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Proteins patterns of eight genera of the Asteraceae family

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Asteraceae family grows as wild plants in the eastern province of Saudi Arabia near the Gulf coast with prevalent high humidity coupled with more moderate temperatures. Eight genera were collected during the flowering season to study genetic diversity according to protein patterns for each plant. The protein patterns showed different numbers of bands, concentrations, molecular weight and intensity. Two common bands were observed to ensure the existence of fixed gene and it can also be considered as a marker of the studied genera. Protein profiles revealed genetic diversity among these species for adaptation to environmental factors. Similarity coefficient was high for *Sonchus oleranceus* and *Senecio desfontainei*, while low for *Launaea capitata* and *Osteospermum vaillantii* among the studied plants. The hierarchical cluster analysis, formed two major clusters, which indicates the existence of genetic diversity among the studied genera. The first cluster was divided into two sub-clusters. Sub-cluster A comprised three species, *Scorzonera papposa*, *Senecio desfontainei*, and *Sonchus oleranceus*; and the second sub-cluster B comprised four species *Anthemis melampodina*, *Echinops hussoni*, *Launaea capitata*, and *O. vaillanti*. The second cluster contained only one species *Artemisia inculta*, which suggests that the Asteraceae family may have more than one evolutionary line.

Key word: Asteraceae, genetic diversity, protein patterns.

INTRODUCTION

The eastern province of Saudi Arabia, near the Gulf coast is rich of wild annual plants especially during the cold seasons (January to April). The climate of Saudi Arabia described by Country Profile: Saudi Arabia (2006) differs greatly between the coast and the interior; high humidity coupled with more moderate temperatures is prevalent along the coast, whereas aridity and extreme temperatures characterize the interior.

Asteraceae is one of the largest families of flowering plants with a world-wide distribution and with more than 1,600 genera and over 24,000 species of herbs, shrubs and trees (Funk et al., 2009). Most Asteraceae (Compositae) family are wild plants in Saudi Arabia. Dempewolf et al. (2008) reported that it is one of the largest families of flowering plants in Saudi Arabia. Also, Al-Farhan (1999) reported that Asteraceae is one of the

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major families in Saudi flora with 222 species and the distribution of life form is closely related to topography and landform (Fakhireh et al., 2012). Some of the Asteraceae plants were and still used in folk medicines and many researchers investigated their medical effects and pharmacological characteristics. Results indicate that some plants are pharmacologically important (Tariq et al., 1987; Elsharkawy et al., 2014), for example *Artemisia*, *Launaea*, *Cichorium*, *Anthemis* and *Sonchus* species.

Most of the taxonomist identifies plant species on the basis of phenotype characters of plants like root, stem and leaf structures (Lifante, 1991). New techniques in molecular biology are considered excellent assessment tools to identify differences among plants. Electrophoresis analysis was used to identify varieties and in evaluating genetic diversity (Sharma and Maloo, 2009). This study aimed to investigate the genetic relations and diversity on the basis of proteins patterns of some wild genera of the Asteraceae family, which grows in the eastern province of Saudi Arabia.

MATERIALS AND METHODS

Description of collected plants

Plants samples of eight genera of Asteraceae family were collected during the flowering season from the central and northern areas of the eastern region of Saudi Arabia. Asteraceae family belongs to order: Campanulales that are generally herbs, rarely woody, and often with latex or oil-passages; inflorescence racemose, with a tendency to form heads. Flowers bisexual or unisexual, regular or zygomorphic, pentamerous with reduction in number of carpels and with one whorl of stamens. Anthers laterally united to form a tube into which the pollen is discharged; style developed into a brush by which the pollen is swept out; ovary inferior, plurilocular, with ∞ -1 ovules in each locule or unilocular with 1 ovule.

All plants described were according to the methods of Migahid (1978) and Mossa et al. (1987).

(1) *Anthemis melampodina*: Collected from the North of Eastern province. Small, annual, ash-coloured, densely grey-woolly desert sand herb. Leaves small pinnatifid, few-lobed or parted with mucronate lobes. Peduncles short not thickened. Heads 2 cm broad. Scales of involucre lanceolate to oblong the outer acute, the inner scarious-tipped. Ray florets white, often red-flushed. Achenes with rounded apex, without auricle. Outer involucral scales prominently scarious-margined, more than in the type.

(2) *Scorzonera papposa*: Collected from the North of Eastern province. Woolly, then glabrescent herb. Leaves often wavy margined, the lower oblong, the upper half clasping, linear. Heads 3 to 4 cm long. Scales of involucre white-margined. Flowers lilac. Achenes 1 cm long, muricate.

(3) *Echinops hussoni*: Stem glabrous, glossy, whitish-yellow. Leaves thin, soft, with flattened margins, with few deep, spiny-tipped lobes, densely cobwebby lanate beneath. Spines few, rather weak. Head with horn-like spines.

(4) *Launaea capitata*: Biennial herb, 5 to 15 cm, high with stiff, stout, scape-like, almost naked, simple or 2-forked stems emerging from a dense rosette of lyrate-cleft leaves. Heads short and thick, nearly sessile, densely-clustered towards the summit of the stem. Involucral scales broad, white-margined. Achenes beakless, broad, yellow, winged, flattened, retuse, short.

(5) *Senecio desfontainei*: Annual sweet smelling herbs, 25 cm high. Leaves fleshy, pinnatifid into linear remotely toothed lobes, the lower leaves tapering into a short petiole, the rest clasping with minute auricles. Heads large, cup-shaped, 1 cm long and broad in loose corymbs with rather long ray florets. Achenes ribbed, hairy.

(6) *Osteospermum vaillantii*: Perennial plants, 40 cm high, glandular-hirsute, richly branched, with simple, entire or few-toothed, half-clasping, oblong-lanceolate leaves, and a few corymbose heads small, peduncled; scales of involucre, linear. Achenes broadly scarious winged.

(7) *Sonchus oleranceus*: Annual, rarely biennial, herbaceous weed. Stems erect, the young branches with glandular hairs, soft, hollow, stem and leaf milky juiced. Leaves alternate, oblong, acute, 10 to 12 cm long, pinnately parted, margins irregularly tooth, the base auriculate and clasping the stem. Yellow flowers in terminal heads; involucre green, 12 to 15 mm long, the involucral bracts in 3 to 4 rows, oblong-lanceolate. Flowers all ligulate, the corolla truncate at the apex, 5-toothed, the lower part tubular, with white hairs. Achenes narrow-margined, 3-nerved and 3-striate, 3 mm long, brown, oblanceolate rugose.

(8) *Artemisia inculta*: Collected from the North of Eastern province. Perennial grey-woolly shrub let with narrow lobed, long leaves. Flower heads brownish, ovoid, erect, densely clustered and sessile. Inner involucral scales linear.

Protein extraction and separation

Leaf samples were used to extract proteins, using Fisher bio-reagents sure-prep RNA/DNA/Protein purification kit. Automated electrophoresis from Bio-Rad with Experion pro260 analysis kit was used for protein separation, scanning and photograph, it is a system that uses a combination of caliper separation technology and sensitive fluorescent sample detection to perform rapid and automated analysis of protein by integrating separation, detection, and data analysis within single platform.

Data analysis

The formula of Nei and Lei (1979) was followed to consider the degree of similarity: $S_{ab} = 2N_{ab} / (N_a + N_b)$, where, N_{ab} = number of bands common to both plants, N_a = number of bands in plants a, and N_b = number of bands in plants b. Dendrogram (hierarchical cluster) was used to construct plant samples according to the average linkage (between groups). The data analysis was done using SPSS-16.0 for Windows statistical package.

RESULTS

Table 1 and Figures 1 and 2 show total protein concentration, molecular weight, and number of bands of collected plants. The protein concentrations do not reveal the number of bands for some plants. Although, *A. melampodina* produced 15 protein bands at concentration of 1301.8 kDa, which is higher than that of *E. hussoni*, which produced 17 protein bands with protein concentration 1216.7 kDa, *L. capitata* produced 13 protein bands with protein concentration of 1155.4 kDa; *S. papposa* produced 8 proteins bands with concentration of 1184.7 kDa; *S. desfontainei* produced 11 proteins bands with concentration of 12114 kDa; *S. oleranceus* produced 10 proteins bands with total concentration of 370.9 kDa; *A. inculta* produced 9 proteins bands with

Table 1. Proteins molecular weight, concentrations and number of bands of eight genera of Asteraceae.

Samples	Molecular weight	Concentration	Number of bands
<i>Anthemis melampodina</i>	108.4	1301.8	15
<i>Scorzonera papposa</i>	89.84	1184.7	8
<i>Echinops hussoni</i>	160.1	1216.7	17
<i>Launaea capitata</i>	147.22	1155.4	13
<i>Senecio desfontainei</i>	173.6	1214	11
<i>Osteospermum vaillantii</i>	56.53	103.4	5
<i>Sonchus oleranceus</i>	127.22	370.9	10
<i>Artemisia inculta</i>	154.48	384.8	9

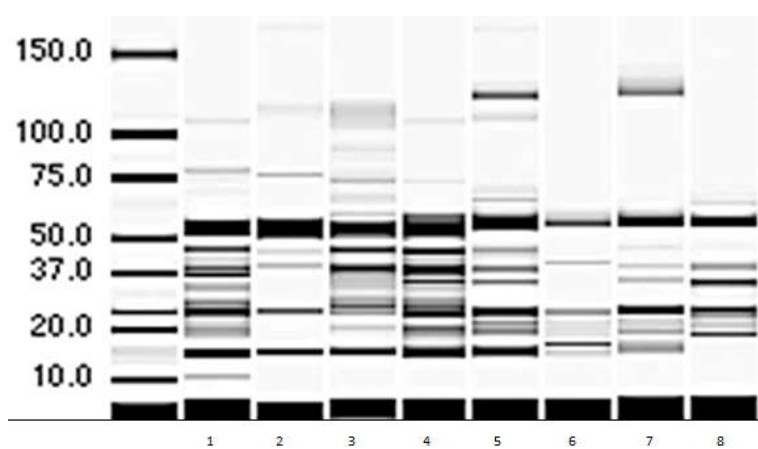


Figure 1. Automated electrophoresis of proteins bands of eight genera of Asteraceae family. 1, *Anthemis melampodina*; 2, *Scorzonera papposa*; 3, *Echinops hussoni*; 4, *Launaea capitata*; 5, *Senecio desfontainei*; 6, *Osteospermum vaillantii*; 7, *Sonchus oleranceus*; 8, *Artemisia inculta*.

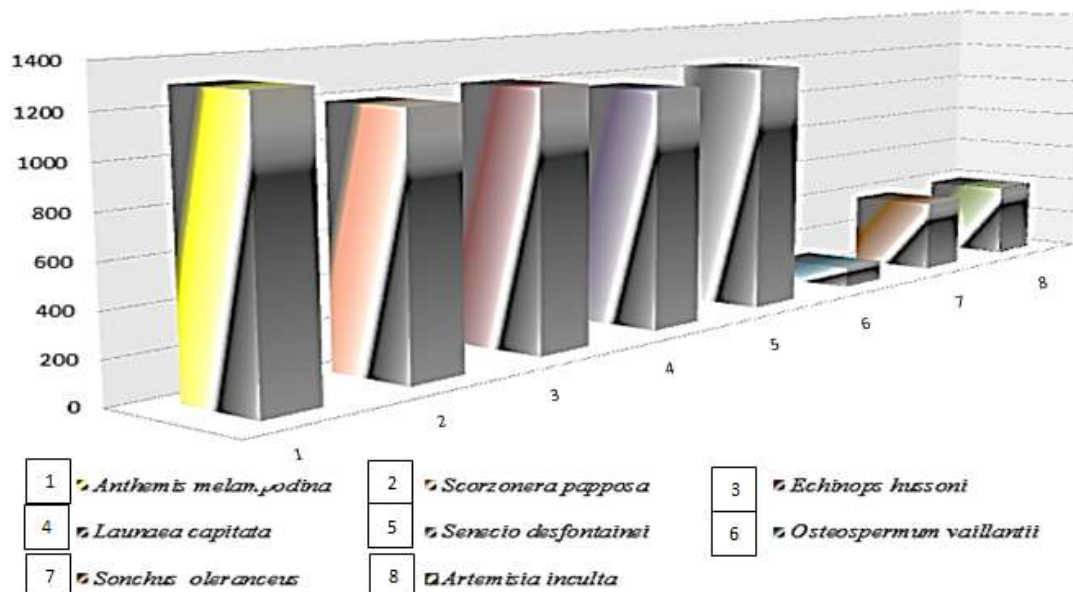


Figure 2. Proteins concentration of some genera of Asteraceae family.

Table 2. Similar degree of eight genera of Asteraceae.

Genera	<i>Anthemis melampodina</i>	<i>Scorzonera papposa</i>	<i>Echinops hussoni</i>	<i>Launaea capitata</i>	<i>Senecio desfontainei</i>	<i>Osteospermum vaillantii</i>	<i>Sonchus oleranceus</i>	<i>Artemisia inculta</i>
<i>Anthemis melampodina</i>	0							
<i>Scorzonera papposa</i>	0.7	0						
<i>Echinops hussoni</i>	0.94	0.64	0					
<i>Launaea capitata</i>	0.93	0.76	0.87	0				
<i>Senecio desfontainei</i>	0.85	0.84	0.79	0.92	0			
<i>Osteospermum vaillantii</i>	0.5	0.77	0.45	0.56	0.63	0		
<i>Sonchus oleranceus</i>	0.8	0.89	0.74	0.87	0.95	0.67	0	
<i>Artemisia inculta</i>	0.75	0.94	0.69	0.81	0.9	0.71	0.94	0

proteins concentration of 384.8 kDa.

In addition, molecular weight showed similar results to the concentrations. There were some plants with low molecular weight and high proteins concentration: *S. papposa* had 89.84 kDa and protein concentration of 1184.7 kDa; *A. inculta* was distinguished with high molecular weight of 154.48 and low protein concentration of 384.8 kDa. The total number of protein bands was 88 without the marker. Results revealed differences among the studied plants in number and intensity of bands, and only two bands were common in every individual plant. The highest number of bands was observed in *E. hussoni* with 17 protein bands, while *O. vaillantii* with five bands had the lowest number of bands.

Table 2 and Figure 3 show the similarity coefficient degree. Data reveals some genera were closer to each other. The highest similarity value was 0.95 between *S. oleranceus* and *S. desfontainei*, and this indicates a close phylogenetically relation, while the lowest similarity value was 0.5 between *A. melampodina* and *O. vaillantii*. The difference in similarity values indicates the existence of genetic diversity. The hierarchical cluster analysis (dendrogram) shows

two major clusters, the first major cluster is subdivided into two sub clusters, sub cluster A contains three genera, *S. papposa*, *S. desfontainei*, and *S. oleranceus*; the second sub cluster B contains four genera *A. melampodina*, *E. hussoni*, *L. capitata*, and *O. vaillantii*. The arrangement of plant under the same sub cluster indicates close protein patterns and genotype, the second major cluster contains one genera plant *A. inculta* (Figure 3).

Table 3 and Figures 4 to 12 show the automated electrophoresis analysis of proteins patterns for every individual plant, separation times of protein band and molecular weight. All bands appeared with different molecular weight, the highest molecular weight was found in *S. desfontainei* and the lowest found in *O. vaillantii*, the two plants were collected from central area of eastern province. Also, Table 4 shows the studied bands, only consistent protein band between 10.0 and 150.0 kDa were considered; the appearance (+) of bands were 44 and the absence (-) was 36. *S. oleranceus* and *S. desfontainei* showed four common bands out of a total number of 10 bands (the highest) and *A. melampodina* and *O. vaillantii* showed three common bands out of total number

of 10 bands; two bands were common in all plants, the first bands appear in all plants with molecular weight range from 25.47 to 25.93 kDa and bands numbers 8, 7, 8, 9, 8, 12, 10, and 11 appear at 28.5 to 28.69 s, the second bands appear at 31.39 to 31.8 s with molecular weight range of 38.82 to 41.07 kDa and bands numbers are 14, 10, 14, 14, 13, 13, 12, and 13 in the order of plants listed in Table 4. Despite of the existing of these two bands in all plants, there were differences in proteins intensity.

DISCUSSION

The total protein concentrations, molecular weight, and intensity revealed variation in gene expression; these variations help plants to adapt to environmental factors. Meena and Shukla (2012) found out that wide range of protein peptides and molecular weight can create additional variability in rice. Automated electrophoresis analysis of protein showed different number of bands, and only for two bands, the first common bands numbers were 8, 7, 8, 9, 8, 12, 10, and 11 and the second bands were 14, 10,

Rescaled Distance Cluster Combine

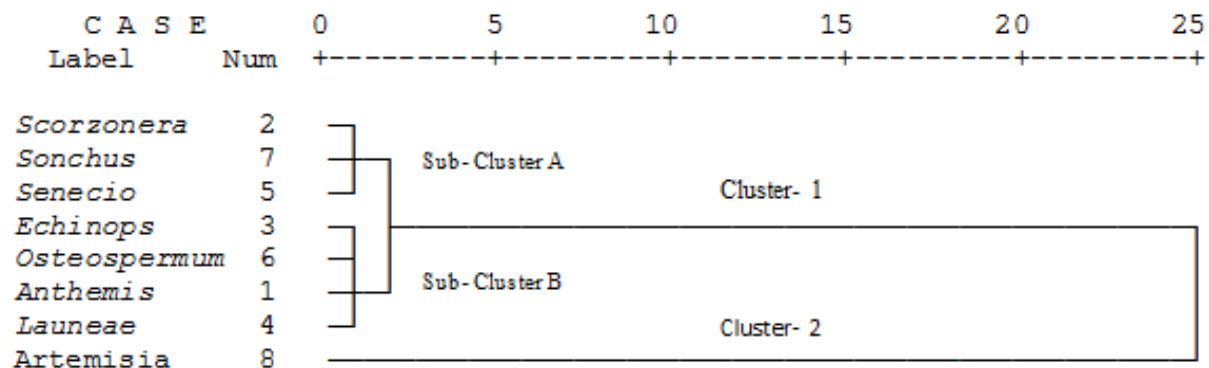


Figure 3. Hierarchical cluster construct for eight plants of Asteraceae. 1, *Anthemis melampodina*; 2, *Scorzonera papposa*; 3, *Echinops hussoni*; 4, *Launaea capitata*; 5, *Senecio desfontainei*; 6, *Osteospermum vaillantii*; 7, *Sonchus oleranceus*; 8, *Artemisia inculta*.

Table 3. Proteins patterns (Number of bands and molecular weight) of eight genera of Asteraceae.

Peak number	Lader		<i>Anthemis melampodina</i>		<i>Scorzonera papposa</i>		<i>Echinops hussoni</i>		<i>Launaea capitata</i>		<i>Senecio desfontainei</i>		<i>Osteospermum vaillantii</i>		<i>Sonchus oleranceus</i>		<i>Artemisia inculta</i>	
	Mig. time (s)	Mol. Wt.	Mig. time (s)	Mol. Wt.	Mig. time (s)	Mol. Wt.	Mig. time (s)	Mol. Wt.	Mig. time (s)	Mol. Wt.	Mig. time (s)	Mol. Wt.	Mig. time (s)	Mol. Wt.	Mig. time (s)	Mol. Wt.	Mig. time (s)	Mol. Wt.
1*	17.15	1.2	10.85	-	10.89	-	10.99	-	10.91	-	11.08	-	10.92	-	11.07	-	10.49	-
2	21.4	6.66	12.59	-	12.55	-	12.63	-	12.79	-	12.79	-	12.94	-	12.64	-	13.08	-
3	22.1	7.56	15.36	-	15.29	-	15.13	-	15.51	-	15.34	-	15.65	-	15.06	-	15.33	-
4	24	10	16.11	-	16.07	-	16.04	-	16.12	-	16.08	-	16.17	-	15.96	-	16.1	-
5	27.25	20	17.15	1.2	17.15	1.2	17.15	1.2	17.15	1.2	16.59	-	17.15	1.2	17.15	1.2	16.53	-
6	28.5	25	22.06	7.51	21.94	7.36	21.92	7.32	21.19	6.38	17.15	1.2	18.37	2.76	21.81	7.18	17.15	1.2
7	31.05	37	24.29	10.9	25.85	15.7	24.51	11.58	21.7	7.05	21.69	7.03	20.71	5.77	26.13	16.56	18.4	2.8
8	33.45	50	25.88	15.78	28.59	25.42	25.86	15.73	22.41	7.95	25.86	15.73	21.13	6.31	27.18	19.78	21.27	6.49
9	37.35	75	27.76	22.06	30.74	35.55	27.45	20.8	25.78	15.49	27.16	19.72	21.74	7.09	27.99	22.94	21.84	7.23
10	40.3	100	28.61	25.51	31.62	40.09	28.65	25.72	27.33	20.33	27.86	22.42	26.46	17.58	28.7	25.93	27.01	19.28
11	45.7	150	29.2	28.31	32.5	44.86	29.04	27.54	28.6	25.47	28.64	25.67	27.82	22.29	30.69	35.32	28.69	25.9
12*	54.55	260	30.15	32.74	33.92	53.01	30.39	33.88	28.93	27.02	30.54	34.62	28.62	25.56	31.55	39.7	30.56	34.69
13	-	-	31.04	36.95	37.63	77.41	30.72	35.46	30.62	34.97	31.42	39.03	31.8	41.07	32.74	46.14	31.52	39.53
14	-	-	31.39	38.82	39.1	89.84	31.4	38.88	31.37	38.73	34.34	55.73	34.47	56.53	34.59	57.31	32.81	46.53
15	-	-	32.68	45.8	54.55	260	32.12	42.79	32.03	42.28	36.01	66.42	54.55	260	40.96	106.1	34.49	56.64

Table 3. Contd.

16	-	-	33.92	52.98	-	-	32.55	45.14	32.49	44.83	41.43	110.5	-	-	43.24	127.2 2	35.83	65.23
17	-	-	34.26	55.21	-	-	34	53.51	33.95	53.2	43.01	125.09	-	-	54.55	260	36.78	71.37
18	-	-	36.54	69.84	-	-	35.01	59.98	37.19	73.96	47.6	173.6	-	-	-	-	45.21	145.48
19	-	-	37.29	74.61	-	-	37.22	74.18	38.45	84.36	54.55	260	-	-	-	-	54.55	260
20	-	-	37.88	79.52	-	-	38.91	88.19	42.07	116.37	-	-	-	-	-	-	65.23	-
21	-	-	41.21	108.4	-	-	39.48	93.09	45.4	147.22	-	-	-	-	-	-	-	-
22	-	-	54.55	260	-	-	41.84	114.2 8	54.55	260	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	46.51	160.0 9	58.82	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	54.55	260	63.23	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	64.26	-	-	-	-	-	-	-	-	-

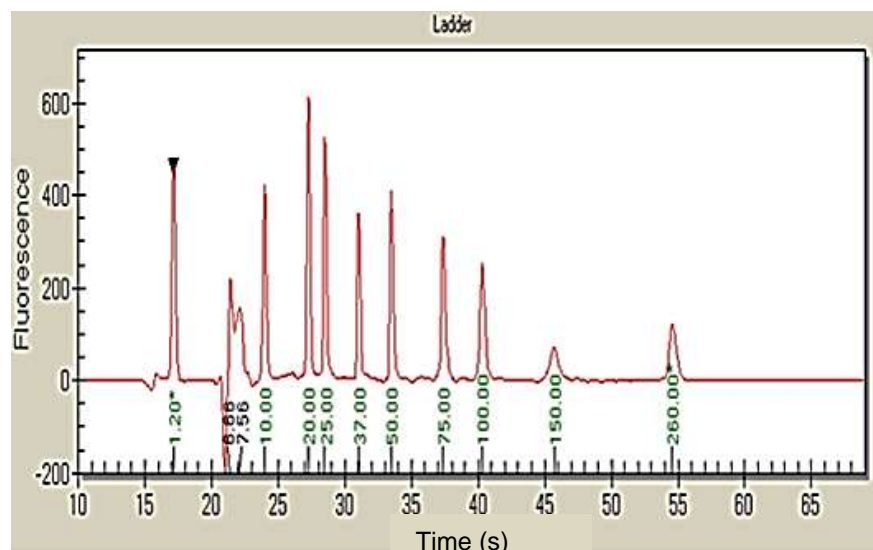


Figure 4. Total proteins bands and molecular weight of the ladder.

14, 14, 13, 13, 12, and 13 among the studied plants. Several researches detected genetic diversity among plants by using electrophoresis

technique (Ehsanpour et al., 2010; Sinha et al., 2012; Alege et al., 2014). The presence of two common bands with different intensity in individual

plants indicates that the gene expression does not vary and gene coding for the protein band is fixed in Asteraceae family. Akinwusi and Illloh (1995)

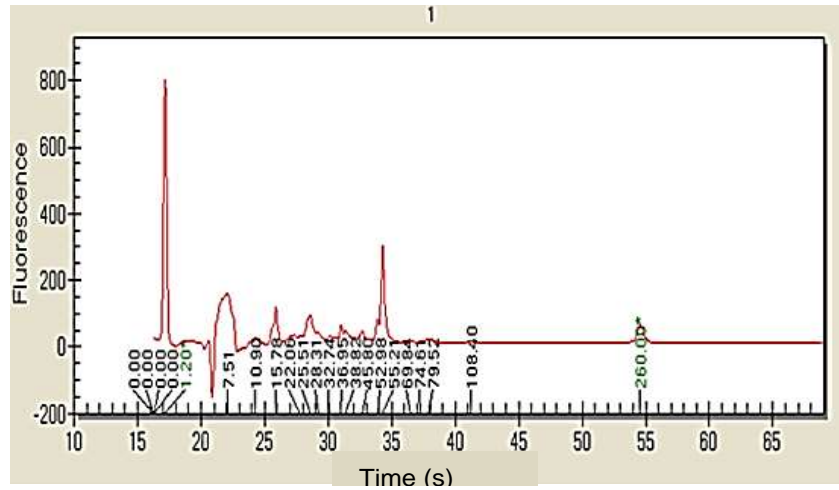


Figure 5. Total proteins bands and molecular weight of *Anthemis melampodina*.

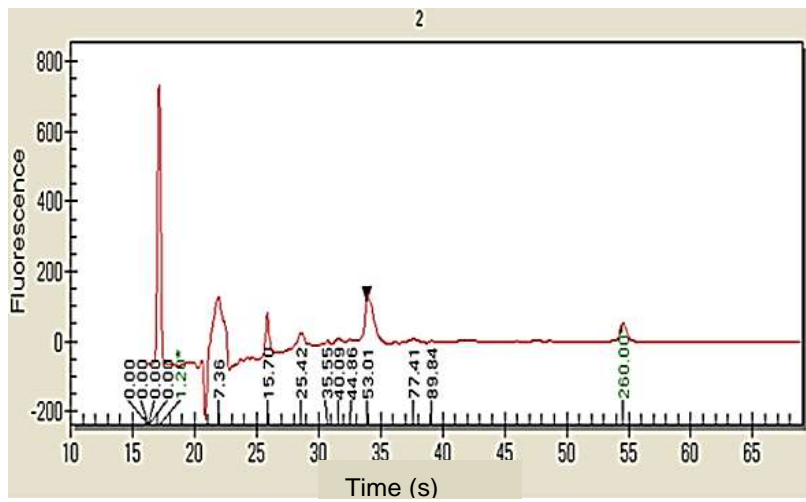


Figure 6. Total proteins bands and molecular weight of *Scorzonera papposa*.

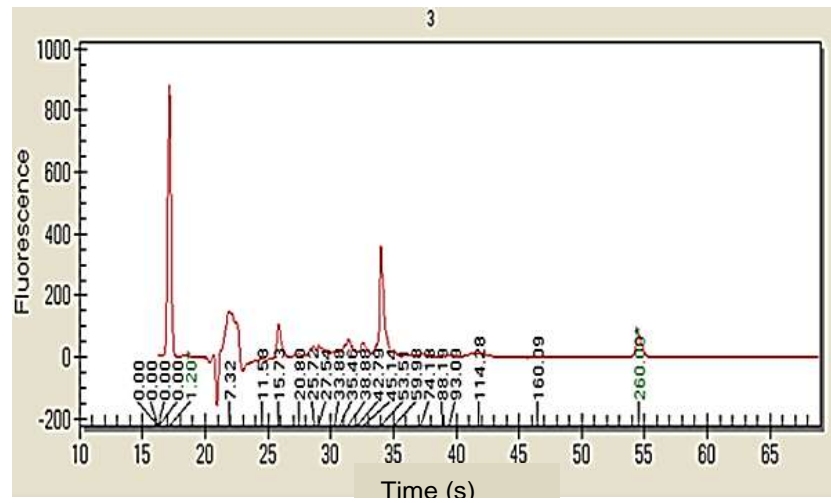


Figure 7. Total proteins bands and molecular weight of *Echinops hussonii*.

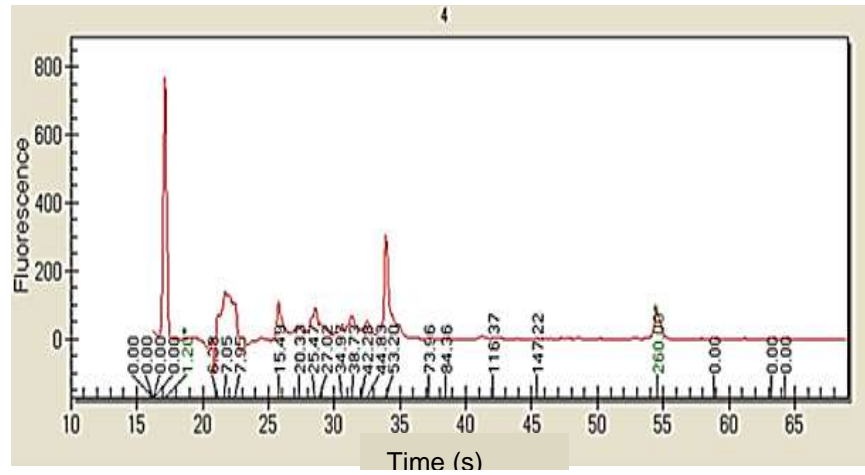


Figure 8. Total proteins bands and molecular weight of *Launaea capitata*.

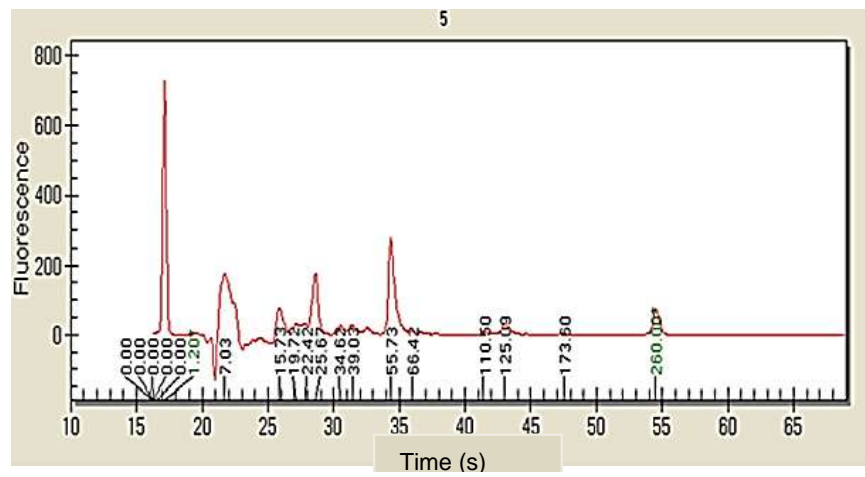


Figure 9. Total proteins bands and molecular weight of *Senecio desfontainei*.

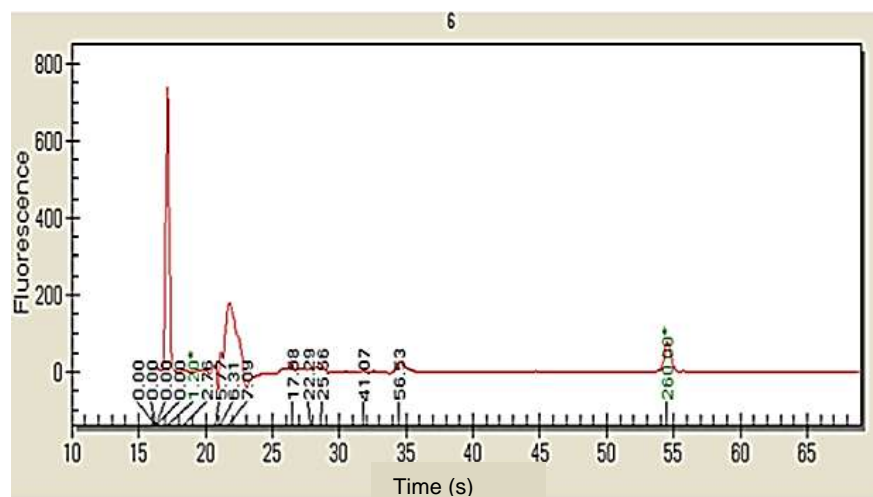


Figure 10. Total proteins bands and molecular weight of *Osteospermum vaillantii*.

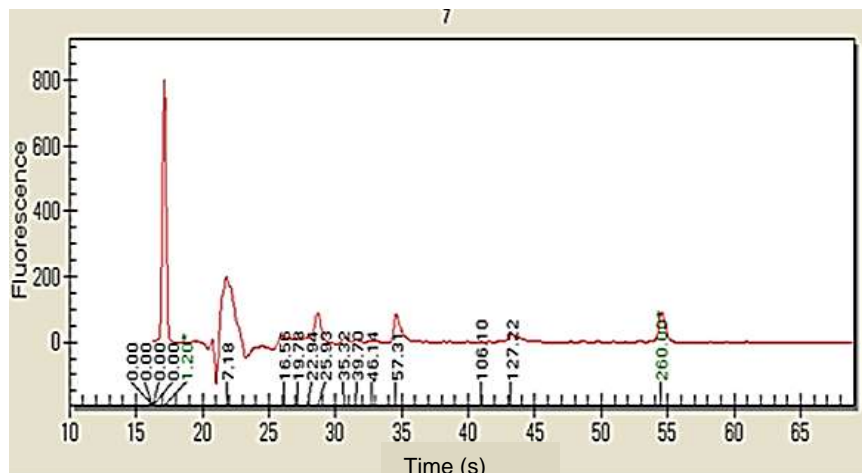


Figure 11. Total proteins bands and molecular weight of *Sonchus oleranceus*.

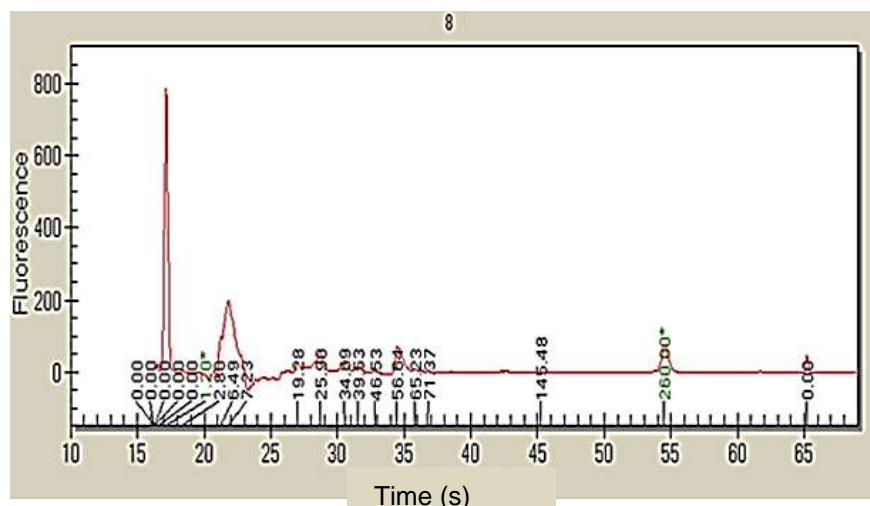


Figure 12. Total proteins bands and molecular weight of *Artemisia inculta*.

attributed that the appearance of a common band in individual plants in population is due to the fact that the gene expression of the protein (enzyme) does not vary in these plants, and there is polymorphism on the basis of differences in protein intensity among genotypes (Munazza et al., 2009). The presence and absence of proteins bands due to genes expression are in response to environmental factors, this difference in genes activation will lead to evolutionary genetic diversity that is reflected in plant phenotype. The similarity coefficient between studied genera, minimum 0.5 and maximum 0.95 indicates that genetic diversity exists within the studied genera of the family (Alege, 2015; Funk et al., 2005). Mossa et al. (1987) and Natarajan (2014) found differences in protein profiles of some *Brassica* species and in soybeans, respectively. The hierarchical cluster of studied plants showed two major clusters, the first major

cluster grouped into two sub-cluster, each genus under the same sub-cluster indicates close genetic affinity and common ancestry (Alege, 2015), while the second major cluster contained only one genus. The presence of two clusters suggests more than one evolutionary line (Alege et al., 2014). Several researches used hierarchical cluster to detect genetic diversity among plants (Irfan et al., 2007; Alege et al., 2014; Bruneau et al., 2001; Amouri et al., 2014).

Conclusion

The result of protein patterns of eight genera of *Astreaeae* revealed genetic diversity that was supported by the hierarchical cluster; the concentrations and intensity with the presence and absence of some proteins

Table 4. Presence (+), absence (-) of proteins bands and the molecular weight of eight genera of Asteraceae.

Mig. Tim (s)	Ladder		<i>Anthemis melampodina</i>		<i>Scorzonera papposa</i>		<i>Echinops hussoni</i>		<i>Launaea capitata</i>		<i>Senecio desfontainei</i>		<i>Osteospermum vaillantii</i>		<i>Sonchus oleraceus</i>		<i>Artemisia inculta</i>	
	Presence /Absence	Mol. Wt	Presence /Absence	Mol. Wt.	Presence /Absence	Mol. Wt.	Presence /Absence	Mol. Wt.	Presence /Absence	Mol. Wt.	Presence /Absence	Mol. Wt.	Presence /Absence	Mol. Wt.	Presence /Absence	Mol. Wt.	Presence /Absence	Mol. Wt.
21.4-21.94	+	6.66	-		+	7.36	+	7.32	+	6.38	+	7.03	+	7.09	+	7.1	+	7.23
22.1-22.41	+	7.56	+	7.51	-		-		+	7.95	-		-		-		-	
24-24.51	+	10	+	10.9	-		+	11.58	-		-		-		-		-	
27.25-27.99	+	20	+	22.06	-		+	20.8	+	20.33	+	22.42	+	22.82	+	22.99	+	19.28
28.5-28.69	+	25	+	25.51	+	25.42	+	25.72	+	25.47	+	25.67	+	25.56	+	25.93	+	25.9
31.39-31.8	+	37	+	38.82	+	40.09	+	38.88	+	38.73	+	39.03	+	41.07	+	39.7	+	39.53
33.45-33.95	+	50	+	52.98	+	53.01	-		+	53.2	-		-		-		-	
37.35-37.88	+	75	+	79.52	+	77.41	+	74.18	+	73.96	-		-		-		-	
40.3-40.96	+	100	-		-		-		-		-		-		+	106.1	-	
45.4-45.4	+	150	-		-		-		+	147.22	-		-		-		+	145.48

bands indicate that the difference between studied plants was due to genotype and the response to environmental factors, and also the appearance of two common bands can be used as a marker for these genera of Asteraceae family.

CONFLICT OF INTEREST

The author has no conflict of interest.

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