Fatty Acid and Amino Acid Profiles in Some Dune Vegetation Species from Istanbul

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Abstract: Dunes are one of the most dynamic ecosystems in the nature and they are considered as a special habitat type hosting valuable genetic resources. Dunes located in Turkey are very rich in terms of plant diversity. Istanbul has special place for the richness of endemic and rare species in its dune vegetations. Biochemical characteristics of some plant species growing in Istanbul dunes, which are under immense threat during the recent years have been examined in the present study. Seed samples and aerial parts of the plants have been analysed for fatty acid and amino acid compositions. The higher concentrations of α-linolenic acid were quantified in Euphorbia paralias (48.24%) and Diplotaxis tenuifolia (24.47%). Linoleic acid concentrations were detected at the higher levels in Xanthium strumarium (68.51%) and Onanthus maritimus (66.62%). Eryngium campestre (44.97%), Cyperus capitus (44.27%) and Eryngium maritimum (41.88%) were found to have high oleic acid concentrations. 20.32% punicic acid was detected in Ecballium elaterium. The higher concentrations of some essential amino acids were observed in the seeds. Proline is the dominant amino acid in the aerial parts of all taxa examined. Significant correlations between amino acid concentrations were calculated. The data obtained in the present study reveal that regarding plants as novel crops can be efficient for utilization in biochemical and biotechnological applications in many fields such as human nutrition, health and other industrial purposes.

İstanbul’da Bazı Kumul Bitkilerinde Yağ Asidi ve Amino Asit Profilleri

Anahtar Kelimeler
İstanbul,
Kumul,
Tohum,
Halofit,
Yağ asidi,
Amino asit

Özet: Kumular, doğadaki en dinamik ekosistemlerden biridir ve değerli genetik kaynakları barındıran özel bir yağ alanı turnu olarak kabul edilirler. Türkiye’de bulunan kumul alanlarda birçok çeşitlilik açısından oldukça zengindir. İstanbul kumul bitkileri de endemik ve nadir türlerin zenginliğini açısından özel bir yere sahiptir. Bu çalışmada, İstanbul kumullarında yetişen ve son yıllarda büyük tehdir altında olan bazı bitki türlerinin biyokimyasal özellikleri incelenmiştir. Tohum örneklerinde ve bitkilerin toprak üstü sürgün kısımlarında yağ asidi ve amino asit bileşimleri analiz edilmiştir. Euphorbia paralias (%48.24) ve Diplotaxis tenuifolia (%24.47) türlerinde yüksek α-linoleneik asit konsantrasyonları ölçülmiştir. Xanthium strumarium (%68.51) ve Onanthus maritimus (%66.62)‘da yüksek linoleik asit düzeyleri tespit edilmiştir. Eryngium campestre (%44.97), Cyperus capitus (%44.27) ve Eryngium maritimum (%41.88) yüksek olı akid asit içeriklerine sahiptir. Ecballium elaterium‘da %20.32 oranında punikik asit saptanmıştır. Taksonların tohumlarında baza esansiyel amino asitlere yüksek düzeylerde rastlanmıştır. Prolin, incelenen tüm taksonların toprak üstü sürgünlerinde basilan olına amino asidir. Amino asit konsantrasyonları arasında anlamlı korelasyonlar gözlemlenmiştir (p<0.05). Elde edilen veriler, söz konusu taksonların yeni ürünler olarak beslenme, sağlıklı ve diğer endüstriyel alanlardaki biyokimyasal ve biyoteknolojik uygulamalarda kullanılabilmesiğini ortaya koymaktadır.

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1. Introduction

Coast ecosystems containing dune areas are important centers of genetic resources because of including unique species adapted extreme abiotic stress conditions. These areas under the anthropogenic pressure highly are getting less day by day and regarding conditions affects the biological diversity negatively. About 2500-3000 halophytic plant taxa live on earth from different phylogenetic origins [1]. This rate equals to 2% of terrestrial plants totally [4]. The plants living in dunes deal with various biotic and abiotic factors that risk their survivals for the adaptation to the extreme edaphic conditions in their habitats. On the other hand, there are various ethnobotanical usage of halophytic plants [2].

They are also used for forage plants, extraction of various medical and industrial products [3]. High diversity of halophytic plants have widespread distribution in Middle East and Anatolia. Approximately 300 halophyte from 150 genera and 40 families including dominantly Poaceae, Chenopodiaceae, Asteraceae, and Fabaceae are contribute the richness of floristic structure of Turkey. *Salsola* L., *Plantago* L., *Limonium* Mill., *Juncus* L. and *Trifolium* L. have the highest number of taxa among halophytes [4,5]. Turkey includes relatively much more dune areas compared to European countries. 845 km of the coast equaling about 10% of the total cost line (6335 km) are dune areas. The total range of 110 different sand dune regions in Turkey is 290,000 decares [6]. There are 75 coastal dune areas in Turkey and 9 of them are within the borders of Istanbul [7]. In general, Istanbul flora has very rich diversity [8,9]. Totally 2512 plant taxa has been identified in Istanbul at the levels of species, subspecies and variety. Total number of the diversity is approximately 2198 at species level [10]. Considering its limited surface area, the plant richness of Istanbul can be explained with its geographical location including Euro-Siberian and Mediterranean phytogeographical regions, geological structure, climatic and topographic conditions and having various habitat types. Dune plants in Istanbul distribute naturally in Terkos-Kasatura coasts, Ağaçlı dunes, Kilyos dunes, Sahilköy-Sile coasts that were accepted as important plant area in the previous projects [11]. Approximately 11.7% of endangered species in Turkey distribute in north dunes [12]. *Isatis arenaria* Azn., *Centaura kilaea* Boiss., *Silene sangaria* Coode&Cullen, *Euphorbia paralias* L., *Carduus pycnocephalus* L. were collected from dune areas in Istanbul including Kilyos, Terkos, Şile and Yeşilköy. Plant specimens have been pressed appropriately and dried. The identification of taxa were carried out properly using keys in the relevant volumes of the “Flora of Turkey and East Aegean Islands” [23,24]. Dried plant specimens and seeds have been kept at about -25℃ for at least one week to be purified from their biological contaminants. Seed samples and aerial parts of *Pancratium maritimum* L., *Otanthus maritimus* (L.) Hoffmanns. & Link, *Echallium elaterium* (L.) A. Rich., *Eryngium maritimum* L., *Cyperus capitatus* Vand., *Xanthium strumarium* L., *Diplotaxis tenuifolia* (L.) DC., *Eryngium campestre* L., *Leymus racemosus* (Lam.) Tzvelev subsp. *sabulosus* (Bieb). Tzvelev, *Jurinea kilaea* Azn., *Centaurea kilaea* Azn., *Silene sangaria* Coode&Cullen, *Euphorbia paralias* L., *Carduus pycnocephalus* L. were collected from dune areas in Istanbul including Kilyos, Terkos, Şile and Yeşilköy. Plant specimens have been pressed appropriately and dried. The identification of taxa were carried out properly using keys in the relevant volumes of the “Flora of Turkey and East Aegean Islands” [23,24]. Dried plant specimens and seeds have been kept at about -25°C for at least one week to be purified from their biological contaminants. Seed samples and aerial parts of the specimens have been kept in a dry place away from light and moisture for amino acid and fatty acid analyzes.

2. Material and Method

Seed samples and aerial parts of *Pancratium maritimum* L., *Otanthus maritimus* (L.) Hoffmanns. & Link, *Echallium elaterium* (L.) A. Rich., *Eryngium maritimum* L., *Cyperus capitatus* Vand., *Xanthium strumarium* L., *Diplotaxis tenuifolia* (L.) DC., *Eryngium campestre* L., *Leymus racemosus* (Lam.) Tzvelev subsp. *sabulosus* (Bieb). Tzvelev, *Jurinea kilaea* Azn., *Centaurea kilaea* Azn., *Silene sangaria* Coode&Cullen, *Euphorbia paralias* L., *Carduus pycnocephalus* L. were collected from dune areas in Istanbul including Kilyos, Terkos, Şile and Yeşilköy. Plant specimens have been pressed appropriately and dried. The identification of taxa were carried out properly using keys in the relevant volumes of the “Flora of Turkey and East Aegean Islands” [23,24]. Dried plant specimens and seeds have been kept at about -25℃ for at least one week to be purified from their biological contaminants. Seed samples and aerial parts of the specimens have been kept in a dry place away from light and moisture for amino acid and fatty acid analyzes.

2.1. Determination of oil content and fatty acids

Total oil content was determined using a Tecator Sxotec System HT (Foss Tecator AB, Horanas Sweden, Sweden). Powdered material (3 g) taken from the bulk of each sample, representing
individual populations, was added to an oil cartridge (W1), and 25–50 mL of diethyl ether was placed in a weighed extraction pot (W2). Extraction was carried out for 15 min with rinsing for 30–45 min. The extracted nutlet meal was thoroughly air dried to remove traces of solvent and dried at 105°C. The pots were placed in desiccators, cooled, and then weighed (W3). The percentage of oil (by weight) was calculated using the equation: %Oil = [(W3 – W2)/W1] × 100. The oil was then transferred to sealed amber glass bottles, which were capped and stored at −18°C until analysis. Fatty acids methyl esters were prepared according to IUPAC standard method [25]. Briefly, 0.1 g of seed oil was screwed into the test tube with a cap and was vortexed by adding 0.5 mL of 2.0 N KOH and 5 mL of heptane. Then, anhydrous sodium sulfate was added to the drying process. After 1 minute, the soaking solution was used directly for gas chromatography (GC, Perkin Elmer, Autosystem GLX, Shelton, USA). Chromatographic separation was carried out using a Supelco SPTM-2380 (30 m 0.25 mm inner diameter, 0.25 mm film thickness) column with a flame ionization detector (FID). The current conditions in the studies are as follows: carrier gas, helium; the flow rate was 0.5 mL / min; injector temperature, 280 °C; detector temperature, 260 °C; the oven temperature program, the first temperature was 120 °C for 2 minutes, increased at 58 °C / min, and kept at 220 °C for 10 minutes. Identification and quantification of FAME by FID response integration were accomplished by comparing the relative retention times of sample peaks with those of authentic standards (Sigma Code No. 189–19, Sigma–Aldrich Co.).

2.2. Determination of total protein and amino acids

Kjeldahl method (AOAC 1990) was used to determine total protein content. The total amount of nitrogen was determined by Kjeldahl analysis and the percentage of nitrogen was multiplied by 6.25 and converted to crude protein. UFLC-UV (Shimadzu) system equipped with a shim-pack XR-ODS (75 mmL. x 3 mm i.d.) was used to determine the amount of amino acids. Samples were hydrolyzed for 24 hours with 6N HCl at 110±1°C for 24 hours and the amino acids were derivatized to PTC (phenylthiocarbamyl) with a PITC (phenylisothiocyanate) reagent and detected with a UV detector at 254 nm. Seventeen components of PTC derivatized amino acid (100 μmol/L each) were analyzed and separated. The temperature was fixed at 40°C. Phosphate buffer/Acetonitrile gradient elution was used as mobile phase at a flow rate of 1.2 mL/min. [13].

2.3. Statistical analyses

Correlation analyses (Pearson) and statistical significance of amino acid and fatty acid data obtained from 10 taxa have been carried out with using SPSS 21 programme. Each value in the tables is the average of duplicate determinations.

3. Results

3.1. Total oil and fatty acid compositions in seeds

In the seeds of taxa examined, total fat (g/100g) amounts, saturated fatty acid, mono-unsaturated fatty acid, poly-unsaturated fatty acid levels (%) are given in the tables. Total amounts of fat are between 1.67 and 38.01%. The highest total fat contents among taxa was detected in Xanthium strumarium and Diplotaxis tenuifolia. The lowest level was observed in Leymus racemosus subsp. sabulosus. In general, palmitic acid (C16:0) and stearic acid (C18:0) values were quantified at high levels in all taxa. Myristic acid(C14:0) concentration was detected at the highest level in Leymus racemosus subsp. sabulosus. Other saturated fatty acid concentrations determined at the low levels generally. It has been observed that the highest concentration of lauric acid (12:0) (0.96%) in Silene sangaria; myristic acid in Leymus racemosus subsp. sabulosus (4.40%); palmic acid in Pancratium maritimum (16.31%); heptadecanoic (C17:0) in Leymus racemosus subsp. sabulosus (7.01%); arachidic acid (C20:0) in Diplotaxis tenuifolia (1.35%); behenic acid (C22:0) in Leymus racemosus subsp. sabulosus (1.0%) and lignoceric acid (C24:0) in Diplotaxis tenuifolia (0.53%). The concentration values of saturated fatty acids in the seeds of taxa was documented in Table 1.

Among mono-unsaturated fatty acids, oleic acid (C18:1n9c) concentrations were detected at high levels generally in all taxa. The highest quantities of eicosanoic acid (C20:1n9) and erucic acid (C22:1n9) were examined in Diplotaxis tenuifolia. It has been observed that other mono-unsaturated fatty acids are at the low levels. The highest concentration of palmitoleic acid (C16:1) in the species Leymus racemosus subsp. sabulosus (0.64%); oleic acid in Eryngium campestr (44.97%); eicosanoic acid (C20:1n9) (6.67%), erucic acid (18.71%) and nervonic acid (C24:1n9) in Diplotaxis tenuifolia (0.59%). This species has a considerable potential for mono-unsaturated fatty acids. The values of mono-unsaturated fatty acids in examined taxa were given in the Table 2.

On the other hand, linoleic acid (C18:2n6) as polyunsaturated fatty acid exhibit the highest concentrations generally in all taxa between 11.40% (in Leymus racemosus subsp. sabulosus) and 68.51% (in Xanthium strumarium). It has been observed that the concentrations of alpha-linolenic acid (C18:3n3) was high in Convolvulus persicus, Diplotaxis tenuifolia, Jurinea kilaea, Silene sangaria, Euphorbia paralias, Echium album subsp. nutans contain the highest punicic acid (C18:3n5) level (20.32%). The other fatty acids were quantified at lower levels generally. Poly-unsaturated fatty acid contents in the seeds of taxa used was documented in Table 3.
The amount of total fatty acid (g/100g) in the seeds of taxa is the range from 1.67 to 38.01. Considering the saturated and unsaturated fatty acid ratios in the species analyzed, it has been observed that saturated fatty acids in total range from 6.51 to 40.22 (%); the ratios of total mono-unsaturated fatty acid is in the range 11.98 to 45.11 (%); total poly-unsaturated fatty acids 12.35 to 72.56(%) and total unsaturated fatty acid ratio range from 24.33 to 90.78(%). In terms of the amount of total oil, the highest level was detected in seeds of *Xanthium strumarium*. Total saturated in

**Table 1. Saturated fatty acid levels (%)**

| Taxa                        | C12:0 | C14:0 | C16:0 | C17:0 | C18:0 | C20:0 | C22:0 | C24:0 |
|-----------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| *Pancratium maritimum*      | 0.01  | 0.23  | 16.31 | 0.08  | 3.08  | 0.34  | 0.12  | 0.08  |
| *Convolvulus persicus*      | 0.22  | 0.81  | 14.18 | 0.09  | 3.32  | 0.64  | 0.34  | 0.18  |
| *Otanthus maritimus*        | 0.01  | -     | 12.19 | 0.09  | 2.23  | 0.46  | 0.16  | 0.11  |
| *Ecballium elaterium*       | 0.01  | 0.15  | 6.93  | 0.05  | 4.70  | 0.14  | 0.02  | 0.03  |
| *Eryngium maritimum*        | 0.06  | 0.08  | 5.26  | 0.03  | 1.65  | 0.18  | 0.06  | 0.04  |
| *Cyperus capitatus*         | 0.02  | -     | 5.55  | 0.04  | 3.20  | 0.35  | 0.51  | 0.13  |
| *Xanthium strumarium*       | 0.01  | 0.06  | 5.39  | 0.04  | 1.90  | 0.09  | 0.47  | 0.15  |
| *Diploptaxis tenuifolia*    | 0.01  | 0.07  | 5.16  | 0.04  | 2.25  | 1.35  | 0.84  | 0.53  |
| *Eryngium campestrum*       | 0.02  | -     | 5.25  | 0.05  | 0.87  | 0.08  | 0.07  | 0.05  |
| *Leymus racemosus*          | 0.35  | 4.40  | 23.5  | 0.93  | 7.01  | 0.85  | 1.00  | 0.38  |

**Table 2. Mono-unsaturated fatty acid levels (%)**

| Taxa                        | C16:1 | C18:1n9c | C20:1n9 | C22:1n9 | C24:1n9 |
|-----------------------------|-------|----------|---------|---------|---------|
| *Pancratium maritimum*      | 0.11  | 30.25    | -       | 0.02    | 0.02    |
| *Convolvulus persicus*      | 0.15  | 27.68    | 0.17    | -       | -       |
| *Otanthus maritimus*        | 0.32  | 14.46    | 0.14    | -       | 0.01    |
| *Ecballium elaterium*       | 0.02  | 11.92    | 0.16    | -       | 0.03    |
| *Eryngium maritimum*        | 0.40  | 41.88    | 0.11    | 0.01    | 0.01    |
| *Cyperus capitatus*         | 0.05  | 44.27    | 0.45    | 0.01    | 0.02    |
| *Xanthium strumarium*       | 0.09  | 21.86    | 0.15    | -       | 0.02    |
| *Diploptaxis tenuifolia*    | 0.34  | 13.25    | 6.67    | 18.71   | 0.59    |
| *Eryngium campestrum*       | 0.33  | 44.97    | 0.08    | 0.11    | 0.02    |
| *Leymus racemosus* subsp. s. | 0.64  | 10.85    | 0.18    | 0.13    | 0.10    |
| *Jurninea kilaea*           | 0.31  | 30.02    | 0.34    | -       | 0.07    |
| *Centarea laeia*            | 0.10  | 17.17    | 0.15    | -       | 0.01    |
| *Silene sangaria*           | 0.21  | 21.04    | 0.32    | 0.03    | -       |
| *Euphorbia paralias*        | 0.14  | 22.02    | 0.55    | 0.04    | 0.05    |
| *Carduus pycnocephalus*     | 0.13  | 17.8     | 0.12    | -       | -       |

**Table 3. Poly-unsaturated fatty acid levels (%)**

| Taxa                        | C18:2n6 | C18:3n6 | C18:3n3 | C18:3n5 | C20:2 | C20:3n3 |
|-----------------------------|---------|---------|---------|---------|-------|---------|
| *Pancratium maritimum*      | 47.64   | -       | 0.22    | -       | -     | -       |
| *Convolvulus persicus*      | 46.93   | 0.32    | 1.34    | -       | 0.03  | 0.01    |
| *Otanthus maritimus*        | 66.62   | -       | 0.11    | -       | 0.03  | -       |
| *Ecballium elaterium*       | 52.11   | -       | 0.10    | 20.32   | 0.03  | -       |
| *Eryngium maritimum*        | 28.69   | 0.06    | 0.28    | -       | -     | -       |
| *Cyperus capitatus*         | 43.42   | -       | 0.18    | -       | 0.12  | -       |
| *Xanthium strumarium*       | 68.51   | -       | 0.12    | -       | 0.01  | -       |
| *Diploptaxis tenuifolia*    | 18.68   | 0.11    | 24.47   | -       | 0.84  | 0.38    |
| *Eryngium campestrum*       | 18.63   | 0.03    | 0.43    | -       | -     | -       |
| *Leymus racemosus* subsp. s. | 11.40   | -       | 0.67    | -       | 0.15  | -       |
| *Jurninea kilaea*           | 29.75   | 0.03    | 4.73    | -       | 0.01  | -       |
| *Centarea laeia*            | 58.24   | 0.01    | 0.24    | -       | 0.01  | -       |
| *Silene sangaria*           | 56.67   | 0.05    | 0.94    | -       | 0.10  | -       |
| *Euphorbia paralias*        | 14.64   | 0.20    | 48.24   | -       | 0.03  | 0.01    |
| *Carduus pycnocephalus*     | 64.61   | -       | 0.17    | -       | 0.01  | 0.03    |

While generally higher concentrations of palmitic, stearic, linoleic and oleic acid are common for all the species, some fatty acids are at higher levels for some species.
The concentration of α-linolenic acid was examined quite high in the species Euphorbia paralias and Diplotaxis tenuifolia. Besides, Ecballium elaterium contains remarkable level of punicic acid.

Similarly, erucic acid exhibits high concentration in Diplotaxis tenuifolia. Investigated taxa from different phylogenetic origins exhibit characteristic fatty acid profiles in the same edaphic conditions.

### 3.2. Total protein and amino acid compositions in seeds and aerial parts

Total protein contents (g/100g) and amino acid concentrations (mg/100g) were analysed in the seeds and aerial parts of taxa. The amount of total proteins were observed between 5.98 to 36.62% in the seeds. While the lowest total protein amount was in the seeds of *Cyperus capitatus*, the highest content was examined in *Xanthium strumarium*. *Diplotaxis tenuifolia* has also higher total protein with 24.16 ratio. Similar amounts of total proteins between 13.97 and 16.40 were detected in the seeds of *Pancratium maritimum*, *Ecballium elaterium*, *Eryngium maritimum* and *Diplotaxis tenuifolia*. On the other hand, total protein amounts of the examined taxa (Table 5) show broad range of this amino acid in *Carduus pycnocephalus* and *Eryngium campestre*. The lowest total protein content in the aerial parts of *Leymus racemosus subsp. sabulosus* and *Carduus pycnocephalus* were found. *Centaurea kilaea*, *Cyperus capitatus*, *Silene sangaria*, *Euphorbia paralias* and *Carduus pycnocephalus* show the lower levels of total protein in their aerial parts between 5.15 and 6.63 (Table 5). On the other hand, considerable differences in the seeds of examined amino acid concentrations among taxa were detected (Table 6). While the concentrations (mg/100g) of aspartic acid are higher in *Pancratium maritimum*, *Ecballium elaterium*, *Xanthium strumarium* and *Diplotaxis tenuifolia*, it has been detected as low levels in the species *Otanthus maritimus*, *Jurinea kilaea*, *Cyperus capitatus*. The ratio of glutamic acid has been quantified at high levels generally. Similar values of glutamic acid were observed in *Ecballium elaterium* and *Eryngium maritimum*, but, remarkable high concentration of this amino acid was detected in *Xanthium strumarium* (8836). Serine amino acid has also been detected at high level in *Xanthium strumarium* and *Diplotaxis tenuifolia*.

### Table 5. Total protein amounts of the examined taxa

| Taxa                              | Total protein amounts (g/100g) |
|-----------------------------------|--------------------------------|
| *Pancratium maritimum*            | 16.49                          |
| *Convulvulus persicus*            | 10.93                          |
| *Otanthus maritimus*              | 7.98                           |
| *Ecballium elaterium*             | 15.18                          |
| *Eryngium maritimum*              | 13.97                          |
| *Cyperus capitatus*               | 5.98                           |
| *Xanthium strumarium*             | 36.62                          |
| *Diplotaxis tenuifolia*           | 24.16                          |
| *Eryngium campestre*              | 10.77                          |
| *Leymus racemosus subsp. sabulosus* | 2.18                           |
| *Jurinea kilaea*                  | 8.17                           |
| *Centaurea kilaea*                | 5.16                           |
| *Silene sangaria*                 | 6.02                           |
| *Euphorbia paralias*              | 6.29                           |
| *Carduus pycnocephalus*           | 6.63                           |

*Analyzes of samples obtained from aerial parts.*

Glycine has been detected at a high level in *Pancratium maritimum*, *Ecballium elaterium*, *Eryngium maritimum*, *Xanthium strumarium* and *Diplotaxis tenuifolia*.

Histidine, one of the essential amino acid was observed at high concentration (1388) in *Xanthium strumarium*. The highest quantities of arginine in *Pancratium maritimum* (1674) and the lowest one in *Otanthus maritimus* (322) show broad range of this amino acid. Alanine and proline have been observed at higher levels in *Pancratium maritimum*, *Diplotaxis tenuifolia* and *Xanthium strumarium*. While threonine as one of the essential amino acid was examined at lowest level (232) in *Eryngium campestre*, the highest quantities (1279) observed in *Xanthium strumarium*. Similar values of tyrosine has been detected in *Otanthus maritimus* and *Cyperus capitatus*. Valine and phenylalanine were found at the higher levels in.
Pancratium maritimum, Diplotaxis tenuifolia, Xanthium strumarium. In Pancratium maritimum, Ecballium elaterium and Eryngium maritimum, the level of leucine and lysine amino acid has been observed at the higher concentrations. Methionine, as one of the essential amino acid exhibits low levels generally in all taxa, but Xanthium strumarium contains this amino acid considerably high concentrations in its seeds (624). Oenanthis maritimus which generally contains other amino acid at low levels comes in the third line after the species Xanthium strumarium, Diplotaxis tenuifolia in terms of methionine content. In general, Xanthium strumarium, Diplotaxis tenuifolia, Ecballium elaterium and Pancratium maritimum have higher concentrations of all amino acid examined parallel with their total protein contents in the seeds.

Considering the aerial parts of taxa, amino acid compositions were quantified at lower levels compared to the results obtained from the seed samples (Table 7). Similar amino acid concentrations have been detected in Centaurea kilaea, Silene sangaria, Euphorbia paralias and Carduus pycnocephalus, lower levels observed in Leymus racemosus subsp. sabulosus. Aerial parts of Silene sangaria have the richest concentrations of amino acid compared to the regarding species examined. Higher concentrations of aspartic acid and glutamic acid have been detected in the aerial parts of Silene sangaria and Carduus pycnocephalus. Close values of serine, glycine and arginine were determined in the aerial parts of taxa except for Leymus racemosus subsp. sabulosus. Generally lower levels of histidine, as an essential amino acid were quantified in the aerial parts of examined taxa, except for Silene sangaria containing higher amount relatively. Aspartic acid, glutamic acid, histidine, threonine, proline and isoleucine were found remarkably higher concentrations in Silene sangaria compare to the aerial parts of other taxa examined. Tyrosine, valine, phenylalanine, leucine and lysine have been detected in the Carduus pycnocephalus at the highest concentrations among the species whose aerial parts have been examined. The concentration of methionine, as one of the essential amino acid has been observed at generally low levels, but it has been measured in Euphorbia paralias at the highest level. Close values range from 185 to 236 (mg /100g) of isoleucine were tested in Centaurea kilaea, Euphorbia paralias, Carduus pycnocephalus and Silene sangaria. As essential amino acids, valine, leucine, phenylalanine and lysine were detected at the highest concentrations in Carduus pycnocephalus.

Proline is the most abundant amino acid in the aerial parts of regarding taxa (Figure 1), contrary histidine, methionine and aspartic acid showed lower levels.

Leymus racemosus subsp. sabulosus has the lowest concentrations of all amino acid generally parallel with its total protein contents. Eryngium maritimum and Eryngium campestre showed generally parallel amino acid profiles (Figure 2), but glutamic acid, glycine and arginine has been found considerably higher levels (Figure 2).

![Figure 1. Concentrations of proline in the seeds of examined taxa](image)

Table 6. Amino acid concentrations (mg/100g dry wt.) for the examined taxa and comparison of the values with FAO/WHO/UNU (1985) estimates of amino acid requirements in children and adults. Each value for total protein and amino acid concentrations is the average of double determinations.

| Taxa              | Asp | Glu | Ser | Gly | His* | Arg | Thr* | Ala | Pro | Tyr* | Val* | Met* | Ile* | Leu* | Phe* | Lys* |
|-------------------|-----|-----|-----|-----|------|-----|------|-----|-----|------|------|------|------|------|------|------|
| P. maritimum      | 142 | 1043| 701 | 1459| 250  | 127 | 1043 | 250 | 127 | 1043 | 250  | 127  | 1043 | 250  | 127  |
| C. persicus       | 774 | 1822| 499 | 655 | 277  | 544 | 467  | 377 | 594 | 89   | 451  | 839  | 662  | 1210 | 1174| 1139 |
| O. maritimus      | 150 | 999 | 339 | 578 | 152  | 475 | 535  | 270 | 502 | 102  | 373  | 723  | 519  | 696  |     |
| E. elaterium      | 1553| 2665| 572 | 1016| 428  | 635 | 640  | 450 | 648 | 35   | 638  | 848  | 1748 |     |
| E. martireium     | 463 | 2725| 507 | 1311| 388  | 709 | 804  | 507 | 750 | 73   | 689  | 1051 | 784  | 1198 |     |
| C. capitisus      | 110 | 621 | 196 | 444 | 74   | 427 | 659  | 260 | 350 | 50   | 166  | 666  | 552  | 89   |     |
| X. strumarium     | 2444| 8836| 2051| 2659| 1388 | 1475| 2204 | 1138| 1801| 624  | 1462 | 2360 | 2202 | 1570 |     |
| D. tenuifolia     | 1457| 4711|1127 |1780 | 792  | 1108| 1908 | 873 |1429 |328  | 1764 | 1173 | 2223 |     |
| E. campestre      | 298 | 1439| 381 | 815 | 132  | 581 | 624  | 390 | 454 | 36   | 788  | 570  | 891  |     |
| J. kilaea         | 151 | 666 | 303 | 661 | 162  | 563 | 739  | 284 | 572 | 64  | 428  | 919  | 634  | 690  |     |
| **2-5 years**     | -   | -   | -   | -   | -    | -   | -    | -   | -   | -    | -    | -    | -    | -    | -    |
| **10-12 years**   | -   | -   | -   | -   | -    | -   | -    | -   | -   | -    | -    | -    | -    | -    | -    |
| **Adult (+18)**   | -   | -   | -   | -   | -    | 90  | -    | 190 | 130 | 130  | 130  | 190  | 190  | 160  |     |

* Essential amino acids.
** FAO/WHO/UNU (1985) estimates of amino acid requirements in children and adults.
†Total amount of tyrosine and phenylalanin.
Significantly positive correlations among investigated taxa based on amino acid concentrations were calculated. Similarly, there is a positive correlations in terms of amino acid contents between *Eryngium maritimum* and *Otanthus maritimus* \((p<0.05)\) (Figure 3). A significant positive correlation was also calculated between leucine and valine in the seeds of all taxa examined \((p<0.05)\) (Figure 4). In general, characteristic compositions of amino acids were determined in taxa from different phylogenetic origins.

**Figure 2.** Comparison of amino acid contents of *Eryngium maritimum* and *Eryngium campestre* species

**Figure 3.** Comparison of amino acid contents of *Eryngium maritimum* and *Otanthus maritimus* species

**Figure 4.** Concentrations of leucine and valine in seeds of examined taxa

**4. Discussion**

**4.1. Evaluation of the results on fatty acids**

Plant genetic resources occurring in extreme habitat conditions with biotic and abiotic stress factors have special importance in terms of containing valuable genes and products for maintaining agricultural production in changing environmental conditions affected with global warming, anthropogenic effects, new pathogens, ever increasing soil salinity in agricultural fields etc. Halophytic vegetation have great potential in finding some useful solutions for the sustainability of crop plants in cultivation areas. Determining diversity and application of conservation strategies, characterisation of genetic resources and understanding adaptation mechanisms by genomic, proteomic and metabolic approaches will provide valuable input and opportunity for improvement of crop plants in the future perspective.

In the present study, it was aimed to determine fatty and amino acid patterns as chemometric parameters in order to observe chemotaxonomic relation and chemical convergence of some halophytes in the similar abiotic stress conditions, in addition to their alternative product potential as novel crops in soil effected soils. Total fat content and fatty acid profiles of seed plants are widely used in chemotaxonomy of various plant groups as intelligible patterns. But, some variations in total content of fat and their fatty acid compositions in the seeds were reported to be related with environmental conditions, spatial distribution, features of growing period, maturation stage of the seeds, intraspecific and taxonomic differentiations effected with genetical characteristics [26]. For example, total amount of fat was found as 10.70 \((\text{g/100g})\) in studies on *Convolvulus tricolor* L. [27]. In the present study, the total fat content of *Convolvulus persicus* L. was detected at the level of 10.65. Similarly, total amount of fat \((\text{g/100g})\) in *Cyperus capitatus* Vand. seeds was determined as 8.20. This ratio was 6.60 in its close relatives *Cyperus rotundus* L. distributing in dune regions [28]. In the current study, total fat \((\text{g/100g})\) in the seeds of *Leymus racemosus* subsp. *sabulosus* was determined as 1.67, parallel with the results on Gramineae, such as *Triticum vulgare* L. with 2.08, *Hordeum vulgare* L. with 1.37 and *Avena sativa* L. with 5.12 total oil as considerably higher level [29,30,31]. These data show that total fat contents of the species at generic level are tend to be similar and suggest that the oil contents may be useful and additional tool at the generic level in taxonomical delineations. On the other hand, high level of unsaturated fatty acid contents of some dune species were reported in a previous studies [13], that may be related with high germination ratio of the seeds in salinity conditions. In our study, generally higher concentrations of unsaturated fatty acid contents were observed. For example, up to 90% saturated fatty acid ratio with high level of poly-unsaturated fatty acid of 68% was detected in *Xanthium strumarium*. Linoleic, oleic and palmitic acid are the most abundant fatty acids individually in all taxa studied from different phylogenetic origins, implying the similar reaction to the same habitat conditions, as may be defined as chemical convergence. The concentrations of linoleic
acid of the seeds analyzed have been observed at high rates. Linoleic acid is known as a fatty acid as strengthening immune system. Conjugate linoleic acid consisting of linoleic acid's isomers coming together causes to show anti-carcinogenic effect along with decreasing insulin resistance in diabetes [32]. Linoleic acid concentration was determined as 68.51% in Xanthium strumarium species and 66.62% in Otanthus maritimus species. Euphorbia paralias (48.24%) and Diploptaxis tenuifoila (24.47%) have considerable amounts of omega-3 alpha linolenic acid having great importance in various metabolic activities and cardiovascular health [33]. The plants growing in the lands contain ω-6 essential fatty acids at the most abundant generally, but they have limited content in terms of ω-3 fatty acids [34]. In our observations, concentrations of omega-6 fatty acids are also tend to be higher in the seeds of dune plants generally, parallel with the other studies [35]. Accumulation of higher quantities of linoleic acid in land plants including halophytes may be related with the down regulation of delta-15 desaturase activity responsible for the transformation of linoleic acid to omega-3 alpha linolenic acid. Considering the mono-unsaturated fatty acid contents in the seeds of examined taxa, oleic acids as ω-9 fatty acid was found at the higher concentrations up to 45% in Eryngium campestre. Oleic acid as remarkable component of sand dune species within this study was reported to prevent the possible arteriosclerosis and decrease the available arteriosclerosis by joining in the structure of HDL(high density lipoprotein) [36]. Besides, it has been revealed that oleic acid decreases the risk of having breast and prostate cancer [37]. It has been found that oleic acid plays a role in the prevention of cardiovascular diseases [38]. Dun species can be evaluated as alternative oleic acid resources and adaptation of oleic acid rich crop plants to the salt affected soils by means of breeding and biotechnological crop improving programs. Besides, punicic acid as an unusual omega-5 fatty acid has been detected at the considerable level up to 20.32% in the seeds of Ecballium elaterium having broad tolerance to various edaphic conditions, corresponding with the previous result at about 22% percent in the seeds [39,40]. Punicic acid, a special conjugate form of linoleic acid is found in the stone of pomegranate at high ratio (64-83%). With oil burning feature, punicic acid has a potential to be used against obesity [41]. It was also reported that punicic acid has an important role in preventing breast cancer [42]. Ecballium elaterium may be evaluated as the raw material for punicic acid extraction in order to fight with obesity, some cancers and for diet practices as functional ingredient. On the other hand, the concentration of erucic acid has been observed in the seeds of Diplotaxis tenuifoila at 18.71 percent. Our observation is highly compatible with the previous studies conducted on Diplotaxis tenuifoila [43]. One of the specific plants of dunes, Cakile maritima Scop. from the same families (Brassicaceae) was reported to have relatively higher level of erucic acid with 23.20% [44]. This fatty acid found abundantly in Brassicaceae can be extracted from the members of halophytic vegetations productively for industrial purposes. Considering the compositions of saturated fatty acids, palmitic and stearic acid were quantified at the higher concentrations in the seeds of all studied taxa compare to the other saturated fatty acids generally. Palmitic acid is used commonly in cosmetic sector [45]. Stearic acid is the valuable ingredient in making water-proof fabric, the production of candle and soap and the cosmetic industry. Palmitic and stearic acids are also found in the structure of organ oil which is used in cosmetic industry as anti-aging and anti-wrinkle features. The fact that palmitic acid is found in animal organism at high concentrations reveals its important functions in lipid metabolism. Panctratium maritimum, Convolvulus persicus and Otanthus maritimus for palmitic acid, and Leymus racemosus subsp. sabulosus and Ecballium elaterium for stearic acid have raw material potential for exploitation of these fatty acids.

4.2. Evaluation of results on amino acids

Different amino acid compositions are effective in developing of proteins with specific characteristics. Composition of amino acids in any protein determined by genomic features is related with its specific structure and functions. Amino acid content and composition as the components of proteomic data are also useful tool in dividing plant groups into different taxonomical level [13]. For example, leucine and alanine concentrations in the grains are reported to be at a lower, but lysine and glycine are at higher levels [46,47]. Taxonomical delimitations of Turkish Quercus taxa were also suggested based on amino acid compositions at infrageneric level [21]. On the other hand, accumulation of some amino acids is related with edaphic conditions. For instance, the higher concentrations of proline in the halophytic plants have been reported as a strategy for struggling with salt stress. Proline is an amino acid which protects cell membranes and proteins by stabilizing them. In the plants, proline can be synthesized in two ways as glutamate and ornithine. Under osmotic stress, the way of glutamate is main source for producing protein [48]. The fact that proline accumulates in cytosol depending on the salt and drought stress in plants has been detected by the previous studies [49]. It has also been detected that proline amino acid accumulates in cytosol at high concentrations as a respond to drought and salt stress in plants. Effects of water stress induced by sodium chloride and mannitol were reported on proline accumulation, photosynthetic abilities and growth characters of eucalyptus [50]. It has been observed that proline concentration increases in the leaves of rice plants which are exposed to drought stress [51]. In many studies, halophytes include proline amino acid at high concentrations. In a study conducted during autumn, proline amino acid has
been found in earthy surfaces of *Pancratium maritimum* by 6.118 mg/100g and in *Cakile maritima* by 4989 mg/100g [52]. Proline constitutes more than 70% of amino acid pool in *Triglochin maritima* L. and *Puccinellia maritime* Parl. [53]. It has also been indicated that accumulation of proline at high concentrations under salty conditions has an importance for providing an osmotic balance. It was also reported that increasing concentrations of proline was detected in the corn with salt application [54].

All these data correspond to the results obtained in the present study and show that level of proline increases under the salt stress.

*Xanthium strumarium* (2204 mg/100g), *Diplotaxis tenuifolia* (1908) and *Pancratium maritimum* (1204) are the species accumulating the highest levels of proline. Besides, lysine, glycine, arginine were reported to be detected at the high concentrations in the xeric species [55], parallel with our observations generally. Significantly positive correlations in investigated amino acid concentrations were calculated in taxonomically related species (Figure 5).

Concentrations of proline, alanine, leucine and valine exhibit parallel accumulation patterns in examined taxa, that may be evaluated as marker amino acids produced by the induced pathways in saline soils.

When we have examined the correlations among 16 amino acids within the scope of statistical data analyses, it is possible to say that almost all the amino acids have shown the increases in parallel with one another. As a result of statistical analyses conducted on amino acids such as glutamic acid, lysine, proline, glycine that are observed at high concentrations in the seeds of the species have been determined to have a positive correlation with each other. A significantly positive correlation between glutamic acid and histidine, leucine and valine has been also detected. A negative correlation between methionine, phenylalanine and arginine have been detected (Figure 5). When the statistical data are examined in general, it has been revealed that there is an important connection among amino acid contents of the species from different taxonomic origins but sharing the same habitats. Regarding correlations and similarities observed in the species may be evaluated as biochemical convergence reflecting induced pathways for the adaptation to saline conditions. Sodium salt in different chemical structures cause accumulation of different materials within halophytic plants. The *Prosopis strombulifera* (Lam). Benth. grown at high concentrations of NaCl has been detected to have proline amino acid in its cytoplasm at a higher concentrations compared to the plants in Na$_2$SO$_4$ [56]. All these data correspond with the findings in the present study generally.

![Figure 5. Correlations between 16 amino acids in seeds of examined taxa](image-url)
The level of methionine has been reported at the concentration of 23.2 (mg/100g) in *Suaeda asparagoides* Miq, which is one of the halophytic plants [57]. In the present study, methionine as an essential amino acid was generally found at lower concentrations compared to other amino acids. But considerably higher concentrations of this amino acid than above species were quantified both in the seed and aerial parts except for *Leymus racemosus* subsp. *sabulosus*. Methionine has been observed in this study at higher levels in *Xanthium strumarium* (624), *Diplotaxis tenuifolia* (328), *Euphorbia paralias* (161) and *Otanthus maritimus* (102).

Considering the salt stress induced accumulation of some amino acids, diversity of halophytic vegetation may be evaluated for exploitations of some nutritionally valuable bioactive substances. It has been detected that the seeds of *Xanthium strumarium*, *Diplotaxis tenuifolia* and *Pancratium maritimum* are very rich in terms of amino acid compositions. For example, glutamic acid (8836 mg/100g) in *Xanthium strumarium*, aspartic acid (1555) in *Ecballium elaterium*, serine (1780) in *Diplotaxis tenuifolia*, valine (1342) in *Pancratium maritimum* and lysine (1210) in *Convolvulus persicus* have valuable product potential as alternative sources of plant based proteins. On the other hand, richness of essential amino acids in a protein is also important in terms of nutritional value. Considering the FAO reference values for amino acids [58], investigated taxa as the members of halophytic vegetation have great potential for human nutrition as alternative plant sources in order to extraction of active amino acid ingredients for the production of functional foods.

Analysing the biochemical compositions of halophytes will provide valuable data for adaptation strategy of the species from the metabolomic point of view. Characteristic accumulation patterns of bioactive substances are also useful parameters for taxonomical delineations. In the present study, investigated taxa adapted to abiotic stress conditions have great potential as alternative agricultural plants. They are also used as genitor through selection and transferring of the appropriate genes for the purpose of adaptation of crop plants to the changing farming ecosystems. Salt resistant plants provide opportunity for preventing drought and salinity and brackishness, maintaining agricultural cultivation in agro ecosystems and farming lands being salted, enabling the adaptation of crop plants to abiotic stress conditions and increasing the yield. It will be useful to create biochemical and genetic databases of halophytes and the other species growing saline soils and various extreme habitat conditions to contribute to sustainable environment and sustainable agriculture vision.

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