, mucronate apex, semicraspedodromous major secondary veins with deflected attachment to midvein, quaternary veins absent, tracheoid idioblasts freely ending veins (Figures 3I and 5I).

Finally, *E. obovata* is included in *Pithyusa* (the sixth sampled section) based on characters of capsule and seeds (Riina *et al.*, 2013). However, our results placed *E. obovata* together with *E. cuneata* (subgenus *Athymalus*, section *Lyciopsis*) in subgroup 10 (Figure 1). *E. obovata* is the only species with palmate and basal acrodromous primary veins (Figure 3 J).

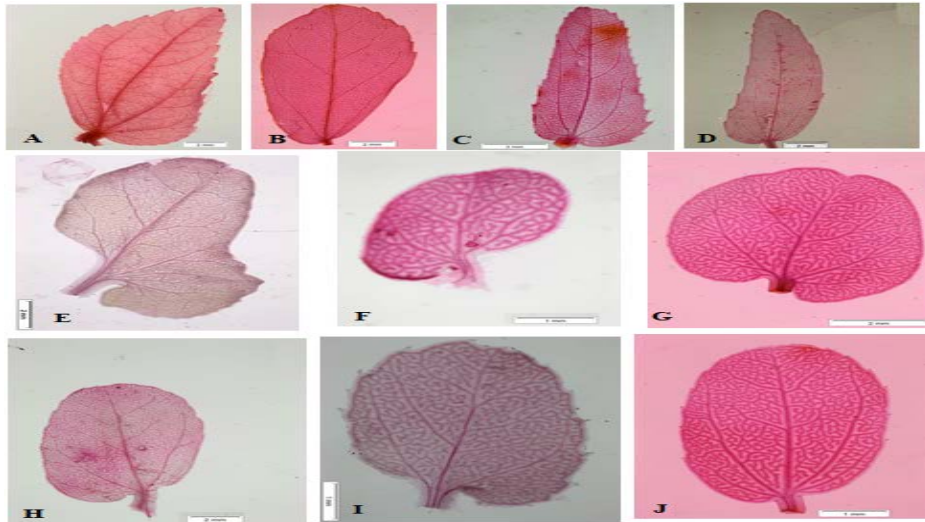


Figure 2: Light microscope micrographs of leaf blade of Euphorbia species, A. *E. hirta*, B. *E. indica*, C. *E. lasiocarpa*, D. *E. hyssopifolia*, E. *E. peplis*, F. *E. granulata* var. *granulata*, G. *E. serpens*, H. *E. forsskaolii*, I. *E. scordifolia*, J. *E. prostrata*.

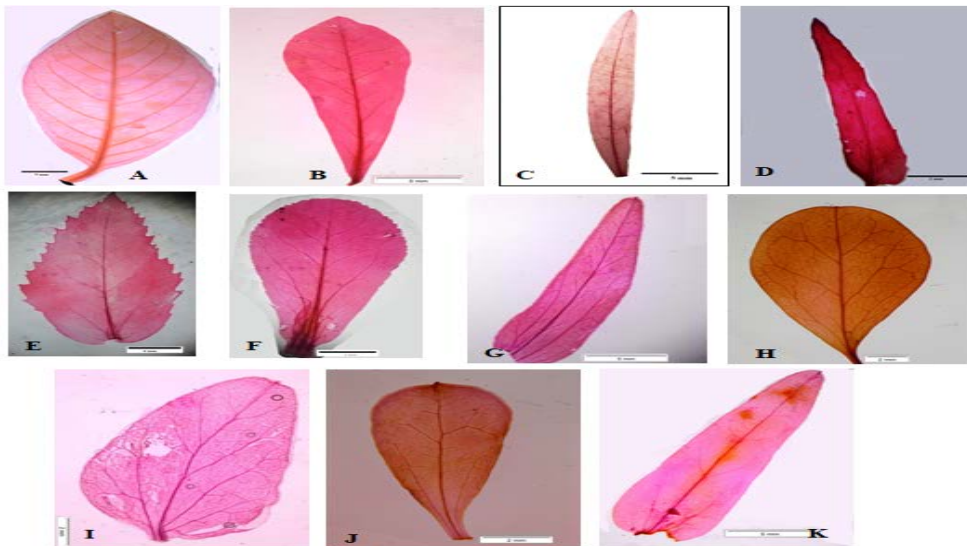


Figure 3: Light microscope micrographs of leaf blade of Euphorbia species, A. *E. heterophylla*, B. *E. cuneata*, C. *E. dendroides*, D. *E. retusa*, E. *E. arguta*, F. *E. helioscopia*, G. *E. dracunculoides*, H. *E. peplis*, I. *E. chamaepeplus*, J. *E. obovata*, K. *E. terracina*.

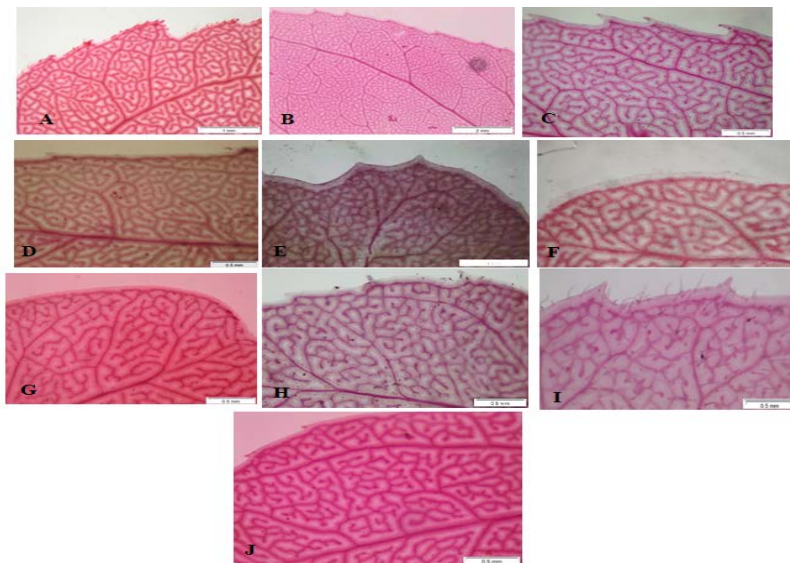


Figure 4: Light microscope micrographs of middle and marginal regions of leaves of Euphorbia species, A. *E. hirta*, B. *E. indica*, C. *E. lasiocarpa*, D. *E. hyssopifolia*, E. *E. peplis*, F. *E. granulata* var. *granulata*, G. *E. serpens*, H. *E. forsskaolii*, I. *E. scordifolia*, J. *E. prostrata*.

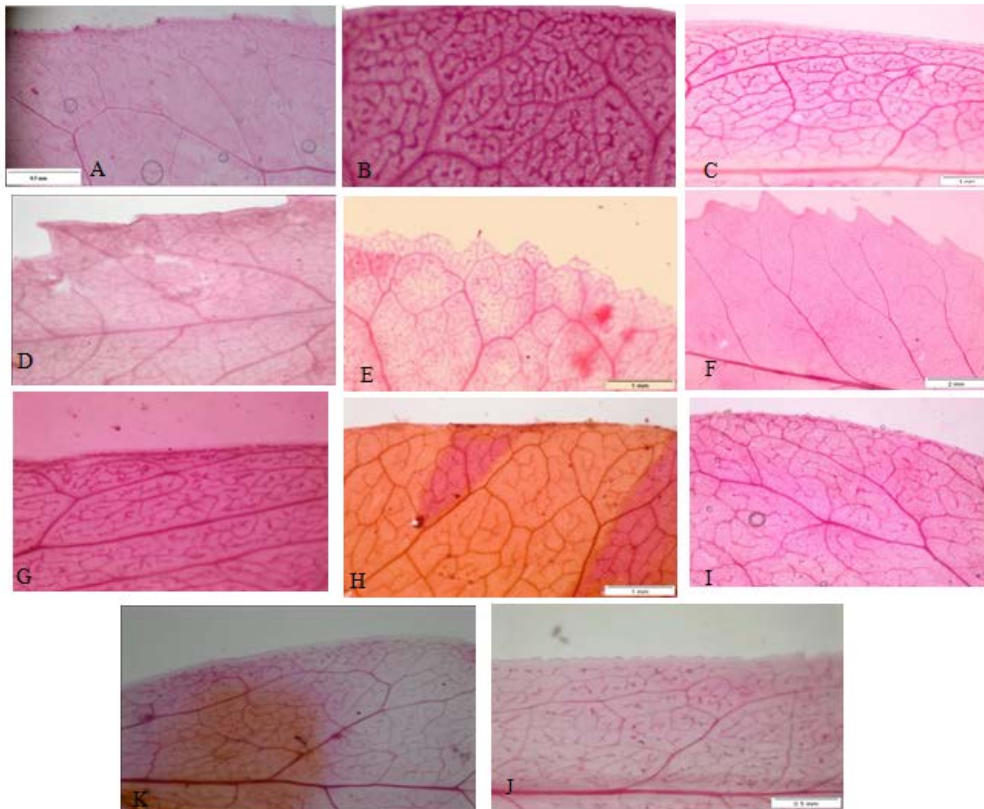


Figure 5: Light microscope micrographs of middle and marginal regions of leaves of *Euphorbia* species, **A.** *E. heterophylla*, **B.** *E. cuneata*, **C.** *E. dendroides*, **D.** *E. retusa*, **E.** *E. helioscopia*, **F.** *E. arguta*, **G.** *E. dracunculoides*, **H.** *E. peplus*, **I.** *E. chamaepeplus*, **J.** *E. obovata*, **K.** *E. terracina*.

6. Conclusion

Since the time of Linnaeus, the identification and reconstruction of relationships between different plants have been based greatly on features of the reproductive organs. Although characters of seed, fruit and flower have proved very useful in plant taxonomy, there are situations in which these organs are not available for study. So, the current study was conducted to assess the importance of leaf morphological characters as well as venation patterns in identification and studying the diversity of 21 taxa of *Euphorbia* in Egypt. Our results are, to some degree, in line with the traditional classification sections of Pax and Hoffmann (1931), especially in placement of *Euphorbia terracina* and *Euphorbia dendroides* in different sections; in addition, our results agree with recently phylogenetic classification in placement of *Euphorbia heterophylla* in distinct section (*Poinsettia*). The arrangement and attachment of leaves, symmetry of base and median part blade, the primary vein and intersecondary veins are considered the most important characters to distinguished taxa at subgeneric and sectional levels, while laminar size, apex and base features of leaf, secondary veins characters, minor secondaries veins, tertiary veins, areolation, tracheoid idioblasts and tooth characters were considered as distinguished characters at species level. Results indicated that leaf architecture features, particularly venation patterns, are genetically fixed and can be used as a good taxonomic tool either in identification or classification of *Euphorbia* species in Egypt.

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