

Comparative morphological and chemical studies on some verbenaceous plants in Egypt

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ABSTRACT

The current research attempts to survey the implications of interweaving macro and micro-morphological characters, and chemical constituents of six taxa belonging to family Verbenaceae namely; *Duranta erecta*, *Gmelina arborea*, *Lantana montevidensis*, *Tectona grandis*, *Verbena hybrid* and *Vitex trifolia* grown in different areas in Egypt, and to come up with valuable taxonomic differentiation among these taxa. Leave shape, apex, base, and venation were included in the macro-morphological investigation, while leaf epidermal cells, stomata, and trichomes were included in the micro-morphological study. The characteristics of the studied taxa were: herbs as *Verbena hybrid*, shrubs as *Duranta erecta*, *Lantana montevidensis* and *Vitex trifolia* and trees as *Gmelina arborea* and *Tectona grandis*. Stem solid, erect in all taxa except in *Verbena hybrid* it was prostrate. Leaves were simple in all taxa except the leaves of *Vitex trifolia*, it was compound. Hairs were unicellular or multicellular, branched or unbranched and glandular or non-glandular. The stomatal types in the studied taxa were diacytic, anisocytic and anomocytic. The main chemical constituents were alkaloids, flavonoids, phenols, tannins, phytosterols and terpenes were found in all taxa except *Vitex trifolia*.

Keywords: Morphological, Hairs, Verbenaceae, phytochemical screening.

INTRODUCTION

Verbenaceae belongs to the class of Dicotyledons. It is a comparatively large family composed of about 35 genera and about 1000 species it is native to tropical and subtropical regions They also extend into temperate lands Gilbert *et al.*, (1994). The economic Verbenaceae species include timber trees such as *Tectona grandis* which is used as a medicine being accessible and inexpensive for combating diseases since times immemorial it also fits the immediate personal need of man (Hickey and King, (1988). They were herbs, shrubs, trees or woody climbers, stems hairy or prickly. Leaves are petiole or sessile commonly opposite occasionally verticillate rare alternate, simple digitately or pinnately divided, margin toothed or entire (Boulos, 2002; Kadereit, 2004; Simpson, 2008 and Cardoso *et al.*, 2021). The stomata patterns were anomocytic, diacytic and paracytic. Trichomes were non-glandular uniseriate filiform and uniseriate glandular capitate, multicellular glandular capitate (Shubhangi, 2013). The most common stomata types were diacytic as found in *Gmelina arborea* and occasionally anomocytic in *Duranta erecta* (Ajuziogu *et al.*, 2018).

Under the phytochemical screening, the plants of genus *Lantana* showed the presence of chemical compounds as phenolic,

flavonoids, alkaloids, tannins, saponins, phytosterols and carbohydrates, while glycosides were absent (Kalita *et al.*, 2011). The phytochemical screening on the plants of genus *Lippia* represented that they have triterpenoids, phenols, flavonoids, phenylpropanoids, glycosides and steroids (Ombito *et al.*, 2014).

Tectona grandis, *Duranta repens*, *Gmelina arborea* and *Verbena hybrid* plants and using Aluminium chloride reagent were Total flavonoid compounds (42.80, 67.20, 27.40 and 57.60 µg) respectively Ali *et al.* (2017).

The current research attempts to investigate the implications of interweaving macro and micro-morphological characteristics and chemical constituents of six species belonging to six genera of family Verbenaceae namely; *Duranta erecta*, *Gmelina arborea*, *Lantana montevidensis*, *Tectona grandis*, *Verbena hybrid* and *Vitex trifolia* grown in different areas in Egypt, and to come up with valuable taxonomic differentiation of these taxa.

MATERIALS AND METHODS

Morphological studies:

Sample of plants:

This research was executed in the Faculty of Agriculture, Department of Agricultural Botany, Al-Azhar University, Nasr city, Cairo,

Egypt in three successive years 2018 to 2020. Six species belonging to 6 genera of Verbenaceae family were collected from three different sites: Al-Azhar University (Az), El-Orman Garden (Or), and flower exhibition at Orman garden (Fl).

Identification

Identification of the collected taxa were achieved by comparing their morphological characters with the characters of the previously identified taxa published by Bailey (1951).

Epidermal features

Epidermal peels of mature foliage leaves and stems were cleared in warm lactic acid and examined microscopically to study the structure of mature stomata patterns, trichomes types and cell surface ornamentation. All photographs were prepared by Nikon Camera on a Carl Zeiss Jena microscope photographs. Multi Variate Statistical Package Program (MVSP) was used to analyze the macro and micro-morphological data according to Sneath and Sokal (1973).

Phytochemical screening

The aerial parts of the plants of six studied taxa were tested phytochemically for the presence of alkaloids, steroids, tannins, saponins and glycosides. The qualitative results are expressed as (+) for presence and (-) for absence.

Techniques used for phytochemically Tests:

For Alkaloids: The alkaloids were tested by Dragendorff's reagent (Potassium bismuth iodide solution) according to Joshi *et al.*, (2013).

For Steroids and Terpenoids: they were tested by Salkowski and Liebermann-Burchard tests according to Ayoola *et al.*, (2008) and Joshi *et al.*, (2013).

For Tannins and Saponins: they were tested according to Banso and Adeyemo, (2006).

For Glycosides: they were tested by Anthraquinone glycoside (Borntrager's test) according to Joshi *et al.*, (2013).

For Phenolic content: it was analyzed using the Folin-Ciocalteu colorimetric method (Chlopicka *et al.*, 2012) with some modifications.

For Flavonoid content (TFC): it was determined using the aluminum colorimetric

method (Stankovic, 2011) with some modifications using quercetin as the standard.

RESULTS AND DISCUSSION

Morphological characters:

Macro morphological characters:

Habitat: All examined species are perennial except *Verbena hybrid* plants, it is annual and herbs (Fig.1-a), shrubs as in *Vitex trifolia*, *Duranta erecta* and *Lantana montevidensis* (Fig.1-b) or trees as in *Gmelina arborea* and *Tectona grandis* (Fig.1- c) (Table 3). These results were in harmony with those obtained by Hickey and King (1988) and Puri (2018).

Stems: Stems of the studied taxa were quadrangular and woody except in *Verbena hybrid*, it was herbaceous (Fig.1- a), erect except in *Verbena hybrid* that was prostrate (Fig.1-a) and glabrous as in *Duranta erecta*, hairy as in *Tectona grandis* or hairy and spiny as in *Lantana montevidensis* (Table 3). The spiny vegetative buds were only in *Duranta erecta* (Fig.1-d). Such results are strengthened by the findings of Boulos (2002), Kadereit (2004), Kumar (2009), Iroka *et al.*, (2015) and Rahman (2016). They observed that the stems were erect, herbaceous or woody hairy or prickly terete or quadrangular.

Leaves: The studied Verbenaceous leaves were simple in taxa except in *Vitex trifolia*, it was compound (Fig.2- e), unlobed and petiolate except in *Verbena hybrid* that was lobed and sessile (Fig.1- a), Upper and lower leaf surfaces were glabrous except in *Lantana montevidensis* and *Gmelina arborea* that were hairy (Table 3). The leaves were pinnately veined in all species except *Vitex trifolia* that was palmately veined (Fig. 2-e). The lower leaf of leaves have four colors; green in most taxa as in *Lantana montevidensis* (Fig. 2-g), grey in *Gmelina arborea* (Fig. 2-f), yellowish in *Duranta erecta* (Fig.1-d) and purple in *Vitex trifolia* (Fig.1-e), heterophyllous showed in *Vitex trifolia* only (Fig. 2-e). Leaf shapes were ovate in *Gmelina arborea* (Fig. 2-f), pinnatifid in *Verbena hybrid* (Fig.1-a) and trifoliate in *Vitex trifolia* (Fig. 2-e). Leaf apex was often acute in *Lantana montevidensis* (Fig. 2-g) and acuminate in *Gmelina arborea* (Fig. 2-f). Leaf margin was complete in *Gmelina arborea* (Fig. 2-f) and serrate in *Lantana montevidensis* (Fig. 2-g). Watson and Dallwitz (1992), Liang and Gilbert (1994), Kadereit (2004) and Tan *et al.*, (2018) reported that the Verbenaceous leaves were simple or 3-foliolate, palmately or pinnately compound, petiolate or sessile, margins entire, dentate, crenate, lobed or incised.

Micro morphological characters:

Epidermal cell wall: Epidermal cell walls on upper and lower epidermal layers were straight in all species except in *Duranta erecta*; it was sinuous walls on the same layers (Fig. 3-b). The results were compatible with Bangar *et al.*, (2011), Adedeji (2012), Shubhangi (2013) and Abhijeet *et al.*, (2017).

Hairs: Several forms of hairs were observed on the surfaces of stem and leaf of the examined plants as follows:

Unicellular nonglandular unbranched hairs in *Duranta erecta* (Fig.4-a),

multicellular nonglandular unbranched hairs in *Vitex trifolia* (Fig.4-b),

sessile gland multicellular hairs in *Gmelina arborea* (Fig.4-c),

unicellular glandular unbranched hairs in *Vitex trifolia* (Fig.4-d) and

multicellular glandular unbranched hairs as in *Gmelina arborea* (Fig.4-e).

Stomata: Many types of stomata were noticed on upper and lower epidermis of leaves of the studied species as follows:

Diacytic in *Vitex trifolia* (Fig.4-a),

Anisocytic in *Verbena hybrid* (Fig.4-b),

anomocytic in *Lantana montevidensis* (Fig.4-c),

tetracytic in *Duranta erecta* (Fig.4-d),

actinocytic in *Tectona grandis* (Fig.4-e) and parallel associated and abnormal stomata on the lower epidermis leaves of *Duranta erecta* (Fig. 4-f) and (Fig.4-g).

These results were in harmony with the finding of Khan *et al.*, (2007), Passos *et al.*, (2009), Bangar *et al.*, (2011), Priya (2015) and Abhijeet *et al.*, (2017) who stated that the trichomes of family Verbenaceae were unicellular or multicellular; branched or unbranched and eglandular or glandular. The stomatal types were diacytic, anisocytic and anomocytic stomata.

From the illustrated previous dendrogram, the investigated species could be divided in to two clusters. The first contains two taxa *Vitex trifolia* and *Verbena hybrid* linked together at similarity (20). The second included two sub clusters; the first sub cluster contains one species *Lantana montevidensis*. The second sub cluster includes four taxa; *Tectona grandis*, and *Gmelina arborea* linked at similarity level 8, while *Duranta erecta* was linked with them at

similarity level 12, and the fourth species *Lantana camara* was linked with them at similarity level 18.

Chemical features

The phytochemical screening on the six species was performed and the results were inserted in Table (4). The results exhibited that the six species have flavonoids, phenols, tannins, phytosterols, and terpenes as the main constituents. On the other side, the alkaloids were found in all the species except *Vitex trifolia*. Also, the saponins were detected in the four species, *Duranta erecta*, *Gmelina arborea*, *Lantana montevidensis* and *Vitex trifolia* and were completely absent in the extracts of the other two species, *Tectona grandis* and *Verbena hybrid*, while the glycosides were found to be absent from the extracts of all the species except *Verbena hybrid*.

The total phenol (TC) and total flavonoids (TF) were assays as equivalent to gallic acid and quercetin, respectively. All the results were summarized in Table (5). it was deduced that all the species are very rich with phenolic contents with the following order: *Duranta erecta* > *Lantana montevidensis* > *Vitex trifolia* > *Verbena hybrid* > *Tectona grandis* > *Gmelina arborea*. Also, the data confirmed that the six species are very rich with flavonoid contents the same sequence of total phenol.

Based upon the above data, the six species exhibited strong correlation via their significant contents of flavonoids tannins, phytosterols, terpenes and phenols. The three species; *Duranta erecta*, *Gmelina arborea*, *Lantana montevidensis*, *Tectona grandis* and *Verbena hybrid* exhibited more correlation via their alkaloid's contents. On the other side, *Duranta erecta*, *Gmelina arborea*, *Lantana montevidensis*, and *Vitex trifolia* showed correlation together by presence of the saponin contents.

Based on the similar characteristics among the investigated species (as illustrated in the previous Dendrogram), they could be divided in to two clusters. A- The 1st cluster contains one species *Vitex trifolia*. The 2nd includes two sub clusters; Sub cluster No.1 contains one species *Verbena hybrid* Sub cluster No.2 includes four taxa *Tectona grandis* at similarity linked with *Gmelina arborea*, *Duranta erecta* and *Lantana montevidensis*.

Dendrogram represented the similarity and dissimilarity according to the morphological and chemical features between 6 species belonging to six genera representing family Verbenaceae.

Based on the similar characteristics among the investigated species (as illustrated in the previous Dendrogram), they could be divided in to two clusters; A- Contains one species *Vitex trifolia* and B- includes two sub clusters; the 1st sub cluster contains one species *Verbena hybrid* and 2nd sub cluster includes four taxa *Tectona grandis* and *Gmelina arborea* linked at similarity level (6), while *Duranta erecta* and *Lantana montevidensis* linked at similarity level (8).

CONCLUSION

According to the morphological and chemical characteristics on six taxa of family, Verbenaceae must be reconsidered *Vitex trifolia* within this family.

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Table 1: Scientific names and collection sites of taxa of family Verbenaceae.

No.	Scientific names	Collection sites	Date of collection
1	<i>Duranta erecta</i> L.	Az	11-2018
2	<i>Gmelina arborea</i> Roxb.	Or	4- 2019
3	<i>Lantana montevidensis</i> (Spreng.) Briq.	Fl	6-2020
4	<i>Tectona grandis</i> L.F.	Or	4-2019
5	<i>Verbena hybrid</i> (Gronal.)	Fl	4-2019
6	<i>Vitex trifolia</i> L.	Az	11-2018

Table 2: Data matrix of observed characters for the examined species listed (28) qualitative and (3) multistate characters of species belonging to family Verbenaceae.

Species, Characters				1	2	3	4	5	6
<u>Qualitative characters: Morphological</u> :Macro morphological characters									
1	Habit	Perennial (+)	annual (-)	+	+	+	+	-	+
2	Stem	Woody (+)/	herbaceous (-)	+	+	+	+	-	+
3	Stem	Erect (+) /	weak (-)	+	+	-	+	-	+
4	" Prostrate	present (+) /	absent (-)	-	-	+	-	+	-
5	Leaf or leaflets	Simple (+) /	compound (-)	+	+	+	+	+	+
6	Leaf or leaflets	Lobed (+) /	unlobed (-)	-	-	-	-	+	-
7	Texture leaf upper	glabrous (+)	hairy (-)	+	+	-	+	+	+
8	Texture leaf lower	glabrous (+)	hairy (-)	+	-	-	-	-	-
9	Leaf petiole	Petiolate (+) /	sessile (-)	+	+	+	+	-	+
10	The reticulate venation,	pinnate (+)/	palmate (-)	+	+	+	+	+	-
11	Leaf color of lower surface	green (+)/	not so (-)	+	-	+	-	+	-
12	Leaf apex	acute (+)/	acuminate (-)	+	-	+	+	+	+
13	Leaf Margin	entire(+)/	serrate (-)	-	+	-	+	-	+
14	Heterophyllus	present (+) /	absent (-)	-	-	-	-	-	+
b- Micro morphological characters:									
15	Epidermal cell wall	Sinuous (+) /	straight (-)	-	+	+	+	+	+
16	Unicellular nonglandular unbranched hair	present (+) /	absent (-)	+	+	+	-	+	+
17	Multicellular nonglandular unbranched hair	present (+) /	absent (-)	+	+	-	+	+	+
18	Unicellular branched hair	present (+) /	absent (-)	+	+	+	-	+	+
19	Multicellular branched hair	present (+) /	absent (-)	+	-	+	-	+	+
20	Sessile gland multicellular hair	present (+) /	absent (-)	-	+	-	-	-	+
21	Unicellular glandular hair	present (+) /	absent (-)	-	-	-	+	-	-
22	Multicellular glandular hair	present (+) /	absent (-)	-	-	-	-	+	+

Stomata patterns									
23	Diacytic	present (+) /	absent (-)	+	+	+	-	-	+
24	Anisocytic	present (+) /	absent (-)	-	-	-	-	+	-
25	Anomocytic	present (+) /	absent (-)	-	-	+	-	-	-
26	Tetracytic	present (+) /	absent (-)	+	-	+	-	-	-
27	Actinocytic	present (+) /	absent (-)	+	+	-	+	-	-
28	Associated parallel stomata	present (+) /	absent (-)	+	-	-	-	-	-
B-Multistate characters: Morphological characters: Macro morphological characters									
Species, Characters				1	2	3	4	5	6
29 – Habit: herbs 1; shrubs 2 and trees 3.				2	3	2	3	1	2
Stem 30 – 30 –Stem Texture: smooth 1, hairy 2 and spiny 3 and spiny3.				1	1	2,3	2	1	1
31- Leaf or leaflets shapes: ovate1; obovate pinnately lobed 3 and trifoliolate 4.				1	1	2	1	3	4

Table 3: The results of the preliminary phytochemical screening of Verbenaceae.

phytochemical screening, Species	Alkaloids	Saponins	Phenols	Flavonoids	Glycosides	Tannins	Phytosterols	Terpens
1- <i>Duranta erecta</i> L.	+	+	+	+	-	+	+	+
2- <i>Gmelina arborea</i> Roxb.	+	+	+	+	-	+	+	+
3- <i>Lantana montevidensis</i> (Spreng.) Briq.	+	+	+	+	-	+	+	+
4- <i>Tectona grandis</i> L.F.	+	-	+	+	-	+	+	+
5- <i>Verbena hybrid</i> (Gronal.)	+	-	+	+	+	+	+	+
6- <i>Vitex trifolia</i> L.	-	+	+	+	-	+	+	+

Key: (+) = present and (-) = absent.

Table 4: Content of phenols and flavonoids in Methanolic extracts of Verbenaceous species.

Species	Total phenols (GAE $\mu\text{g}/\text{mg}$)	Total flavonoids (QuerE $\mu\text{g}/\text{mg}$)
1- <i>Duranta erecta</i> L.	698.50	119.39
2- <i>Gmelina arborea</i> Roxb.	255.91	36.03
3- <i>Lantana montevidensis</i> (Spreng.) Briq.	482.59	88.71
4- <i>Tectona grandis</i> L.F.	321.47	55.02
5- <i>Verbena hybrid</i> (Gronal)	354.81	62.86
6- <i>Vitex trifolia</i> L.	396.48	69.19

**Figure 1:** (a - d) Show types of stem: a -*Verbena hybrid*, b-*Vitex trifolia*, c - *Gmelina arborea* and d-*Duranta erecta*.

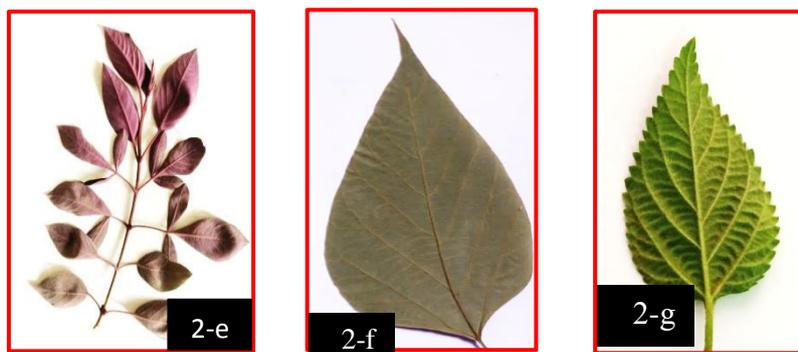


Figure 2: (e - g) Show type of leaves. e- *Vitex trifolia*, f- *Gmelina arborea* and g- *Lantana montevidensis*.

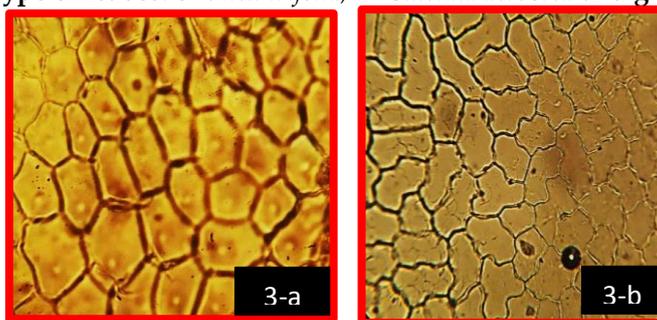


Figure 3: (a and b) Show shapes of epidermal cell walls (x 100). a- *Tectona grandis* and b- *Duranta erecta*.

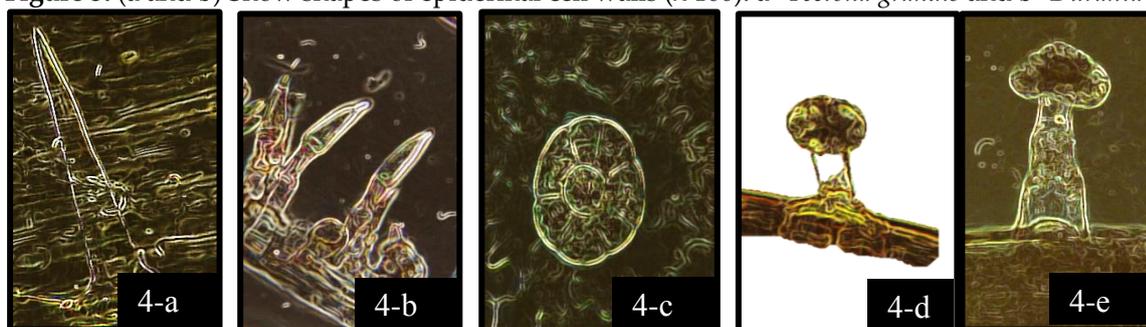


Figure 4: (a- e) Types of hairs (x 400). a- *Duranta erecta*, b- *Vitex trifolia*, c- *Gmelina arborea*, d- *Vitex trifolia* and e- *Gmelina arborea*.

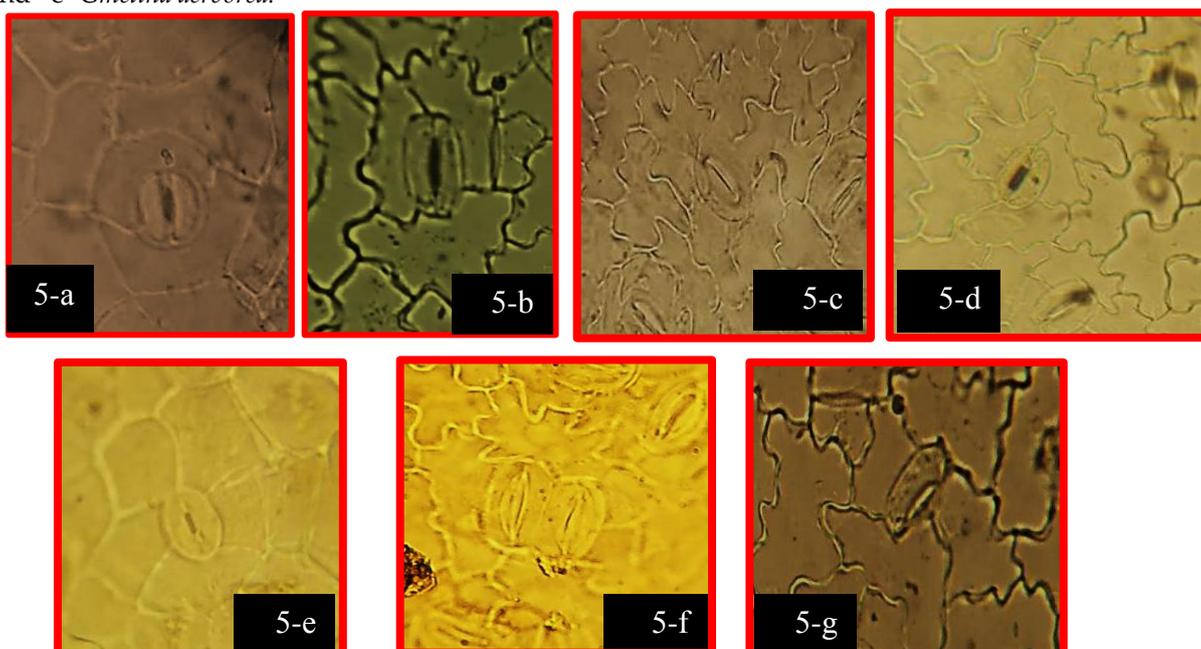


Figure 5: (a- g) Types of stomata (x 400).a- *Vitex trifolia*, b- *Verbena hybrid*, c- *Lantana montevidensis*, d- *Duranta erecta*, e- *Tectona grandis* f and g- *Duranta erecta*.

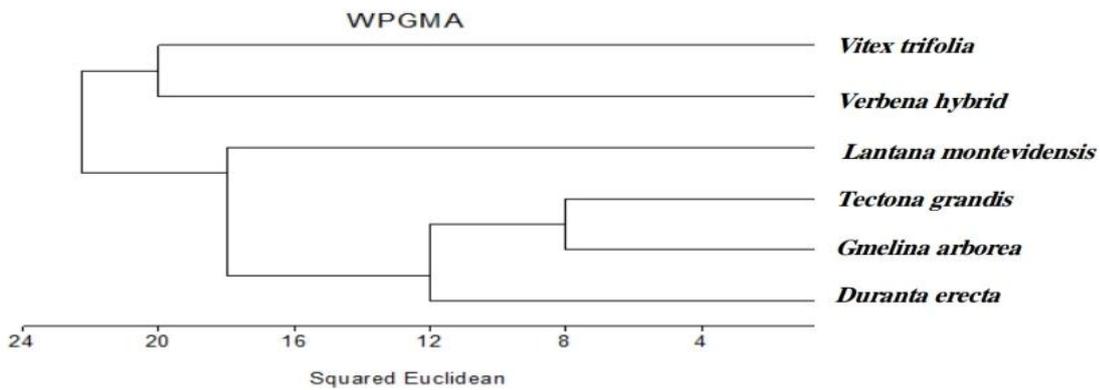


Figure 6: Dendrogram representing the similarity according to the morphological features of Verbenaceae species.

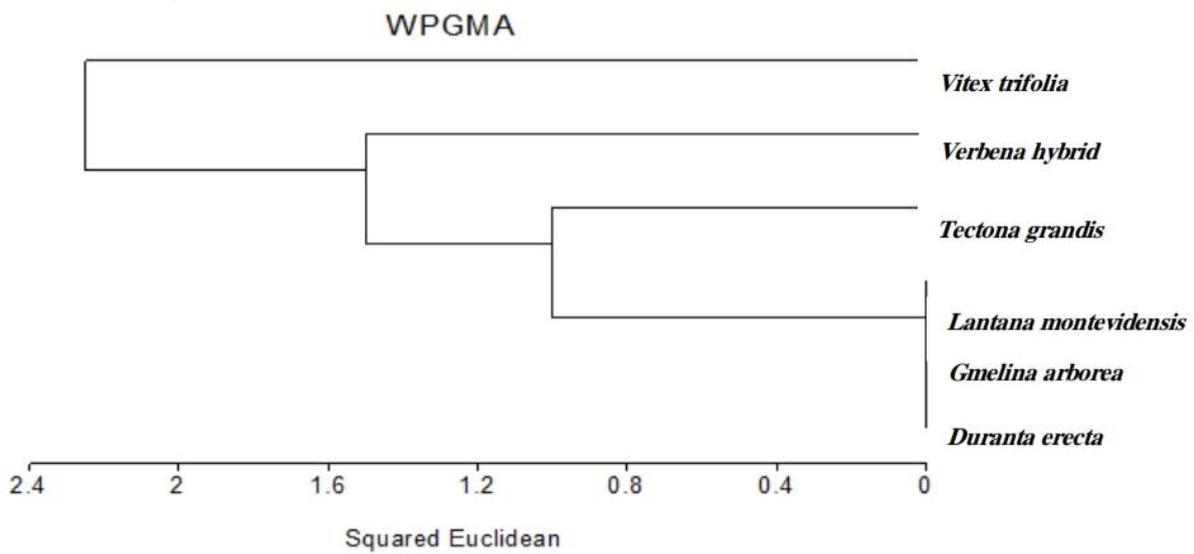


Figure 7: Dendrogram representing the similarity and dissimilarity according to the chemical features between 6 taxa belonging to family Verbenaceae.

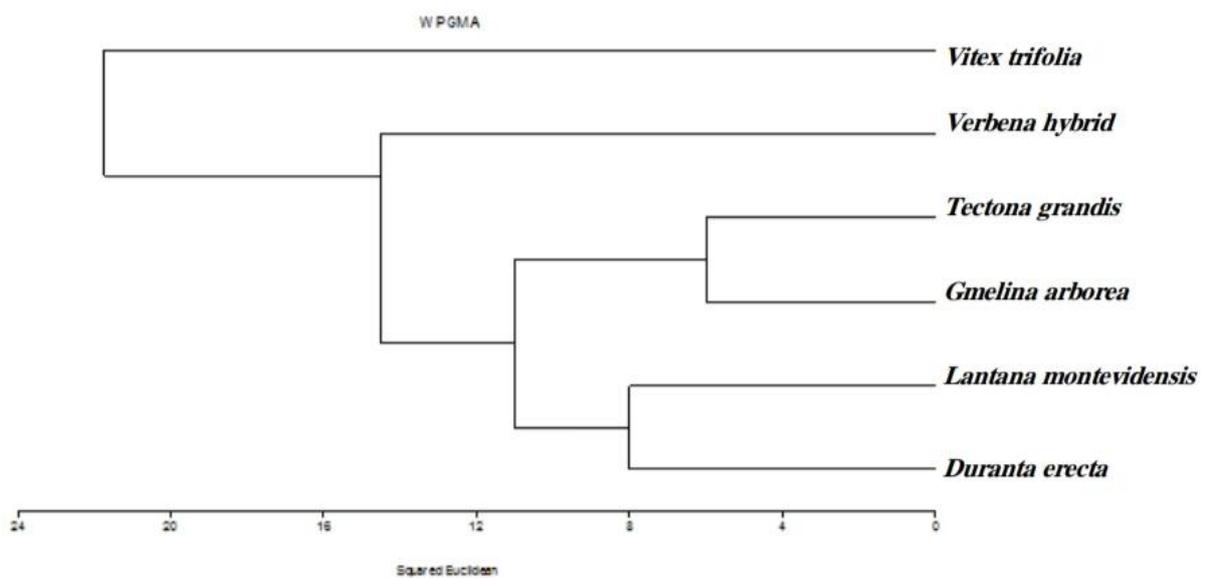


Figure 7: Dendrogram representing the similarity and dissimilarity according to the morphological and chemical features between 6 species belonging to six genera representing family Verbenaceae.

دراسات مورفولوجية وكيميائية مقارنة على بعض نباتات الفصيلة الفربيونية في مصر

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الملخص العربي

أجريت هذه الدراسة على 6 أنواع تمثل 6 أجناس تتبع الفصيلة الفربيونية . جُمعت هذه العينات النباتية الممثلة لتلك الأنواع من مناطق مختلفة في مصر. دُرست هذه النباتات من حيث طبيعة النمو والشكل الظاهري للجذور، والسيقان والأوراق. تم عمل سلخات في طبقة البشرة للأوراق وذلك لدراسة الأنماط المختلفة من الثغور والشعيرات. كما تم دراسة بعض الصفات الكيميائية لهذه النباتات. وأظهرت النتائج أن الأنواع محل الدراسة نباتاتها أما أعشاب حوليه كما في *Verbena hybrid* أو شجيرات كما في *Vitex trifolia* أو أشجار كما في *Gmelina arborea*. سيقان تلك النباتات قائمة قوية مربعة في المقطع العرضي. الأوراق كانت بسيطة في كل نباتات الأنواع المدروسة ما عدا النوع *Vitex trifolia* فالأوراق كانت بسيطة ومركبة ثلاثية. الشعيرات وحيدة الخلية أو عديدة الخلايا قد تكون غدية أو غير غدية. الثغور متعامدة وغير متكافئة وغير مميزة ورباعية أو شعاعية. كما أظهرت النتائج وجود المجاميع الكيميائية الآتية: الفينولات والفلافونيدات والجليكوسيدات والتانينات والفيتوسترولات والتربينات وأيضا وجود القلويدات في كل نباتات الأنواع المدروسة باستثناء *Vitex trifolia* L. ومن تحليل النتائج المتحصل عليها من الدراسة باستخدام برنامج (MVSP) تبين أن *Vitex trifolia* L. كان أكثر اختلافاً عن باقي الأنواع محل الدراسة من خلال دراسة صفاته المورفولوجية والكيميائية.

الكلمات الاسترشادية: الشكل الظاهري، الشعيرات الفربيونية، المسح الكيميائي النباتي.