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Amino Acid Patterns in the Aerial Parts of *Echium* L. and *Anchusa* L. Growing in the Sand Dunes of Turkey

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Two species species of *Echium* and two of *Anchusa* growing naturally in the sand dunes were analysed for total protein and amino acid compositions in their aerial parts. Total protein contents were detected between 7.55% (*Echium italicum*) and 12.97% (*Anchusa officinalis*). Valuable concentrations of the essential amino acids were detected in all species generally. The highest quantities of the amino acids were obtained from *A. officinalis*, while the lowest levels were detected in *E. italicum* except for aspartic acid and glutamic acid. Broad range of concentrations for leucine (544-1497 mg / 100 g) and lower levels in methionine (127-289 mg / 100 g) as essential amino acids were observed in all species. Leucine (1497 mg / 100 g), alanine (1339 mg / 100 g), phenylalanine (1016 mg / 100 g), threonine (935 mg / 100 g), proline (935 mg / 100 g), glycine (907 mg/ 100 g) and valine (859 mg / 100g) exhibited critical levels in *A. Officinalis*. Concentrations of some amino acids including aspartic acid, glutamic acid, phenylalanin, histidine, gycine and serine suggested discriminative patterns at generic level. Significant differences were calculated ($p<0.001$) in amino acid quantities of the species as additional chemotaxonomical markers. Essential amino acids were found at considerable levels compared to FAO reference values. Investigated species as alternative rich sources of essential amino acids may be evaluated as new crops which can be cultivated in saline soils and improving of the agricultural crops in the field.

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1. INTRODUCTION

Boraginaceae with ca. 100 genera and 2000 species in the tropical and temperate regions of the world is a very large family in the plant kingdom. As an important origin center of this family, Turkey comprises about 32 genus and 315 species distributed in various habitat conditions [1]. The ratio of endemism is very high at specific level (35%). The Mediterranean region is an especially well-represented area for Boraginaceae having great diversity. Apart from taxonomical studies based on its morphological, micromorphological and anatomical features of some vegetative and generative organs in addition to palinological observations, phylogenetic relationships in the tribe Boragineae were also suggested on the basis of comparative sequencing of the trnL intron of the plastid genome and of the ITS1 region of the nuclear ribosomal DNA [2]. In general, the family contains some plant-based toxins such as pyrrolizidine alkaloids associated with harmful effects in both humans and animals [3,4]. In the framework of this study, genera *Echium* and *Ancusa* have broad range of distribution in Anatolia with various ecological populations as variable species. In addition to traditional taxonomical observations, few studies on Turkish accessions of these genera were conducted to determine fatty acid profiles of the seeds [5-7] and populational molecular variations [8]. The genus *Echium* with ca.60 species has large distribution zones in West Asia, Europe, North and South Africa and Macaronesian islands with high endemism ratio, as well as parts of East Asia [9,10]. Nine species in the flora of Turkey distribute almost throughout Turkey in limestone slopes, fallow fields, sund dunes, steppe, disturbed ground and vast place between sea level to 1950 m. Therefore, broad variations in morphological features were reported [1]. It contains high concentrations of unusual fatty acids including gamma linolenic (18:3n6) and stearidonic acid (18:4n3) having in some cases therapoetic effects like cardiovascular and otoimmune disease [11,8]. On the other hand, *Anchusa* includes about 40 species distributing in Europe, North Africa, South Africa and Western Asia. Fifteen *Anchusa* species were recorded in the Flora of Turkey growing native in different habitats in Turkey. Several *Anchusa* species are used as analgesic, demulcent, sedative and hypotensive purposes in the folk medicine practices of many countries with valuable contents of unsaturated fatty acids [12], triterpene glycosides and flavonoids [13]. *Anchusa officinalis* L. is a variable species for some morphological features and covering very large distributional areas in Anatolia. Delimitation of the informal groups of *A. officinalis* was recommended using fatty acid profiles as additional biochemical markers [12]. Besides, *Anchusa undulata* subsp. *hybrida* is widespread in the sand dune vegetations and coastal zones having rich diversity of halophytes classified as extremophile species, inhabiting extremely salinized and arid environments. Thirty percent of the Earth's surface is severely affected by increased salinization constraining also crop production. Many halophytes highly productive under saline conditions which can tolerate high NaCl concentrations more than 200 mM are considered as alternative potential nutritional sources with high nutritional value for cultivation in arid environments of the poorer regions of the world. Approximately 300 halophytic taxa were reported from Turkey [14] which can be evaluated as raw material for the production of valuable compounds and improving of agricultural crops in the salt affected soils. Wide range of saline environments naturally occurring in Anatolia include great diversity in species and ecological varieties well adapted to extreme conditions. Investigated species growing also in the saline habitats as facultative halophytes show broad diversity in Anatolian gene pool and have great potential as new crops for the production of bioactive substance such as unusual long chain unsaturated fatty acids including gamma linolenic acid (18:6n3) and stearidonic acid (18:3n4), and essential amino acids which are indispensible compounds in nutritional requirements. In our recent study carried out on the sand dune vegetation species, high potential of some halophytic species for the production of unsaturated fatty acids was revealed [15]. On the other hand, non-random systematic variations of total protein amino acid composition observed in various plant groups are valuable taxonomic tool [16-18]. Amino acid profiles of different organs exhibite some characteristic differences useful for specific delineations [19-21]. But, ecological conditions affect the level of amino acid concentrations by inducing the production of some adaptive proteins. For example, changeable patterns of free amino acid concentrations and crude protein contents was reported under salinity stress conditions [22] compared to the non-stress (control) with respect to plant species and seasonal variation [23]. Similarly, higher salinity in *Cirrenalia pygmea*, a mangrove fungus led to an increase in the amino acid pool size and the number of amino acids produced [24]. Valuable essential amino acid contents such as lysine and methionine in highly soluble globulin storage protein of halophytic *Batis maritima* was also reported [25]. Regarding all species with high tolerance capacity to various habitat conditions have great potential to produce considerable fitomass in halophytic vegetations. In the present study, two species of *Echium* and two of *Anchusa* as facultative halophytic species were analysed for total protein and amino acid compositions of the aerial parts in order to observe their alternative nutritional source potential in saline conditions and contribute their taxonomical delimitations using amino acid patterns.

2. MATERIALS AND METHODS

Five to six individual specimens of *Echium italicum* L., *Echium plantagineum* L., *A. undulata* L. subsp. *hybrida* (Ten.) Coutinho and *A. officinalis* L. were collected from their natural distribution areas in A1 and A2 grid squares in north-west part of Turkey between June and August 2014. Collected specimens were used as herbarium material and identified using the Flora of Turkey and collection of specimens from Istanbul University, Faculty of Science (ISTF) herbarium. Voucher specimens were deposited in Botany section of Faculty of Science of the University. Aerial parts of each samples were ground into meal and dried. Kjeldahl method (AOAC 1990) was used for analysing the total protein amount. Total nitrogen was determined by Kjeldahl analysis and converted to crude protein by multiplying the percent nitrogen by 6.25. Three different samples for total protein and amino acid determinations were assayed. UFLC-UV (Shimadzu) system equipped with an shim-pack XR-ODS (75 mmL. x 3 mm i.d.) was used for quantitation of the amino acids. The samples according to standard method were hydrolysed at 110±1℃ with 6N HCl for 24 h and the amino acids are derivatized to PTC (phenylthiocarbamyl) by a PITC (phenyl isothiocyanate) reagent, and detected by a UV detector at 254 nm. Seventeen components of the PTC derivatized amino acid (100 µmol/L each) are analyzed and separated. The temperature was held in 40ºC. Phosphate buffer/Acetonitrile gradient elution was used as mobile phase with 1,2 mL/min flow rate. Identification and quantification of amino acids by UV were accomplished by comparing the relative retention times of sample peaks with those of amino acid mix stock standard. Statistical analyses of the experimental results, including multivariate and hierarchical cluster analysis using average linkage (between groups), were carried out with p = 0.05 indicating significance (SSPS 11.5). The results obtained were presented in the Table 1.

3. RESULTS

Analytical data derived from the samples showed some variation for crude protein and amino acid composition (Table 1). Total protein amounts between 7.55 and 12.97% in studied taxa were obtained. The highest level of total protein was detected in *A. officinalis*, while the lowest measurement value observed in the aerial parts of *E. italicum*. *A. undulata* subsp *hybrida* and *E. plantagineum* exhibited similarly moderate levels for protein contents. Statistically siginificant differences were observed for each individual amino acid concentrations among taxa. Aspartic acid, glutamic acid, phenylalanin, histidine, gycine and serine suggested characteristic profile at generic delimitation. Critical higher values were found in alanine, phenylalanine and leucine for all species. The highest total quantities of amino acids were generally detected in *A. officinalis* parallel with its total protein percent except for aspartic acid, glutamic acid and arginine, while the lowest levels were found in *E. italicum*. Non essential amino acids such as aspartic acid, glutamic acid, glycine, serine, proline and arginine showed statistically significant differences between both species of *Echium*. Beside, all amino acid quantities exhibited statistically significant differences between *Anchusa* species.

Significant differences were calculated on the quantities of amino acids among the taxa. In the dendrogram constructed with amino acid data, *E. plantagineum* and *A. undulata* subsp *hybrida* clustered togather. *A. officinalis* placed with this group in the same main clad, while *E. italicum* occured in separate branch. On the other hand, very rich concentrations of essential amino acids were observed in the present sudy. The amounts of all essential amino acids from examined taxa are generally sufficient compared to FAO reference values for the requirements of all age groups [26]. *A. officinalis* is the best sources for the highest quantities of total protein and essential amino acids compared to the other taxa examined here.

Table 1. Total protein percents and amino acid concentrations (mg/100 g 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 triplicate determinations

Taxa / amino acid (mg/100 g)	*E. italicum*	*E. plantagineum*	*A.undulata* subsp. *hybrida*	*A. officinalis*	2-5 years	10-12 years	Adult (18+ years)
Total protein (g/100 g)	7.55±0.53	10.16±0.96	10.18±1.12	12.97±0.93	-	-	-
L-Alanine*	1141±5	913±3	1026±6	1339±8	-	-	-
L-Aspartic acid*	372±3	290±3	178±2	197±3	-	-	-
L-Methionine*	127±2	250±4	211±3	286±4	250 [a]	220[a]	170[a]
L-Glutamic acid*	639±8	605±7	325±4	348±5	-	-	-
L-Phenylalanin*	372±4	671±7	713±7	1016±11	630 [b]	220[b]	190[b]
L-Lysine*	306±3	517±5	524±6	817±10	580	440	160
L-Histidine*	154±3	174±3	219±6	306±4	-	-	-
L-Tyrosine*	218±3	422±5	480±4	694±4	630 [b]	220[b]	190[b]
L-Glycine*	395±4	565±8	727±6	907±8	-	-	-
L-Valine*	334±4	637±6	666±9	859±7	350	250	130
L-Leucine*	544±6	1068±12	1127±18	1497±15	660	440	190
L-Isoleucine*	297±5	585±6	582±6	782±8	280	280	130
L-Threonine*	671±6	627±7	685±5	935±9	340	280	90
L-Serine*	306±4	390±5	547±4	680±7	-	-	-
L-Proline*	307±3	612±7	751±7	935±12	-	-	-
L-Arginine*	306±4	390±4	268±3	374±4	-	-	-

[a]*Total amount of methionine and cysteine.*
[b]*Total amount of tyrosine and phenylalanin.*
** Significantly different among the taxa analysed*

4. DISCUSSION

4.1 Protein and Amino Acid Composition in Taxonomic Identification

Proteins having great diversity and different pathways in any plant groups as characteristic macromolecules in taxonomy are genetically controlled and little effected from environmental conditions [27,28]. It was reported that valuable informations comparing the protein contents of homolog organs of different plant taxa could be provided in taxonomical studies [27]. Beside, amino acids are important biological compounds that are associated with peptides and proteins, and occupy an important position in the food and pharmaceutical industries. The global amino acid composition of a protein, although a cruder variable than sequence, is nevertheless informative and has been correlated with protein structural class [29]. Different compositions of amino acids reflect the specific characteristics of a protein, showing also the basis for a structural protein taxonomy well related to the biological classification. Variation of protein amino acid pattern from high taxonomic categories to cultivars in different plant groups have taxonomically intelligible patterns [16-18,20-33]. In cereal grains, leucine and alanine levels are lower in festucoids than in other grasses, while those of lysine and glycine are higher. Chloridoid grasses, in the middle of grass classifications, have intermediate levels of leucine and alanine. In dicotyledone leaves there is patternisation of quantitative data on isoleucine, lysine, cystine, phenylalanine, alanine, aspartic acid, glutamic acid, glycine and serine. It was stated the morphological descriptors and amino acid composition analysis to be complementary methods for the characterisation of cultivars [31,34]. Contrary, no significant diversity in amino acid patterns were reported from ecologically different populations [30,35] and cultivars [36] in some species, but interspecific variations are notable. The variability of protein content and amino acid compositions within a same species may result from biochemical mutations in addition to phenotypic variations. In this sudy, it is probable that highly variable concentrations of amino acids may be of significance for delineations of *Echium* and *Anchusa* species at generic, specific and infraspecific levels as a diagnostical parameter. Some amino acids such as aspartic acid, glutamic acid, phenylalanin, histidine, gycine and serine exhibited discriminative patterns for generic delimitation. It was stated that the amino acid composition of unfractionated leaf protein is controlled by genetic rather than by environmental factors [37]. Both species of *Echium* can be delineated with each others based on total protein and amino acid quantities. Generally higher total protein and amino acid concentrations in *E. plantagineum* than *E. italicum* were observed except for alanine, aspartic acid, glutamic acid and threonine. Beside, all analytical results for total protein and amino acid concentrations showed higher values in *A. officinalis* than *A. undulata* subsp *hybrida*. Obtained results revealed that these parameters may be useful tool at specific delineations within *Echium* and *Anchusa* as discriminative proteomic data. On the other hand, highly similar total protein and amino acid compositions in *E. plantagineum* and *A. undulata* subsp. *hybrida* may reflect their proteomic convergence in the similar saline environments. Because both species generally distribute within broad range of habitat conditions including fields, banks, waste grounds, rocky slopes and sand dunes in the coastal zones of Turkey as native species and weeds in the fields. Invasive nature of *E. plantagineum* is also known in some country such as Australia, California and Oregon. Beside, three informal groups were recommended for *A. officinalis* in the flora of Turkey based on the colors of corolla, sizes of calyx and features of inflorescence. This species with high intraspecific variations in morphological and biochemical characteristics [12] has also very large distributional patterns in Anatolia showing its broad genetic base adapted various habitat conditions. *A. officinalis* clustered with *E. plantagineum* and *A. undulata* subsp. *hybrida* in the dendrogram reflecting similar aspect of amino acid accumulations for genetically adaptation to extreme environments. Apart from the others, *E. italicum* exhibited different total protein and amino acid profile. High level of micromorphological, biochemical and genetic polimorphisms were also reported in this species as a cosmopolite, which shows strong adaptation potential to various climate and soil conditions [8].

4.2 Environmental Conditions on Protein and Amino Acid Composition

Different compositional patterns of amino acids may reflect adaptation capacity to saline habitats with different expression levels of stress proteins. Some amino acids such as proline, glycine, arginine and lysine were reported to be accumulated in xerophytic species than mesophytic ones [30]. The highest concentrations of regarding amino acids in addition to leucine, alanine, phenylalanin, threonine and valine that may be related to the stress proteins encoded with stress tolerant genes in saline conditions such as sodium transporters, antioxidant enzyme genes etc. were found in *Echium* and *Anchusa*. In a study carried out on halophyte *Suaeda asparagoides*, lysine and methionine which are essential amino acids were detected with the value of 215 mg / 100 g and 23,2 mg / 100 g, respectively [22]. Remarkably higher quantities of lysine (306-817 mg / 100 g) and methionine (127-286 mg / 100 g) were measured in the investigated species here. Comparative proteome analyses of leaf tissue in halophyte *Cakile maritima* treated with 100 mM NaCl-salinity revealed different abundance changes of a specific set of 44 proteins being involved in energy metabolism such as photosynthesis and respiration [38]. Different sodium salts may cause different solute accumulation in the halophyte. *Prosopis strombulifera* grown in high NaCl concentrations have relatively higher osmotic potential than plants grown in Na_2SO_4 (at 49 days) and increased synthesis of proline, pinitol and mannitol in the cytoplasm [39]. In *Phragmites australis* (Cav.) Trin. ex Steud., amino acid contents increased significantly up to four-fold from 0 to 10 parts per thousand salinity. Furthermore, this increase was caused by up to 200-fold increase of proline and 11-fold increase of glutamine at 10 parts per thousand salinity in rhizome and leaves [40]. The highest levels of soluble protein indicating the possible intervention of amino acids in the regulation of intracellular osmotic pressure have been reported in *Atriplex* sp. and *Suaeda fruticosa* [41]. An increase in the amino acid pool size and the number of amino acids produced in *Cirrenalia pygmea*, a mangrove fungus was reported in higher salinity conditions. Furthermore, higher concentrations of acidic amino acids with increasing salinity and existence of some amino acids only such as glycine, histidine, threonine, arginine and valine have been revealed in this fungus exposed to high salinity conditions [24]. Saline soils may induce the increased quantities of some amino acids and occuring of additional ones in the methobolic pathways of the halophytic species. Therefore, the species adapted saline habitat conditions may be good alternative sources for exploitations of essential amino acids.

4.3 Advantages and Disadvantages of *Echium* and *Anchusa* as Food and Fodder Sources

In the present study, the highest levels of amino acids were leucine (1497 mg / 100 g), alanine (1339) and phenylalanin (1016). Nutritive potential value of the investigated species have significant importance as rich sources of essential amino acids. Particular focus is given to the lysine requirements for adults, since this indispensable amino acid is most likely to be limiting in the cereal-based diets, characteristic of populations in large areas of the developing world [42,43]. Considerable higher levels of lysine compared to the cereals were observed in all species here (306-817 mg / 100 g). It was declared that total seed protein in barley is reported to be quantitatively inherited [44] and high lysine content is due to the recessive monogenic effect [45]. Different cereals such as barley and in particular wheat have low content of essential amino acids such as lysine, methionine and threonine, and are of poor nutritional value [46]. Obtained results for total protein contents [47,46] and essential and non-essential amino acids [48] from aerial parts of the investigated species growing in saline soils are highly comparable with wheat, rice, corn, barley, oats, sorghum, millet, and acorn [20]. Regarding species have also potential to produce high levels of dry mass. It has been reported that the most productive species have yielded 10–20 tons/ha of biomass on seawater irrigation, producing yields equivalent to conventional crops [49]. Although no single plant would generally provide humans with adequate levels of all essential amino acids, investigated species as promising natural sources can be used as raw materials for the production of essential amino acids and prepared with other foods contributing to useful amounts of the amino acids to the diet. All specimens herein can be compared favourably with the FAO standard [26]. As a disadvantage from feed and nutritional points of view, regarding species contain pyrrolizidine alkaloids (PA) which are toxic secondary plant compounds expressed as a chemical defense strategy. Therefore, regarding seconder metabolit having genotoxic and carcinogenic effect can be eliminated with the suitable processes in nutritional production of dry mass and amino acids [50]. Examined species as new crops can be also cultivated in saline soils for commercial production of long chain unsaturated fatty acids such as gamma linolenic acid (18:6n3) and stearidonic acid (18:3n4) as unusual fatty acids having various health benefits [7,8,11]. In general, the plant species growing in saline ecosystems have great potential for accumulation of various bioactive substance and for valuable solutions against probable salinisation and aridity in the cultivated areas for maintaining of agricultural production. The plantation of salt tolerant plants in saline sodic soils as a bioreclamation method showed an effective decline in soil salinity. Broad diversity of sand dune ecosystems comprising 2% of terrestrial plant species have also been regarded as alternative new sources for production of new crops in saline environments. During the last century, a loss of about 70% of dune ecosystems for European coasts has been calculated as a result of urbanization, industrialization, and tourism activities [51]. Over-exploitation results in a degraded and even lost natural ecosystem. In Turkey, 79% of coastal dunes of west Black Sea region of Turkey and many saline areas that have yet to be surveyed are under immense threat and declining fast [52].

5. CONCLUSION

Some habitats with extreme conditions such as saline ecosystems have rich diversity which can be evaluated as plant genetic resources containing valuable allels to maintain and increase crop productivity in the face of changing environments and climate, and unforeseen risks in the agricultural production. Medicinal plants growing in the sand dunes are also promising sources for obtaining pharmacologically active substances. Halophytes might be valued for their potential as cash crops themselves. The economic benefits that society receive from the natural functioning of the beach and coastal dunes are very high. Conservation of this vulnarable ecosystems most affected by anthropogenic factors and introduction of invasive species, and preparing their molecular databases for the characterisation of the germplasms are primary purposes. Turkey with ca. 300 halophytic taxa has great potential for biosaline agriculture in production of oil, amino acids, various bioactive derivatives etc. and improving of the agricultural crops in the fields.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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