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Phytochemical Studies On Some Species Of Polygonaceae In Egypt.

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ABSTRACT

The present study aimed to investigate and reassess the taxonomic relationships of 9 species in Polygonaceae family; *Persicaria senegalensis* (Meisn.) Soják., *Persicaria salicifolia* (Brouss. ex Willd.) Assenov., *Rumex dentatus* L., *Rumex vesicarius* L., *Calligonum polygonoides* L., *Antigonon guatimalense* Meisn., *Antigonon leptopus* Hook. & Arn., *Ruprechtia laxiflora* Meisn. and *Ruprechtia salicifolia* (Cham. & Schltdl.) C.A. Mey. using electrophoresis of storage seed protein and analysis of fatty acids composition of seed oils. Clustering analysis was used as a tool in the identification and in evaluation of the taxonomic relationships among the studied species. The total number of recorded protein bands was 21 bands varied from plant to another ranging between 9–15 for each species. Protein molecular weight ranged from 4.5-93 kDa. There were 4 common bands in all studied species. *Rumex dentatus* was characterized by the presence of the largest number of protein bands. The oil extracts of the studied species included 7 saturated fatty acids and 4 unsaturated fatty acids. Palmitic acid, linoleic acid and arachidic acid were the main fatty acids while myristic acid, palmetoleic acid, margrinic acid and ligoceric acid were lowest. The 32 characters of phytochemical analysis (21 of seed protein bands and 11 fatty acids) were employed in cluster analysis. The dendrogram produced indicated that the 9 species could be grouped into two major clusters. Each represent a subfamily. The first cluster represented the subfamily Polygonoideae and divided into two sub groups. The second cluster represented the subfamily Coccoloboideae and divided into two groups. The numerical cluster analysis of seed protein electrophoresis and fatty acids compositions considered Polygonoideae and Coccoloboideae as two separate subfamilies.

Keywords: Seed protein electrophoresis, Fatty acid analysis, Polygonaceae.

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INTRODUCTION

Polygonaceae is a family of flowering plants known as the knotweed family. The name is based on the genus *Polygonum* which refers to the many swollen nodes the stems of some species. Polygonaceae is a morphologically diverse family that contains nearly 1,200 species in 48 genera. Growth form varies from small herbs or cushion plants to shrubs, lianas, and trees over 20m tall (Freeman and Reveal 2005).

The family is taxonomically divided in two subfamilies, Polygonoideae and Eriogonoideae. The subfamily Polygonoideae includes five tribes: Calligoneae, Fagopyreae, Persicariae, Polygoneae and Rumiceae (Burke *et al.* 2010). There are 46 genera with 1100 species in the world. In Egypt, The family is represented by 28 species belonging to seven genera (Boulos, 1999).

Dammer (1893) subdivided the family into three subfamilies: Rumicoideae, Polygonoideae and Coccoloboideae where Eriogonoideae recognized as a tribe within Polygonoideae. Jaretsky (1925) named two subfamilies: Eriogonoideae and Polygonoideae, including Coccoloboideae within the latter. Roberty and Vautier (1964) divided the family again into three subfamilies, but this time the groups were Polygonoideae, Eriogonoideae, and Calligonoideae. Reveal (1989), Brandbyge (1992) and Freeman and Reveal (2005) used the division proposed earlier by Jaretsky. However, a previous study using the chloroplast gene *rbcL* showed that there was no support for the two subfamily circumscription, since a monophyletic Eriogonoideae was nested within Polygonoideae (Lamb-Frye and Kron 2003).

Plants belonging to this family are known to produce a large number of biologically important secondary metabolites, such as flavonoids, anthraquinones, steroids (Fukuyama *et al.*, 1983) and alkaloids (Korulkin and Muzychkina 2015). The most characteristic compounds of the Polygonaceae are polysaccharides, phenylpropane glycosides and stilbenes (Li-shuang *et al.*, 2006).

The electrophoresis analysis of storage proteins found in the seeds has been recognized as a powerful tool of experimental taxonomy in detecting interspecific variations and evaluating interspecific relationships. Seed proteins are usually considered as the immediate products of the genome and are less affected by the environmental conditions (Przybylska 1995 and Przybylska *et al.*, 2000). Consequently, the high stability of protein characters particularly those of seeds, makes them a powerful tool in elucidation the origin, evolution and relationship of the taxa, (Davis and Heywood 1963 and Ladizinsky and Hymowitz 1979). The presence or absence of protein bands can be used as a diagnostic character for a group of taxa or on a certain taxon (Ladizinsky and Hymowitz 1979).

The genetic relationship can be traced by the study of fatty acids. Satisfactory results were obtained by using fatty acids in constructing the genetic relationship between the wild and the cultivated *Gossypium* species in Egypt (Amer 1999). These compounds were also used to differentiate between the different ecotypes of *Balanites aegyptica* (Amer *et al.*, 2002), *Artemisia monosperma* (Khafagi and Mohamed 2002) and on Apocynaceae (Thirumala Reo *et al.*, 1984, Halim *et al.*, 1990, Abbott *et al.*, 1990, Daulatabad *et al.*, 1992, Hichri *et al.*, 2003, Moustafa *et al.*, 2007, Khanzada *et al.*, 2008, Sathya *et al.*, 2010, Augustus and Seiler 2011). Few phytochemical studies are found in the literature describing the chemical constituents of Polygonaceae (Abou Elfotouh *et al.*, 2013).

The aim of the present study was to investigate the seed storage protein and fatty acids composition of seed oil to reassess the taxonomic relationships of 9 species of Polygonaceae in the light of their taxonomic treatment.

Materials and Methods

The present study is based on 9 species of Polygonaceae collected fresh from different localities in Egypt (Table I). The studied materials were identified by means of comparison with specimens kept in the herbarium of the Flora and Phytotaxonomy Research Department (CAIM). In addition to keys of Bailey (1949), Lindley (1932), Hutchinson and Dalziel (1963), Täckholm (1974), Davis (1975), and Boulos (1999). The voucher specimens were deposited in the herbarium of the Flora and Phytotaxonomy Research Department (CAIM).

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

Species	Locality and date
<i>Persicaria senegalensis</i> (Meisn.) Soják	El- Qaluobea, Marc 2018
<i>Persicaria salicifolia</i> (Brouss. ex Willd.) Assenov.	El- Fayoum, Marc 2019
<i>Rumex dentatus</i> L.	El- Qaluobea, Marc 2018
<i>Rumex vesicarius</i> L.	El- Qaluobea, Marc 2018
<i>Calligonum polygonoides</i> L.	Alexandria, June 2018
<i>Antigonon guatimalense</i> Meisn.	Mazhar Garden, 20/ 5/ 2018.
<i>Antigonon leptopus</i> Hook. & Arn.	Mazhar Garden, 20/ 5/ 2018.
<i>Ruprechtia laxiflora</i> Meisn.	Mazhar Garden, 20/ 5/ 2018.
<i>Ruprechtia salicifolia</i> (Cham. & Schltdl.) C.A. Mey.	Mazhar Garden, 20/ 5/ 2018.

Table (2): The taxonomic treatment of the studied species in the system of **A. Engler** according to **Melchior (1964)**.

Subfamily	Tribe	Species
Polygonioideae	Persicarieae	<i>Persicaria senegalensis</i>
		<i>Persicaria salicifolia</i>
	Rumiceae	<i>Rumex dentatus</i>
		<i>Rumex vesicarius</i>
	Atraphaxidinae	<i>Calligonum polygonoides</i>
Coccoloboideae	Coccolobeae	<i>Antigonon guatimalense</i>
		<i>Antigonon leptopus</i>
	Triplariaceae	<i>Ruprechtia laxiflora</i>
<i>Ruprechtia salicifolia</i>		

The Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was prepared according to the method of Laemmli (1970). Gel- electrophoresis was conducted at room temperature with 40 mA. Current per slab during stacking gel and 80 mA. during separation for 4 to 5 hr. After electrophoresis, the gel was stained in 50ml of staining solution. The stained gel was detained and photographed. Total bands for each species were scored and their molecular weight (M.W) was calculated using the protein marker as a standard.

The fatty acids composition of the seed oil was determined by gas chromatography (GC) as fatty acid methyl esters (FAME). FAME was prepared by cold methylation method according to (IOC 2001). The analysis of fatty acid methyl esters was made according to (AOAC 2005) official method of analysis. A chromatographic analysis was performed in anaglient 6890 equipped with DB23 capillary column (60m x 0.32 mm x 0.25 Mm). The FAME was injected into the GC system and Fatty acid standers were used to identify the peaks.

The relationship between the studied species has been analyzed using cluster analysis of SPSS program version 22.

RESULTS AND DISCUSSIONS

Seed Protein Electrophoresis

The results of the electrophoresis pattern analysis of seed proteins of the studied species of Polygonaceae are presented in **Table (3)** and **Figure (1)**.

The bands were detected with different molecular weights ranged from 100 K. Da. to 4.5 K. Da. The total number of bands about 21 varied from plant to another. Protein bands ranging between 9– 15 for each species. The highest number of protein bands (15) was found in *Rumex dentatus* while the lowest number (9) was recorded in *Calligonum polygonoides*.

There were four common bands existed in all examined species (No. 12, 14, 20 and 21; mol. wt. 23.6 K. Da., 18.7 K. Da., 6.0 K. Da. and 4.5 K. Da. respectively). Bands (No. 1 and 17 mol. wt. 93 and 12.3 K. Da. respectively) was recorded in all studied species except for *Calligonum polygonoides* only.

On the other hand *Rumex dentatus* only had a specific band (No. 16; mol. wt. 13.5 K. Da.). *Antigonon guatemalense*, *Antigonon leptopus*, *Ruprechtia laxiflora* and *Ruprechtia salicifolia* showed four common bands (No. 4, 13, 18 and 19; mol. wt. 70, 20, 10 and 8.2 K. Da. respectively). *Persicaria senegalensis*, *Persicaria salicifolia*, *Rumex dentatus* and *Rumex vesicarius* recorded three common bands (No. 6, 7 and 8; mol. wt. 49, 43 and 38 K. Da. respectively).

Table (3): SDS-PAGE of total seed protein banding of the studied species

NO. of bands	M. wt. (K Da.)	<i>Persicaria senegalensis</i>	<i>Persicaria salicifolia</i>	<i>Rumex dentatus</i>	<i>Rumex vesicarius</i>	<i>Calligonum polygonoides</i>	<i>Antigonon guatemalense</i>	<i>Antigonon leptopus</i>	<i>Ruprechtia laxiflora</i>	<i>Ruprechtia salicifolia</i>
1	93	1	1	1	1	0	1	1	1	1
2	82	0	1	0	0	1	0	0	0	0
3	76	0	0	1	1	0	0	0	1	0
4	70	0	0	0	0	0	1	1	1	1
5	55	0	0	0	0	1	1	1	1	1
6	49	1	1	1	1	0	0	0	0	0
7	43	1	1	1	1	0	0	0	0	0
8	38	1	1	1	1	0	0	0	0	0
9	36	0	0	1	1	1	1	1	1	1
10	30	0	1	1	1	1	0	0	0	0
11	25	1	1	1	1	1	0	0	0	0
12	23.6	1	1	1	1	1	1	1	1	1
13	20	0	0	0	0	0	1	1	1	1
14	18.7	1	1	1	1	1	1	1	1	1
15	15.4	0	0	1	0	0	1	1	1	1
16	13.5	0	0	1	0	0	0	0	0	0
17	12.3	1	1	1	1	0	1	1	1	1
18	10	0	0	0	0	0	1	1	1	1
19	8.2	0	0	0	0	0	1	1	1	1
20	6.0	1	1	1	1	1	1	1	1	1
21	4.5	1	1	1	1	1	1	1	1	1

1= Present 0= Absent

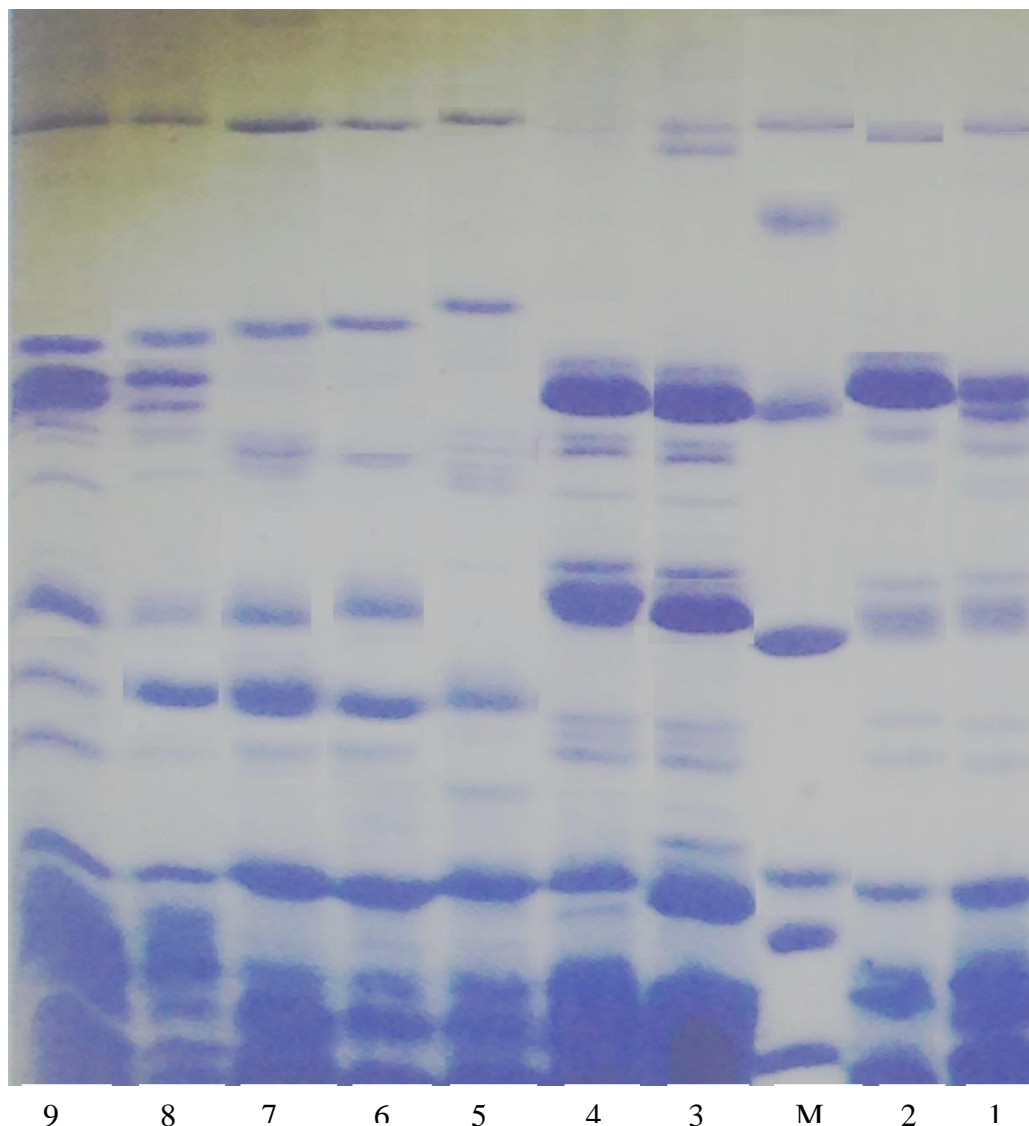


Fig. 1: The SDS-PAGE Gel of 14 species of Polygonaceae

- | | | |
|--|--|---------------------------------------|
| 1- <i>Persicaria senegalensis</i> | 2- <i>Persicaria salicifolia</i> | 3- <i>Rumex dentatus</i> |
| 4- <i>Rumex vesicarius</i> | 5- <i>Calligonum polygonoides</i> | |
| 6- <i>Antigonon guatimalense</i> | 7- <i>Antigonon leptopus</i> | 8- <i>Ruprechtia laxiflora</i> |
| | 9- <i>Ruprechtia salicifolia</i> | |

Fatty Acid Analysis

The fatty acids content of the total lipids of the studied species were analyzed as very low value (less than 1%), low value (1-10%), moderate value (10- 20%) and high value (more than 20%). The content of total lipids in the studied species recorded its maximum value (20.01%) in *Ruprechtia salicifolia* and its minimum value (15.32%) in *Rumex dentatus*. Most studied species recorded values from 19.93% to 15.50%. The oil extracts of the studied species recorded seven saturated fatty acids and four unsaturated fatty acids were isolated (**Table 4 & 5**).

The content of Myristic acid ranged between very low to low content in most studied species and absent in *Ruprechtia laxiflora* and *Ruprechtia salicifolia*. Palmitic acid and Linoleic acid recorded high content in *Persicaria senegalensis*, *Persicaria salicifolia*, *Rumex dentatus*, *Rumex vesicarius* and *Calligonum polygonoides* while recorded moderate values in the reminders. Palmetoleic acid, Margaric acid, Ecosenic acid, Beheic acid and Ligoceric acid showed very low to low values in all studied species. Stearic acid and Oleic acid contents were low in all studied species.

Arachidic acid content was low in *Persicaria senegalensis*, *Persicaria salicifolia*, *Rumex dentatus*, *Rumex vesicarius* and *Calligonum polygonoides* while was high in the rest.

Table (4): Fatty Acids isolated from the Studied Species

Fatty Acids	Code	Molecular Formula	Common Name	Systematic Name	Molecular Weight
Saturated Fatty Acids	C14:0	C14H28O2	Myrisitic acid	Tetradecanoic acid	228
	C16:0	C16H32O2	Palmitic acid	Hexadecanoic acid	256
	C17:0	C17H34O2	Margarinic acid	Heptadecanoic acid	270
	C18:0	C18H36O2	Stearic acid	Octadecanoic acid	284
	C20:0	C20H40O2	Arachidic acid	Eicosanoic acid	312
	C22:0	C22H44O2	Behenic acid	Docosanoic acid	340
	C24:0	C24H48O2	Ligoceric acid	Tetracosanic acid	386
Unsaturated Fatty Acids	C16:1	C16H30O2	palmetoleic acid	9- Hexadecanoic acid	254
	C18:1	C18H34O2	Oleic acid	9- Octadecenoic acid	282
	C18:2	C18H32O2	Linoleic acid	9,12- Octadecadienoic acid	280
	C20:1	C20H38O2	5-Ecosenic acid	Trans-Ecosenic acid	310

Table (5): Fatty Acid Composition of Seed Oils in the Studied Species

Fatty acids \ Species	Total Oil %	Myrisitic acid	Palmitic acid	Palmetoleic acid	Margarinic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Ecosenic acid	Behenic acid	Ligoceric acid
<i>Persicaria senegalensis</i>	15.50	1.06	31.22	0.28	0.14	2.92	8.83	24.63	9.50	1.21	0.15	0.31
<i>Persicaria salicifolia</i>	16.41	1.40	30.91	0.42	0.58	2.28	8.87	22.95	9.65	1.60	0.27	0.50
<i>Rumex dentatus</i>	15.23	0.96	34.11	0.31	0.16	2.42	7.19	21.66	8.62	1.28	1.61	0.06
<i>Rumex vesicarius</i>	17.32	0.98	33.92	0.16	0.11	1.03	7.11	21.33	9.44	1.17	1.11	0.04
<i>Calligonum polygonoides</i>	15.84	1.02	33.67	0.86	1.09	1.56	8.02	22.49	7.86	176	2.57	0.20
<i>Antigonon guatimalense</i>	18.06	0.60	19.58	1.07	1.23	5.31	4.82	19.76	26.55	0.61	0.40	4.60
<i>Antigonon leptopus</i>	18.4	0.51	19.13	1.15	1.23	5.85	4.18	19.18	26.60	0.68	0.28	4.20
<i>Ruprechtia laxiflora</i>	19.	0.0	17.8	1.8	0.9	6.5	5.5	18.	24.	0.2	0.2	2.05

	93		4	0	6	6	0	56	35	7	7	
Ruprechtia salicifolia	20.01	0.0	17.70	1.86	0.93	7.32	5.52	16.05	24.63	0.48	0.48	2.05

Numerical Analysis

All the 32 characters from seed protein electrophoresis and fatty acid composition of the seed oil for the 9 species of polygonaceae were used for numerical analysis by using cluster analysis of SPSS version 22.

The method of clustering analysis was used as a tool in the identification and in taxonomic relationships among the studied species of polygonaceae.

Seed Protein Electrophoresis

The results of clustering particularly analysed by the agglomeration of Schedule measure similarity, using average linkage between groups (Fig. 2) showed that species were grouped into two major clusters. The first one represented the subfamily Polygonoideae and divided into two sub clusters. The first one included one species *Calligonum polygonoides* represented the tribe Atraphaxidinae. The second sub cluster divided into two groups. The first group contained *Persicaria senegalensis* and *Persicaria salicifolia* represented the tribe Persicarieae. The second group included *Rumex dentatus* and *Rumex vesicarius* represented the tribe Rumiceae. The second cluster represented the subfamily Coccoloboideae and divided into two groups. The first group contained one species *Ruprechtia laxiflora* while the second group contained *Antigonon guatimalense*, *Antigonon leptopus* and *Ruprechtia salicifolia*.

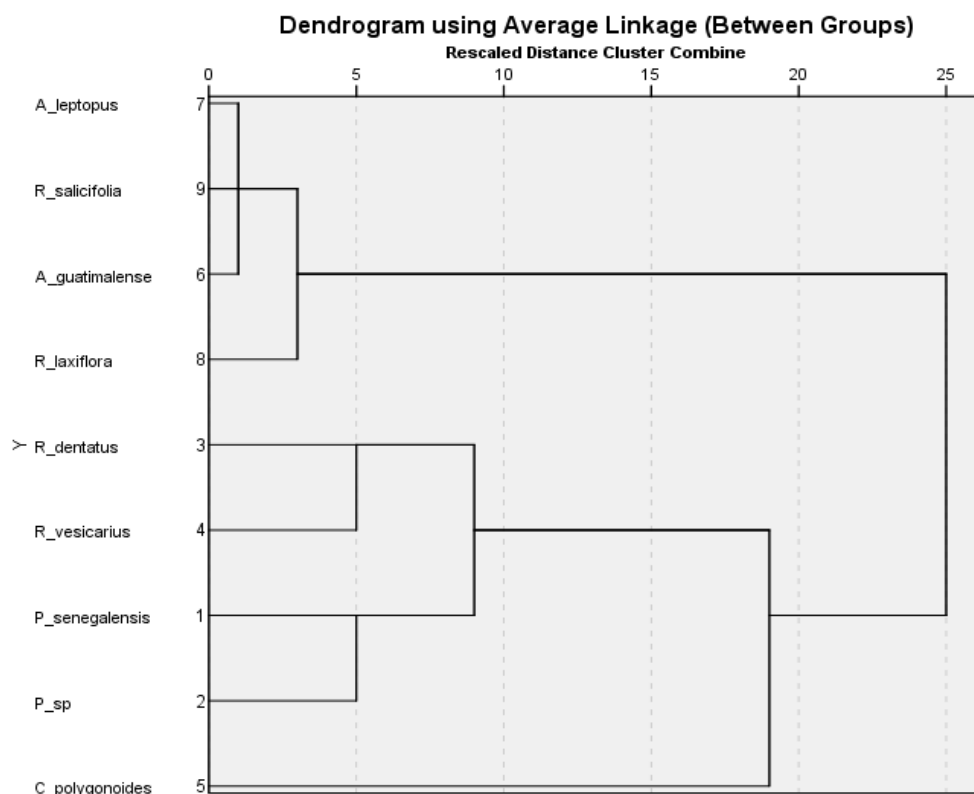


Fig. 2: Dendrogram showing the interrelationships between the studied species based on 21 characters of seed protein by using SPSS Program.

Table (6): Proximity matrix showed similarity value of the studied species based on seed protein electrophoresis

	<i>Persicaria senegalensis</i>	<i>Persicaria salicifolia</i>	<i>Rumex dentatus</i>	<i>Rumex vesicarius</i>	<i>Calligonum polygonoides</i>	<i>Antigonon guatimalense</i>	<i>Antigonon leptopus</i>	<i>Ruprechtia laxiflora</i>	<i>Ruprechtia salicifolia</i>
<i>Persicaria senegalensis</i>	1.00	0.920	0.302	0.324	0.290	0.196	0.112	0.104	0.147
<i>Persicaria salicifolia</i>	0.920	1.00	0.331	0.356	0.255	0.142	0.162	0.112	0.132
<i>Rumex dentatus</i>	0.302	0.331	1.00	0.884	0.254	0.113	0.156	0.183	0.186
<i>Rumex vesicarius</i>	0.324	0.356	0.884	1.00	0.213	0.132	0.154	0.106	0.122
<i>Calligonum polygonoides</i>	0.290	0.255	0.254	0.213	1.00	0.167	0.143	0.075	0.104
<i>Antigonon guatimalense</i>	0.196	0.142	0.113	0.132	0.167	1.00	0.826	0.340	0.865
<i>Antigonon leptopus</i>	0.112	0.162	0.156	0.154	0.143	0.826	1.00	0.310	0.851
<i>Ruprechtia laxiflora</i>	0.104	0.112	0.183	0.106	0.075	0.340	0.310	1.00	0.423
<i>Ruprechtia salicifolia</i>	0.147	0.132	0.186	0.122	0.104	0.865	0.851	0.428	1.00

Fatty Acid Analysis

The fatty acids content of the lipid of the studied species were analyzed as (1) very low value (less than 1%), (2) low value (1-10%), (3) moderate value (10- 20%) and (4) high value (more than 20%) as shown in (Table 6).

The result of clustering analysed by the agglomeration of measure similarity, using average linkage between groups (Fig. 3) showed that species were grouped into two major clusters. The first one represented the subfamily Polygonoideae and divided into two sub clusters. The first one included one species *Calligonum polygonoides* represented the tribe Atraphaxidinae. The second sub cluster divided into two groups. The first group contained *Persicaria senegalensis* and *Persicaria salicifolia* represented the tribe Persicarieae. The second group included *Rumex dentatus* and *Rumex vesicarius* represented the tribe Rumiceae. The second cluster represented the subfamily Coccoleboideae and divided into two groups. The first group contained *Ruprechtia laxiflora* and *Ruprechtia salicifolia* represented the tribe Triplarideae. The second group contained *Antigonon guatimalense* and *Antigonon leptopus*. represented the tribe Coccolebeae.

Table (7): The fatty acid content of the studied species

Fatty Acids \ Species	Myristic acid	Palmitic acid	Palmetoleic acid	Margarinic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Ecosenic acid	Behenic acid	Ligoceric acid
<i>Persicaria senegalensis</i>	2	4	1	1	2	2	4	2	2	1	1
<i>Persicaria salicifolia</i>	2	4	1	1	2	2	4	2	2	1	1
<i>Rumex dentatus</i>	1	4	1	1	2	2	4	2	2	2	1
<i>Rumex vesicarius</i>	1	4	1	1	2	2	4	2	2	2	1
<i>Calligonum polygonoides</i>	2	4	1	2	2	2	4	2	2	2	1
<i>Antigonon guatimalense</i>	1	3	2	2	2	2	3	4	1	1	2
<i>Antigonon leptopus</i>	1	3	2	2	2	2	3	4	1	1	2
<i>Ruprechtia laxiflora</i>	1	3	2	1	2	1	3	4	1	1	2
<i>Ruprechtia salicifolia</i>	1	3	2	1	2	1	3	4	1	1	2

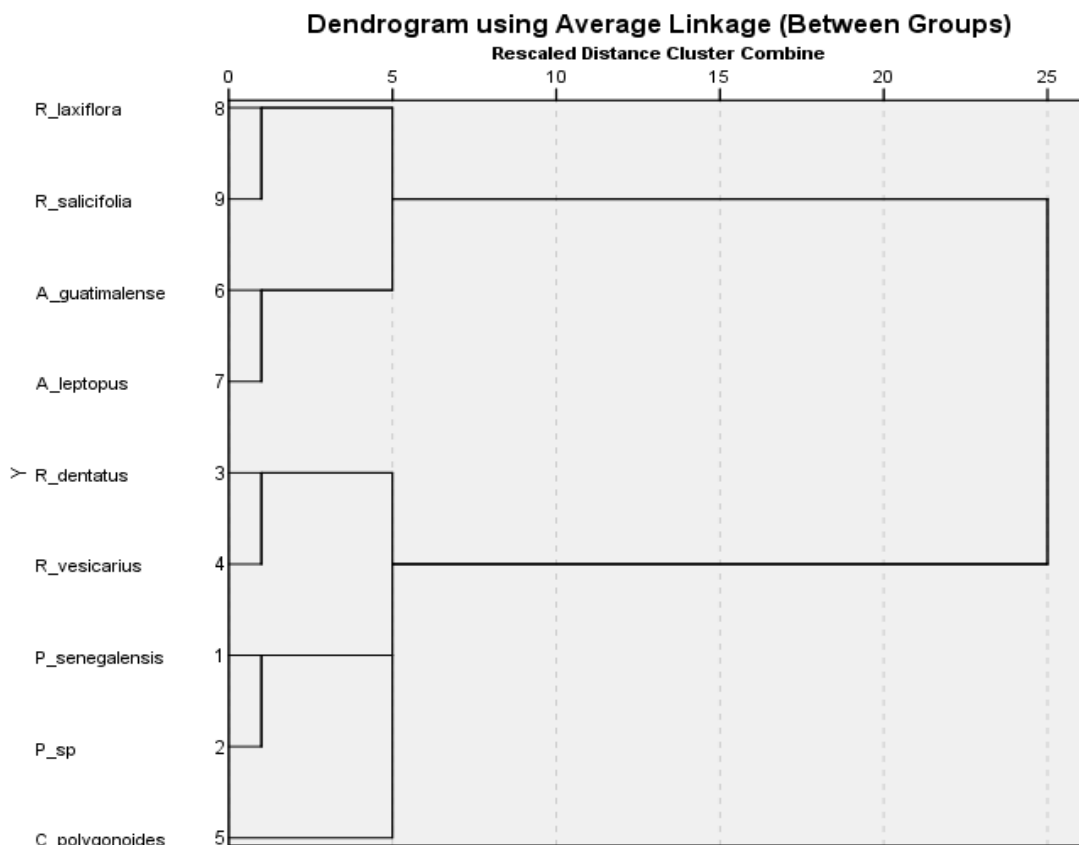


Fig. 3: Dendrogram showing the interrelationships between the 9 species of Polygonaceae based on 11 characters of fatty acids analysis by using SPSS Program.

Table (8): Proximity matrix showed similarity value of the studied species based on fatty acids analysis

	<i>Persicaria senegalensis</i>	<i>Persicaria salicifolia</i>	<i>Rumex dentatus</i>	<i>Rumex vesicarius</i>	<i>Calligonum polygonoides</i>	<i>Antigonon guatemalense</i>	<i>Antigonon leptopus</i>	<i>Ruprechtia laxiflora</i>	<i>Ruprechtia salicifolia</i>
<i>Persicaria senegalensis</i>	1.00	0.960	0.901	0.324	0.881	0.484	0.484	0.524	0.524
<i>Persicaria salicifolia</i>	0.960	1.00	0.901	0.356	0.881	0.484	0.484	0.524	0.524
<i>Rumex dentatus</i>	0.901	0.901	1.00	0.971	0.890	0.484	0.484	0.524	0.524
<i>Rumex vesicarius</i>	0.901	0.901	0.971	1.00	0.890	0.484	0.484	0.524	0.524
<i>Calligonum polygonoides</i>	0.881	0.881	0.890	0.890	1.00	0.412	0.412	0.408	0.408
<i>Antigonon guatemalense</i>	0.484	0.484	0.484	0.484	0.412	1.00	0.961	0.891	0.891
<i>Antigonon leptopus</i>	0.484	0.484	0.484	0.484	0.412	0.961	1.00	0.891	0.891
<i>Ruprechtia laxiflora</i>	0.524	0.524	0.524	0.524	0.408	0.891	0.891	1.00	0.955

Combination of Seed Protein and Fatty Acids Analysis

The 32 characters states of phytochemical analysis (21 of seed protein bands and 11 fatty acids) were employed in cluster analysis. The dendrogram produced from using SPSS analysis used similarity (Fig.4) showed that species could be grouped into two major clusters.

The results of clustering analysed by the agglomeration of measure similarity, using average linkage between groups showed that species were grouped into two major clusters. The first one represented the subfamily Polygonoideae and divided into two sub clusters. The first one included one species *Calligonum polygonoides* represented the tribe Atraphaxidinae. The second sub cluster divided into two groups. The first group contained *Persicaria senegalensis* and *Persicaria salicifolia* represented the tribe Persicarieae.

The second group included *Rumex dentatus* and *Rumex vesicarius* represented the tribe Rumiceae. The second cluster represented the subfamily Coccoleboideae and divided into two groups. The first group contained *Ruprechtia laxiflora* and *Ruprechtia salicifolia* represented the tribe Triplarideae. The second group contained *Antigonon guatimalense* and *Antigonon leptopus* represented the tribe Coccolebeae.

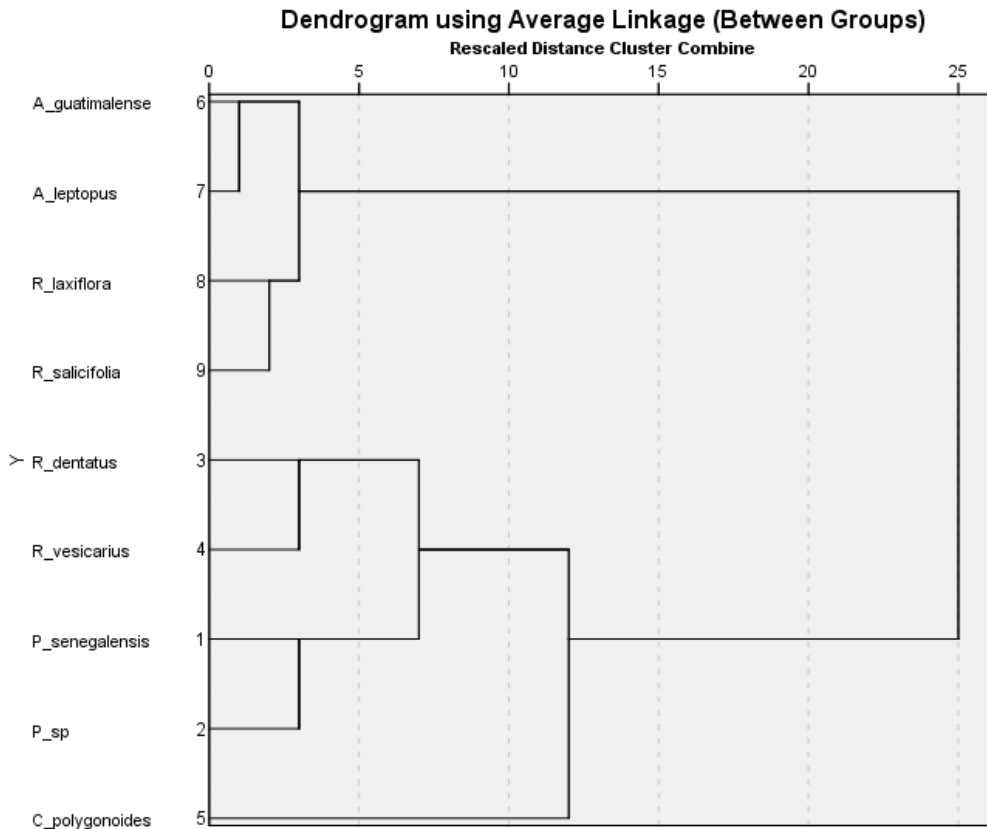


Fig. 4: Dendrogram showing the interrelationships between 9 species of Polygonaceae based on 32 characters of seed protein and fatty acids analysis by using SPSS Program.

Table (9): Proximity matrix showed similarity value of the studied species based on seed protein electrophoresis and fatty acids analysis

	<i>Persicaria senegalensis</i>	<i>Persicaria salicifolia</i>	<i>Rumex dentatus</i>	<i>Rumex vesicarius</i>	<i>Calligonum polygonoides</i>	<i>Antigonon guatimalense</i>	<i>Antigonon leptopus</i>	<i>Ruprechtia laxiflora</i>	<i>Ruprechtia salicifolia</i>
<i>Persicaria senegalensis</i>	1.00	0.972	0.904	0.928	0.846	0.663	0.663	0.630	0.630
<i>Persicaria salicifolia</i>	0.972	1.00	0.890	0.921	0.872	0.611	0.611	0.576	0.600
<i>Rumex dentatus</i>	0.904	0.890	1.00	0.969	0.831	0.602	0.602	0.602	0.524
<i>Rumex vesicarius</i>	0.928	0.921	0.969	1.00	0.858	0.619	0.619	0.610	0.608

<i>Calligonum polygonoides</i>	0.846	0.872	0.831	0.858	1.00	0.693	0.693	0.633	0.652
<i>Antigonon guatimalense</i>	0.663	0.611	0.602	0.619	0.693	1.00	0.976	0.908	0.908
<i>Antigonon leptopus</i>	0.663	0.611	0.602	0.619	0.693	0.976	1.00	0.908	0.908
<i>Ruprechtia laxiflora</i>	0.630	0.576	0.602	0.618	0.633	0.908	0.908	1.00	0.985
<i>Ruprechtia salicifolia</i>	0.630	0.600	0.595	0.608	0.652	0.908	0.908	0.985	1.00

CONCLUSION

The results of seed protein electrophoresis and fatty acids analysis considered Polygonoideae and Coccoleboideae as two separate subfamilies. This study supported the systems of Melchior (1964), Sanchez *et al.*, 2011 and Schuster *et al.*, 2015 while was not agreed with the systems of Jaretzky (1925), Reveal (1989), Brandbyge (1992) and Freeman and Reveal (2005) in including Coccoleboideae within Polygonoideae.

REFERENCES

- [1] Abbott, T. P.; Peterson, R. E.; Tjarks, L. W.; Doris, M. P. and Bagby, M. O. (1990): Major extractable components in *Asclepias linaria* (Asclepiadaceae) and *Ilex verticillata* (Aquifoliaceae), two potential hydrocarbon crops. *Economic Botany* 44 (2): 278- 284.
- [2] Abou Elfotouh, M. A.; Shams, K. A.; Anthony, K. P.; Shahat, A. A.; Ibrahim, M. T.; Abdelhady, N. M.; Abdel Azim, N. S.; Hammouda, F.M.; El-Missiry, M. M. and Saleh, M. A.(2013): Lipophilic Constituents of *Rumex vesicarius* L. and *Rumex dentatus* L., *Antioxidants* 2:167-180.
- [3] Amer, W. M. (1999): Egyptian cotton: relict *Gossypium herbaceum* L. in Egypt. *Bulletin of Faculty of Science. Assiut University.* 28(2-D): 161- 172.
- [4] Amer, W. M.; Soliman, M. M. and Sheded, M.M. (2002): Biosystematic studies for *Balanites aegyptiaca* (Balanitaceae) population in Egypt. *Flora Mediterranea*, 12: 353- 367.
- [5] AOOAC (2005): Association of official agriculture Chemists. *Official methods of analysis*, Washington. DC.
- [6] Bailey, L. H. (1949): *Manual of cultivated plants*. The Macmilan company, New York. P.: 347- 351.
- [7] Boulos, L. (1999): *Flora of Egypt*. Al Hadara Publishing, Cairo, Egypt. vol.1: 21-34.
- [8] Brandbyge, J. (1992): *Reforestation de los Andes ecuatorianos con especies nativas*. CESA, Quito, Ecuador. p.: 90.
- [9] Dammer, U. (1893): Polygonaceae. In *Die natürlichen Pflanzenfamilien vol III*, ed. A. Engler and K. Prantl. Leipzig, Germany: Engelmann. P.: 1-36.
- [10] Daulatabad, C. D.; Mulla, G. M.; Mirajkar, A. M. and Hosamani, K. M. (1992): *Cryptolepis buchnani* seed oil: A rich source of keto fatty acid. *Journal of the American Oil Chemists' Society* 69 (2): 188-189.
- [11] Davis, P.H. and Heywood, V. (1963): *Principles of Angiosperm Taxonomy*. Oliver and Boyd. Edinburgh and London.
- [12] Davis, P. H. (1975): *Flora of Turkey*. (6): 158-174. Edinburgh.
- [13] Freeman, C. C. and Reveal. J. L. (2005): Polygonaceae. in *Flora of North America* vol. 5, ed. Flora of North America Editorial Committee. New York: Oxford University Press. P.: 216–218.
- [14] Fukuyama, Y.; Sato, T.; Miura, I.; Asakawa, Y. and Takemoto, T.(1983). *Hydropiperoseide*, a novel coumaryl glycoside from the root of *Polygonum hydropiper*, *Phytochemistry*, 22(2): 549–552.
- [15] Halim, A. F.; Zaghloul, A. M. and Ebaid, K. A. (1990): *Lupeol long- chain fatty acid esters and other lipid constituents from Cynanchum acutum* L. Fam. Asclepiadaceae in Egypt. *J. Pharm. Sci.* 31:99- 105.
- [16] Hichri, F.; Ben Jannet, H. and Mighri Z. (2003): *Antibacterial activities of a few prepared derivatives of oleanolic acid and of other natural triterpenic compounds*. *Comptes Rendus Chimie* 6(4):473.
- [17] Hutchinson, J. and Dalziel, J. M. (1963): *Flora of west tropical Africa*. London, Vol. II: 51- 103.
- [18] IOC (2001): *International Olive Conference. Preparation of fatty acids methyl esters*. COI/ T20/ DOC No. 19.
- [19] Jaretzky, R. (1925): *Beiträge zur systematik der Polygonaceae unter berücksichtigungdes oxymethyl-anthrachinon-vorkommens*. *Feddes Repertorium Specierum Novarum Regni Vegetabilis* 22: 49–83.

- [20] Khafagi, A. A. and Mohamed, A. H. (2002): Morphological and chemical characteristics of *Artemisia monosperma* seeds from Egypt. *Journal of the Faculty of Education*. 27: 479- 489.
- [21] Khanzada, S. K.; Shaikh, W.; Kazi, T. G.; Sofia, S.; Kabir, A.; Usmanghani, K. and Kandhro, A. A. (2008): Analysis of fatty acid, elemental and total protein *Calotropis procera* medicinal plant from Sindh, Pakistan. *Pak. J. Bot.* 40 (5): 1913- 1921.
- [22] Korulkin, D. Y.u. and Muzychkina, R. A. (2015). Correlation of Structure and Antiviral Activity of Alkaloids of *Polygonum* L. Plants Growing in Kazakh-stan *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering* Vol:9, No:7.
- [23] Ladizinsky, G. and Hymowitz, T. (1979): Seed protein electrophoresis in taxonomic evolutionary studies. *Theor. App. Genet.* 54: 145-151.
- [24] Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature* 22: 680- 685.
- [25] Lamb-Frye, A.S. and Kron, K.A. (2003): Phylogeny and character evolution in Polygonaceae. *Syst Bot* 28:326–332.
- [26] Li-shuang, L.V.; Gu, X.; Ho, C. T. and Tang, J. (2006). Stilbene glycosides from the roots of *Polygonum multiflorum* Thunb and their in vitro antioxidant activities. *Journal of Food Lipids*, 13 : 131–144.
- [27] Lindley, J. (1932): *Flora medica; A botanical account of all the more important plants used in medicine*. Longman, London. P.: 527- 545.
- [28] Melchior, H. (1964). *A. Engler's Syllabus Der Pflanzenfamilien*, Berlin, Nikolassa. vol.1: 75- 79.
- [29] Moustafa, A. M. Y.; Khodair, A. I. and Saleh, M. A. (2007): Phytochemical investigation and toxicological studies of lipid constituents isolated from *Leptadenia pyrotechnica*. *J. Ph. and Toxi.* 2 (8): 681- 697.
- [30] Przybylska, J. (1995): Some examples of the use of electrophoretic protein analysis in taxonomic investigations of leguminous plants. *J. Applied Genet.* 36: 255- 271.
- [31] Przybylska, J.; Zimnia- Przybylska, Z. and Krajewski, P. (2000): Diversity of seed globulins in *Lathyrus sativus* L. and some related species. *Genet. Res. Crop Evol.* 47: 239- 246.
- [32] Reveal, J. L. (1989). The Eriogonoideae flora of California (Polygonaceae: Eriogonoideae). *Phytologia* 66: 295–414.
- [33] Roberty, G. S. and Vautier, M. (1964): Les genres de Polygonacées. *Boissiera* 10: 7–128.
- [34] Sathya, A.; Ramasubramaniam, R. and Brindha, P. (2010):
- [35] Pharmacognostical, Phytochemical and GC-MS Investigation of Successive extracts of *Gymnema sylvestre* R.Br. *Journal of Pharmacy Research*, 3(5): 984-987.
- [36] Sharawy, S. M. (2013): Taxonomic relationships of some taxa of sub family Asclepiadoideae (Apocynaceae) as reflected by morphological variations and polymorphism in seed protein and RAPD electrophoretic profile. *Inter. J. Bot.* 9(1): 18- 29.
- [37] Täckholm, V. (1974): *Students' flora of Egypt*. 2nd ed. Cairo Univ. Publication, Corporative Printing Co., Beirut. P.: 58- 68.