Bioactive Compounds of *Salicornia europaea* L Aboveground Organs of East Kazakhstan Flora

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Article

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Laura Kazhygeldieva [1], Lyazzat Orazzhanova [1], Binur Mussabayeva [1,2]*, Alfira Sabitova [1] and Batiyash Silybayeva [3]

[1] Shakarim University, Glinky Str., 20A, 071412 Semey, Kazakhstan

[2] Astana International University, Kabanbay Batyr Ave, 8, 010017 Astana, Kazakhstan

[3] Alikhan Bokeikhan University, Mangilik el Str., 11, 070000 Semey, Kazakhstan

* Correspondence: mussabayevabinur@gmail.com; Tel.: (+77076208586)

Abstract

This work presents the study results of the phytochemical profile and antioxidant activity of aboveground organs of the East Kazakhstan population of *Salicornia europaea* L. The chemical composition of the plant sample was studied using a complex of modern analytical methods, including HPLC, GC-MS, IR-Fourier spectroscopy, and elemental analysis. It was found that the content of flavonoids was 2.40 ± 0.02 mg QE/g of dry raw materials, and the content of polyphenols was 6.73 ± 0.03 mg GAE/g. The antioxidant activity (ABTS test) reached 7.85±0.04 mg TE/g. The concentration of fat-soluble and water-soluble vitamins was: C - 1.27 ± 0.12 mg/100 g, A - 1.16 ± 0.11 mg/100 g and E - 3.89 ± 0.38 mg/100 g. The IR characterization of plant raw materials and ash was carried out, the indicators of the elemental composition (TC, TOC, TIC, TN, TS) were determined. The totality of the data obtained indicates the phytochemical potential of *Salicornia europaea* L., which grows in the territory of Eastern Kazakhstan, and substantiates the prospects of its use in the development of cosmetic and cosmeceutical products.

Keywords: chemical composition; *Salicornia europaea* L; flavonoids; phenolic compounds; antioxidant activity; vitamins

1. Introduction

Currently, there is a significant increase in interest in biologically active substances (BAS) of plant origin in the pharmaceutical and cosmetic industries. This is due to their diverse chemical composition, polytropic physiological action, and high biocompatibility [1,2]. In accordance with the recommendations of the World Health Organization, herbal sources are considered as an important and promising raw material base for obtaining pharmaceutically and cosmeceutically significant BAS [3].

In the context of the search for highly effective plant antioxidants, researchers are particularly interested in plants adapted to extreme environmental conditions. Such stress-tolerant species are able to accumulate in high concentrations secondary metabolites with complex molecular organization and pronounced physiological activity (phenolic acids, flavonoids, terpenoids, etc.).

Among plants adapted to extreme environmental conditions, halophytes, salt–resistant species, are of particular interest. The increased salinity of the soil causes an oxidative explosion in such plants, which stimulates the accumulation of BAS with high antioxidant potential. The use of halophyte species is consistent with the principles of "green chemistry", focused on the use of renewable resources of extreme ecosystems [4,5].

One of the most promising halophytes is European soleros (*Salicornia europaea* L.), which is characterized by high environmental stability and the ability to form a specific chemical composition, including a variety of bioactive compounds.

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Salicornia europaea L. belongs to the genus *Salicornia*, the *Amaranthaceae* family (formerly part of the *Chenopodiaceae* family) [6,7]. It is a low-growing shrub 10-40 cm high. The shoots are jointed, fleshy, cylindrical in shape, bright green in color, gradually turning red by the flowering period. The leaves are reduced and presented in the form of small scaly formations fused with the shoot. The flowers are small, nondescript, sessile, collected three at a time in the axils of the segments; the fruits are small nuts with a single seed. This morphology reflects the adaptation of the plant to the extreme conditions of saline habitats, where the moisture-retaining and ion-regulating function of tissues ensures its survival [8,9] (Figure 1).

Figure 1. Salicornia europaea L.

This species is widespread in Europe, Asia, and North Africa and occurs in the salt marshes of the Republic of Kazakhstan [10,11].

The phytochemical composition of plants of the genus Salicornia has repeatedly been the subject of review studies emphasizing the high content of secondary metabolites of various chemical nature [12–15] systematized data on the presence of flavonoids, phenolic acids, saponins, sterols, and other classes of metabolites in *S. europaea*. A more detailed analysis of polyphenolic compounds of plants of the genus Salicornia is presented in the review by [12]. Using GC–MS and LC–MS/MS, fatty acids were identified, among which oleic acid (36.55%) [1], α-tocopherol (2.1 ± 0.3 mg/100 g) [16] dominates, as well as more than 90 bioactive compounds related to saponins, flavonoids, phenols, chlorogenic acids, terpenoids, sterols, lignans, aldehydes, tocopherols, etc. [16].

The presence of these BAS determines a wide range of biological activity of *S. europaea,* including antioxidant, anti-inflammatory [17], antibacterial [18], immunomodulatory [19,20] and other properties [15,21,22].

The territory of Kazakhstan, characterized by an arid climate, high soil salinity and water scarcity, creates favorable conditions for the growth of *S. europaea* in significant areas. However, despite the widespread distribution, pronounced bioactive properties and high ecological plasticity, studies of the phytochemical composition of Kazakhstani populations of *S. europaea* remain fragmentary and practically undisclosed to date. The available works of Russian scientists are mainly focused on the applied and technological aspects of its use. So, [23] showed the possibility of using *Salicornia* from the Turkestan region as a partial substitute for table salt. [24] considered *Salicornia* as a promising component of marine aquaponics [23,24].

Data on the chemical composition of S. europaea, which grows in Eastern Kazakhstan, are presented in the work [25], where phenolic and volatile compounds were identified. However, a

 doi:10.20944/preprints202605.1100.v1

quantitative assessment of the elemental composition, vitamin content, and antioxidant activity for East Kazakhstan *S. europaea* has not been conducted before.

The analysis of the patent database (Table 1) shows that Kazakhstan's patent activity in relation to *S. europaea* is limited mainly by agrotechnical solutions. There are no national patents related to the chemical composition, biological activity and processing technology of *S. europaea* L.

Table 1. Foreign and Kazakhstani patents for *Salicornia europaea* L.

Country	Patent number	Year	Summary
International (PCT, WIPO)	WO2008/143414 A1	2008	The preparation of a salt substitute from plants of the genus Salicornia (including *S. europaea*) and the method of its production are considered. An aqueous extraction process for processing aboveground biomass is described to produce a product containing a reduced amount of NaCl and an increased content of mineral elements (K, Mg, Ca) and amino acids. The product is intended for food and nutraceutical use as an alternative to table salt.
China	CN102783630A	2012	The method of obtaining "vegetable salt" from *S. europaea*: blanching, grinding, filtration, vacuum drying; application in the food and cosmetics industry.
European Patent / USA	EP2144516B1 / US20100304000A1	2012 / 2010	Production of salt from plants of the genus Salicornia and its use as a substitute for table salt in dietary nutrition.
China	CN104274355A	2015	Anti-aging cosmetic composition based on S. europaea extract and reed rhizome (*Phragmitis Rhizoma*); inhibition of collagenase, stimulation of collagen synthesis.
Russia	RU2726544C2	2018	It refers to the food industry, in particular to a substitute for edible salt for use in food products made from the plant *Salicornia*
Kazakhstan	KZ 35814	2022	A method for cultivating S. europaea under artesian irrigation conditions; developed by the Southwestern Research Institute of Animal Husbandry and Crop Production

The unique combination of the sharply continental climate and high salinity of the soils of Eastern Kazakhstan can contribute to the formation of special chemotypes of *Salicornia europaea* L with a unique quantitative content of BAS.

The aim of this work is a comprehensive study of the chemical composition and assessment of the antioxidant potential of the aboveground organs of *Salicornia europaea* L, native to Eastern Kazakhstan.

2. Results

 doi:10.20944/preprints202605.1100.v1

In our work [26], we presented data on the content of total carbon (TC), organic carbon (TOC), inorganic carbon (TIC), as well as total nitrogen (TN) and sulfur (TS) for *S. europaea* L samples growing in Eastern Kazakhstan. However, their detailed characteristics were not given. This paper provides an interpretation of the previously obtained results (Figure 2).

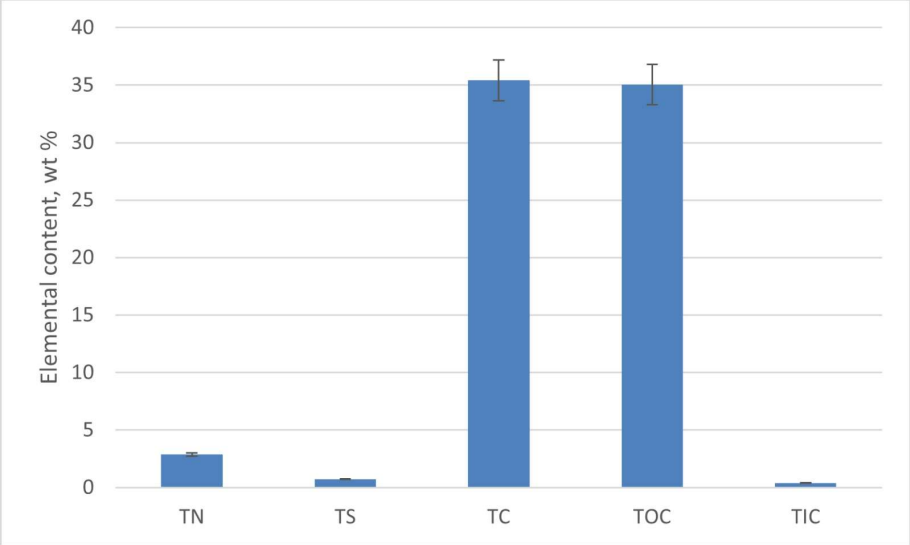


Figure 2. The content of TC, TOC, TIC, TN and TS in the aboveground part of *Salicornia europaea* L.

26 organic compounds were identified in the ethanol extract of *S. europaea* L by GC-MS analysis, (Figure 3, Table 2).

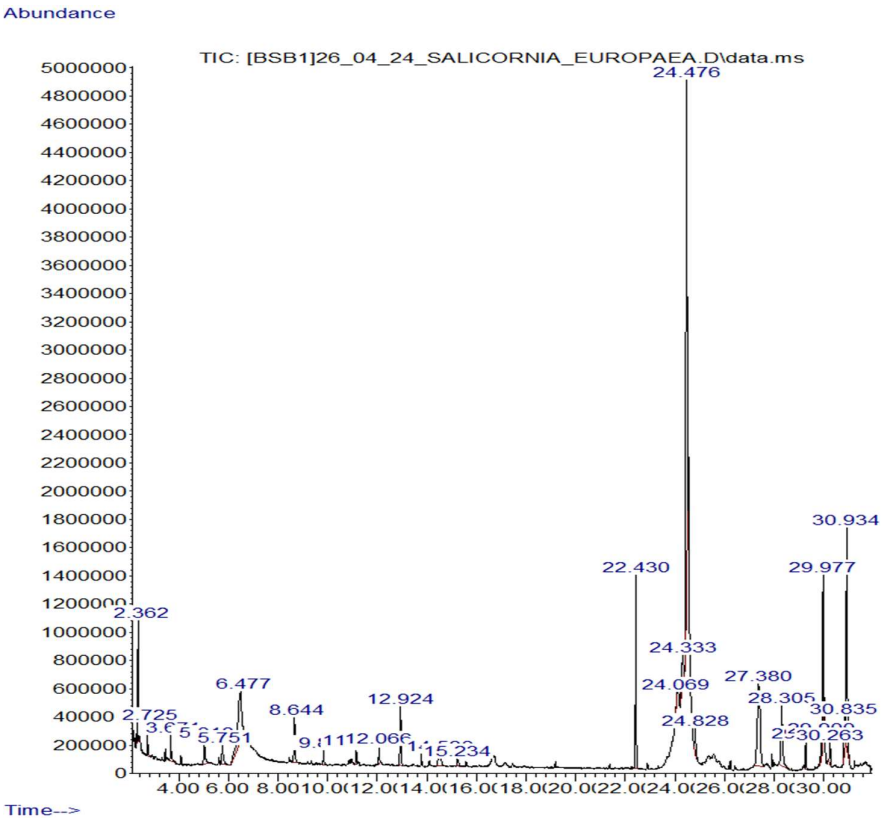
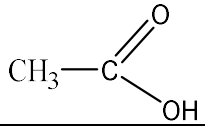
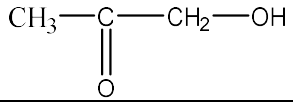
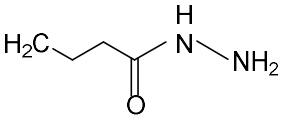
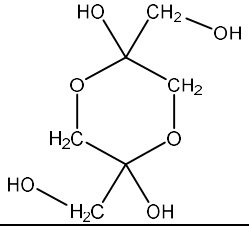
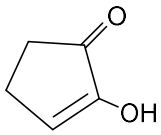
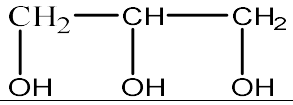
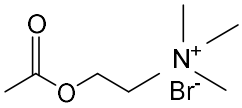
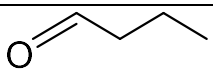
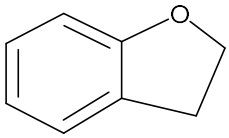
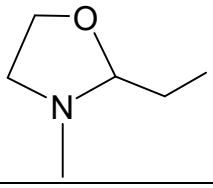
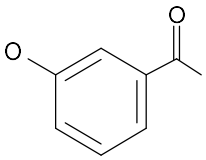
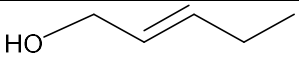


Figure 3. Chromatogram of *Salicornia europaea* L ethanol extract.

 doi:10.20944/preprints202605.1100.v1

Table 2. GC mass spectrometric analysis data for ethanol extract of *Salicornia europaea* L.

Peak	R. time	Area, %	Name	MF The molecular formula	The structural formula	Mw
1	2.362	3.67	Acetic acid	$C_2H_4O_2$		60,05
2	2.725	1.04	1-hydroxy- 2-Propanone	$C_3H_6O_2$		74,08
3	3.671	1.16	Butyric acid hydrazide	$C_4H_{10}N_2O$		102,14
4	5.013	1.46	1,3-Dihydroxyacetone dimer	$C_6H_{12}O_6$		180,16
5	5.751	1.26	2-Cyclopenten-1-one, 2-hydroxy-	$C_5H_6O_2$		98,1
6	6.477	9.45	Glycerin	$C_3H_8O_3$		92,09
7	8.644	2.42	Acetylcholine bromide	$C_7H_{16}BrNO_2$		226,11
8	9.832	0.56	Butanal	C_4H_8O		72,11
9	11.131	0.70	Benzofuran, 2,3-dihydro-	C_8H_8O		120,15
10	12.066	0.70	Oxazolidine, 2-ethyl-3-methyl-	$C_6H_{13}NO$		115,17
11	12.924	2.69	Ethanone, 1-(3-methoxyphenyl)-	$C_9H_{10}O_2$		150,17
12	14.530	1.73	2-Penten-1-ol, (E)-	$C_5H_{10}O$		86,13

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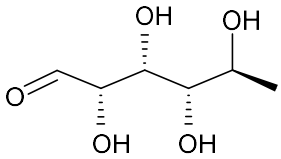
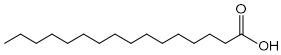
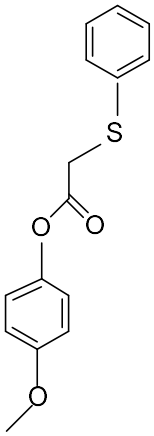
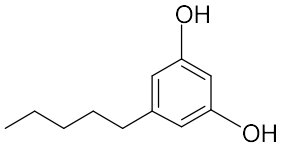
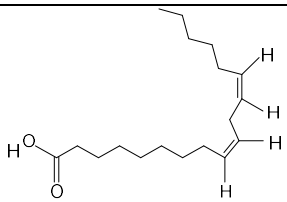
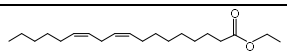
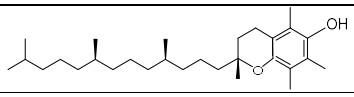
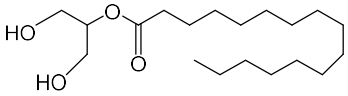
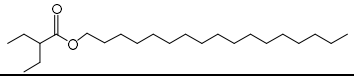
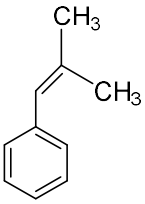
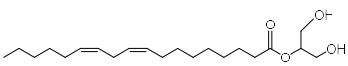
13	15.234	0.56	L-Mannose, 6-deoxy-	$C_6H_{12}O_5$		164,16
14	22.430	7.34	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		256,42
15	24.069	0.68	Phenylthioacetic acid, 4-methoxyphenyl ester	$C_{15}H_{14}O_3S$		274,3
16	24.333	2.98	1,3-Benzenediol, 5-pentyl-	$C_{11}H_{16}O_2$		180,24
17	24.476	24.82	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$		280,4
18	24.828	0.98	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$		308,5
19	27.380	10.87	Vitamin E	$C_{29}H_{50}O_2$		430,71
20	28.305	4.97	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$		330,5
21	29.284	1.04	2-Ethylbutyric acid, heptadecyl ester	$C_{23}H_{46}O_2$		354,6
22	29.900	0.80	Benzene, (2-methyl-1-propenyl)-	$C_{10}H_{12}$		132,20
23	29.977	5.44	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{38}O_4$		354,52

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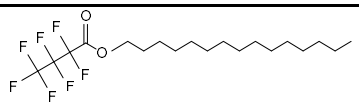
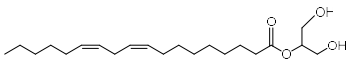
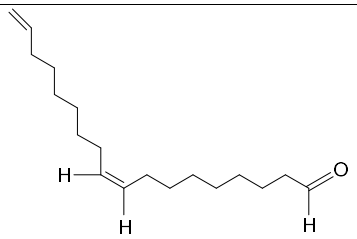
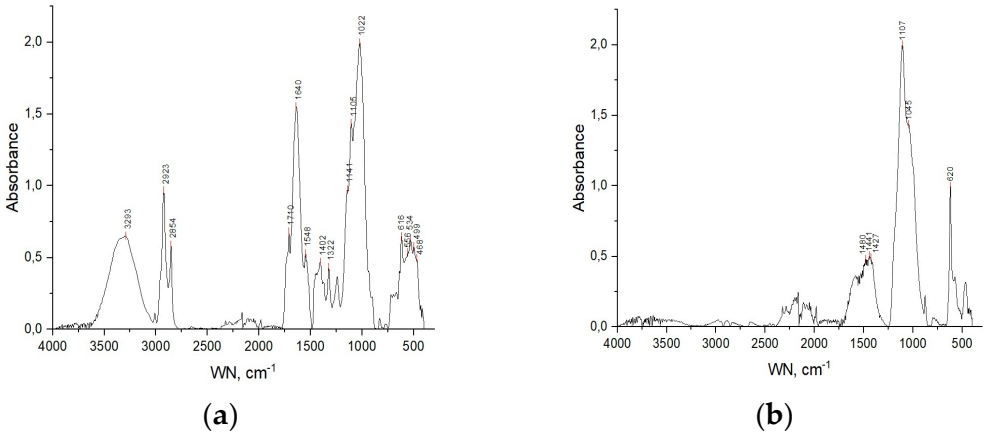
24	30.263	0.74	Pentadecyl heptafluorobutyrate	$C_{19}H_{31}F_7O_2$		424,4
25	30.835	1.47	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{38}O_4$		354,52
26	30.934	10.46	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$		264,4

The largest contribution to the chemical profile of the extract was made by 9.12-octadecadienoic acid (Z,Z) (24.82%), vitamin E (10.87%), 9.17-octadecadienal (Z) (10.46%), glycerin (9.45%) and n-hexadecanoic acid (7.34%).

Fourier transform IR spectroscopy of the crude sample and the ash fraction of the plant sample under study (Figure 4, Table 3) made it possible to identify the functional groups of *S. europaea* adapted to the conditions of Eastern Kazakhstan. Figures 5a and 5b show the IR spectra of the raw sample and the ash fraction of *S. europaea* L.



(a) (b)

Figure 4. IR spectra of the crude sample (a) and the ash fraction (b) of *Salicornia europaea* L.

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Figure 5. Cartographic diagram of the Salicornia europaea L. collection route (Abai region, Kazakhstan).(- the place of collection of plant material).

Table 3. Characteristics of the main absorption bands in the IR spectra of *Salicornia europaea* L and its ash fraction.

Sample	Wave number, cm^{-1}	Type of oscillation	Functional group/compound type	References
Crude sample	3293	ν(O-H) и ν(N-H)	-OH groups of polysaccharides (cellulose, hemicellulose), phenols; -NH group of amines	[27,28]
	2923–2854	ν_{as}, ν_s (C-H)	CH_2/–CH_3 groups of lipids, waxes, and fatty acids	[28,29]
	1710	ν(C=O)	C=O groups of fatty acids and pectins	[30,31]
	1640	Amide I, ν(C=O)	Peptide bond, proteins	[32]
	1548	Амид II, ν(N-H), δ(C-N)	Proteins, amino acids	[30,31]
	1402	$\nu_s(COO^-)$	Organic acid COO^- ions	[28]
	1322	δ (O-H) и δ(C-O)	Polysaccharides, components of amide III	[33]
	1141-1022	ν(C-O-C) и ν(C-O)	Glycoside bonds of cellulose and hemicellulose	[34]
Ash fraction	1480, 1441, 1427	$\nu(CO_3^{2-})$	Carbonates of alkaline earth metals	[35,36]
	1107, 1045	ν(Si-O-Si), ν(Si-O)	Metal silicates, SiO_2	[35,37,38]
	620	δ(M–O)	Metal oxides and silicates	[39]

The results of liquid chromatography with mass spectrometric detection (LC-MS-TOF) indicate the presence of a wide range of secondary metabolites in the plant raw materials of *S. europaea* L. 22 key secondary metabolites were identified, the structure of which was confirmed based on accurate m/z values and regulatory patterns (Table 4).

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Table 4. Data from high-performance liquid chromatography of a sample of *Salicornia europaea* L aboveground organs.

№	RT [min]	Observed m/z	Fragmentation	M_F	Putative Compound	Compound Class
1	3.40	301.0930	121.0278, 139.0383	$C_{13}H_{17}O_8$	Isotachioside	Benzoates
2	4.03	367.0648	137.0230, 181.3157, 261.6907	$C_{16}H_{15}O_{10}$	Syringic acid hexoside	Benzoates
3	4.68	329.0878	123.0437, 167.0338, 173.0286, 226.0909	$C_{14}H_{17}O_9$	Vanillic acid hexoside	Benzoates
4	4.80	299.0771	137.0230, 179.0341, 313.2883	$C_{13}H_{15}O_8$	Hydroxybenzoic acid hexoside	Benzoates
5	5.45	179.0339	135.0437, 179.0339	$C_9H_7O_4$	Caffeic acids	HCA-derivatives
6	5.95	195.0651	135.0434, 136.0518, 153.3114, 180.0419, 195.0657	$C_{10}H_{11}O_4$	Ferulic acid	HCA-derivatives
7	6.12	283.0822	137.0230, 171.8092, 239.0922	$C_{13}H_{15}O_7$	Benzoic acid hexaside	Benzoates
8	6.43	355.1035	134.0360, 193.0497, 217.0502, 355.1041	$C_{16}H_{19}O_9$	Feruloyl hexoside	HCA-derivatives
9	6.57	325.0929	145.0282, 163.0388, 265.0722, 325.1295	$C_{15}H_{17}O_8$	Coumaroyl hexoside	HCA-derivatives
10	6.94	167.0342	108.0201, 123.0437, 152.0102, 167.0338	$C_8H_7O_4$	Vanillic acid	Benzoates
11	7.93	337.0934	128.7437, 158.3876, 191.0555, 251.7633, 277.0750, 290.0354, 337.0975	$C_{16}H_{17}O_8$	Coumaroylquinic acid	HCA-derivatives
12	9.25	579.2084	166.0260, 181.0496, 208.0365, 327.0515, 402.1319, 505.0917	$C_{28}H_{35}O_{13}$	Syringaresinol hexoside	HCA-derivatives
13	9.65	463.0883	151.0023, 271.0247, 300.0275, 301.0352, 313.1665	$C_{21}H_{19}O_{12}$	Quercetin-3-hexoside	Flavonoids
14	9.67	301.0347	167.9164, 183.1018, 201.1121, 269.0459, 285.0405, 286.0486, 301.2022	$C_{15}H_9O_7$	Quercetin	Flavonoids
15	10.16	549.0888	151.0023, 271.0247, 300.0276, 332.3157	$C_{24}H_{21}O_{15}$	Quercetin-3-malonylhexoside	Flavonoids
16	10.17	505.0988	151.0023, 255.0296, 271.0247, 300.0275, 301.0354, 343.0373, 453.3864	$C_{23}H_{21}O_{13}$	Quercetin-3-acetylhexoside	Flavonoids
17	10.34	243.0294	207.1019, 225.1126, 243.1234	$C_{13}H_7O_5$	Trihydroxyxanthone II	Xanthones
18	10.51	243.0296	181.1225, 207.1019, 225.1126, 243.1234	$C_{13}H_7O_5$	Trihydroxyxanthone I	Xanthones

 doi:10.20944/preprints202605.1100.v1

19	10.55	209.0813	165.1275, 209.1187	$C_{11}H_{13}O_4$	Sinapic alcohol	HCA-derivatives
20	10.96	533.0940	284.0326, 285.0404, 341.0675	$C_{24}H_{21}O_{14}$	Kaempferol-3-malonylhexoside	Flavonoids
21	11.06	299.0191	179.0531, 199.0392, 243.0305 271.0249, 299.0191	$C_{15}H_7O_7$	1,2,4,5-tetrahydroxy-7-hydroxymethyl-9,10-anthraquinone	Anthraquinones
22	11.74	257.0452	151.0023, 166.0252, 242.0581	$C_{14}H_9O_5$	Dihydroxymethoxyxanthone	Xanthones

The studied sample of *S. europaea* L is characterized by the presence of phenolic compounds and flavonoids. Thus, the total content of polyphenols was 6.73 ± 0.03 mg GAE/g, and the total content of flavonoids was 2.40 ± 0.02 mg QE/g of dry raw materials. The value of antioxidant activity according to the ABTS method was 7.85 ± 0.04 mg TE/g, which indicates the presence of compounds with antioxidant potential.

For the first time, we have obtained data on the accumulation of fat-soluble vitamins in *S. europaea* growing in Eastern Kazakhstan. The results of determining the vitamin content in *S. europaea* L samples are presented in Table 5.

Table 5. Quantitative content of vitamins in *Salicornia europaea* L.

Vitamin	Content, mg/100 g of dry matter
Vitamin A (β-carotene, provitamin A)	1,16±0,11
Vitamin C (ascorbic acid)	1,27±0,12
Vitamin E (α-tocopherol)	3,89±0,38

3. Discussion

An analysis of the elemental composition of *S. europaea* L showed a predominance of organic matrix (TOC 35.03 ± 0.65%), while the proportion of inorganic carbon is insignificant (TIC 0.39 ± 0.02%). This indicates a high proportion of carbonaceous metabolites in the biomass of aboveground organs and a small contribution of mineral carbon compounds to the structure of shoots (absence of carbonate inclusions), which is typical for halophytes growing in soils with a neutral or slightly alkaline environment [40]. This plays a positive role for the release of BAS.

The TN content was 2.88 ± 0.25%, which significantly exceeds the values given for vegetative organs of *S. europaea* from Korean populations (1.22-1.78%) [40], as well as for plants grown under controlled salinity conditions (0.41-0.58%) [41]. The high TN content, as well as the ratio C/N=12.3 in the East Kazakhstan sample, indicates the active synthesis of nitrogen-containing osmolytes in response to osmotic stress caused by high concentrations of chlorides and sulfates in the soil [42].

An increased TS content (0.72 ± 0.05%) characterizes the presence of sulfur-containing components in plant tissue, including structural and metabolic compounds [43], which is typical for the East Kazakhstan region under conditions of sulfate salinity [44]. No direct data on the sulfur content in *S. europaea* were found for comparison, which underlines the originality of the results obtained.

Thus, the results of the analysis of the elemental composition of *S. europaea* L growing in Eastern Kazakhstan show a unique adaptive population strategy, expressed in the following aspects: high nitrogen potential, optimal stoichiometric ratio of C:N, specific accumulation of sulfur. This contributes to the pronounced metabolic plasticity of *S. europaea* L and makes it a valuable source of BAS for the bio-industry in Eastern Kazakhstan.

The chemical profile of the studied plant raw materials is characterized by the predominance of fatty acids and their derivatives, which is consistent with previously published data for *S. europaea*

 doi:10.20944/preprints202605.1100.v1

[45] and other representatives of the genus *Salicornia* [19,46]. In particular, according to GC-MS analysis, linoleic acid and palmitic acid are the main components in *S. europaea* seed oil [47]. Earlier, in [25] the components of essential oils (volatile terpenes) in *S. europaea* from the same geographical range were identified. However, there is no data on the content of fatty acids and vitamins in this work. This may be due to the methodological features of hydrodistillation, which does not allow the extraction of non-volatile lipophilic compounds. Thus, our study provides for the first time a detailed characterization of the lipophilic fraction of a sample of *S. europaea* native to Eastern Kazakhstan. At the same time, linoleic acid (24.82%) is the absolute dominant, since it exceeds the values for the vegetative organs of other populations of soleras [19]. Palmitic acid (7.34%) and its derivative (Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester – 4.97%) also make a significant contribution to the lipophilic pool. The alpha-tocopherol content (10.87%) in the studied sample significantly exceeds the data of [1] for *S. europaea* of other populations (2.1 ± 0.3 mg/100 g). The high accumulation of vitamin E in the East Kazakhstan population can be explained by the plant's adaptation to abiotic stress, since α-tocopherol is the main lipophilic antioxidant that stabilizes membranes and neutralizes singlet oxygen [48].

In addition to lipophilic components, low molecular weight compounds and alcohols, including glycerol, were found in the extract, which are described in the literature as metabolites potentially involved in the adaptation of halophytes to salt stress [19]. The significant glycerol content (9.45%) confirms that the studied population is under severe osmotic stress caused by high salinity in the soils of Eastern Kazakhstan. In general, the data obtained represent for the first time the characteristic of the lipophilic fraction of East Kazakhstan *S. europaea*: high accumulation of alpha-tocopherol, linoleic acid and glycerol. These results show the promise of the studied raw materials as a source of lipophilic antioxidants for cosmeceutical applications.

The IR spectral profile of crude sample demonstrates a high concentration of organic compounds exhibiting osmotic and antioxidant activity. The presence of intense absorption bands of amide I and amide II indicates an active protein metabolism aimed at the synthesis of stress proteins. Also, the high intensity of the absorption bands at 1710 and 2923-2854 cm^{-1} indicates the dominance of lipid and pectin fractions, which contribute to the water retention effect in conditions of strong salinity. The analysis of the ash residue indicates the presence of a carbonate-silicate matrix in the test sample, which confirms the biogenic accumulation of calcium and silicon compounds.

Thus, the results of IR spectroscopy showed that *S. europaea* L, growing in salinized soils of Eastern Kazakhstan, functions as a biological storage of proteins, lipids and pectins, which converts salts available from the soil into biogenic forms and organic protective complexes.

The metabolic profile of *S. europaea* L is characterized by a wide range of structural diversity, including benzoic and hydroxycinnamic acid derivatives, flavonoids, xanthones, and anthraquinones. The main phenolic pool is formed by derivatives of benzoic acid (vanillic acid, syringic acid, hydroxybenzoic acid) and hydroxycinnamic acid (ferulic acid, coumaric acid). The detection of glycoside and esterified derivatives, including *vanillic acid hexoside, syringic acid hexoside, feruloyl hexoside, coumaroyl hexoside, coumaroylquinic acid*, also indicates a strategy for accumulating metabolites into a "soluble reserve", maintaining the stability of redox homeostasis [49–52]. *Sinapic alcohol* in combination with the photoprotective properties of hydroxycinnamates indicates the occurrence *S. europaea* L processes of lignification and protection of the photosynthetic apparatus under conditions of intense insolation and salt stress.

Flavonoid composition *S. europaea* exhibits high structural complexity, dominated by quercetin derivatives (quercetin-3-hexoside, quercetin-3-malonylhexoside, quercetin-3-acetylhexoside) and kaempferol-3-malonylhexoside. Acetylated derivatives of flavonoids contribute to the neutralization of reactive oxygen species (ROS) and minimize oxidative damage to cellular structures [53].

Of particular interest is the detection of xanthone compounds (trihydroxyxanthone I, trihydroxyxanthone II and dihydroxymethoxyxanthone), as well as the anthraquinone derivative (1,2,4,5-tetrahydroxy-7-hydroxymethyl-9,10-anthraquinone). Xanthones are rare in higher plants, and their presence in S. europaea emphasizes the uniqueness of the secondary metabolism of this

 doi:10.20944/preprints202605.1100.v1

population. These compounds together with anthraquinones provide a synergistic effect, expanding the antimicrobial and cytoprotective potential of the studied raw materials [54–58].

The identified metabolic markers demonstrate the biological activity of *S. europaea* in East Kazakhstan due to the synergistic action of flavonoids (direct-acting antioxidants), xanthones and anthraquinones. A specific profile of metabolites can be formed as a result of salt stress and insolation, which indicates the adaptive plasticity of this species.

The obtained values TPC of *S. europaea* L correlate with the literature data [59], while they are high for such a soft extractant as water used in our study. According to [60], the extraction efficiency of phenolic compounds is influenced by the composition of the extractant: the harder the extractant, the higher the TPC values.

An analysis of the literature data on TFC and ABTS in *S. europaea* also shows the dependence of the flavonoid content on the stage of plant development, the extraction method, and the standard used for expressing results (QE and CE). Thus, in studies [60] using UAE for *S. europaea* L, it was found that an increase in the proportion of ethanol in the extractant leads to an increase in the total content of flavonoids, with the maximum TFC values being ≈8-9 mg QE/g DW. The value of TFC obtained in this study is 2.40 ± 0.02 mg QE/g DW for an aqueous extract of aboveground organs. *S. europaea* L confirms the presence of a flavonoid fraction in the raw material under study. At the same time, direct quantitative comparison of our data between other studies is limited due to the use of different standards (QE and CE), as well as differences in extraction conditions.

According to [61], the antioxidant activity of ABTS exhibits a pronounced dependence on extraction conditions and can vary in a wide range from 2.23 to 10.96 mg TE/g, depending on the solvent used. When using ultrasonic extraction with aqueous alcohol solutions, the antioxidant activity of ABTS can reach 21.44 ± 1.07 mg TE/g DW.

In our study, the high antioxidant activity of ABTS at moderate concentrations of TPC and TFC indicates the high functional effectiveness of the extract. In our opinion, this result is provided not only by phenolic compounds and flavonoids, but also by a pronounced lipophilic fraction (vitamin E and linoleic acid) identified by the GC-MS method. This set of metabolites is probably a response to sodium sulfate salinity and extreme temperatures in the East Kazakhstan region.

Vitamins A, C, and E are important biologically active compounds with pronounced antioxidant properties [62–65]. The studied sample is characterized by a moderate content of vitamins A, C, and E. The content of vitamin A significantly exceeds the literature data (0.01 mg/100 g), which may be due to differences in growing conditions and analysis methods [66]. The vitamin C content is close to the values given in [66] (1.25 mg/100 g c V.), but is low. The reason for the low vitamin C content may be a decrease in ascorbate synthesis in conditions of drought and high insolation, typical for Eastern Kazakhstan [67]. The vitamin E content in the test sample is in the range typical for other species of soleros, for example, *Salicornia ramosissima* [63]. At the same time, the level of α-tocopherol is dominant among the studied complex. The dominant role of α-tocopherol in antioxidant protection is confirmed by the ratio of vitamin E to β-carotenoid (≈3.4:1). Thus, the results obtained confirm the modern concept that, under conditions of abiotic stress, halophyte metabolism is directed towards the accumulation of tocopherols as the most effective lipophilic antioxidants [48].

4. Materials and Methods

4.1. Sampling and Sample Preparation

The aboveground organs of *S. europaea* were collected in September 2024 in the vicinity of the village of Kindikty (Aksuat district, Abai region) (Figure 5). The choice of this location is due to the need to obtain environmentally friendly plant raw materials that are not subject to man-made pollution.

The botanical identification was carried out on the basis of morphological features according to regional definitions - Flora of Kazakhstan [10] and the Plants of the World Online (POWO) database [68].

 doi:10.20944/preprints202605.1100.v1

Selection criteria: the phase of maximum vegetative development, absence of damage and signs of phytopathology. Harvesting was carried out manually in dry weather, in the phase of maximum vegetative development of the plant.

After purification from impurities and pre-freezing, the collected raw materials were freeze-dried (SCIENTZ-12N Freeze Dryer, China) at a temperature of -50 °C to a constant weight. The dried material was ground to a particle size of about 0.5-1 mm and stored in glass jars with sealed stoppers.

4.2. Organic Elemental Analysis

The content of total carbon (TC), organic carbon (TOC), inorganic carbon (TIC), total nitrogen (TN) and total sulfur (TS) was determined using a CNS Vario Max element analyzer (Elementar Analysensysteme, Germany). The samples were previously dried at 105 °C and ground in an agate Pulverisette 2 mill (Fritsch, Germany).

TC [mass. %] was determined by catalytic combustion at 1140 °C, and TOC [mass %] was determined by thermal decomposition at 550 °C. The TIC content [mass %] was calculated from the difference between TC and TOC. All measurements were performed in two repetitions, followed by statistical processing.

4.3. Gas Chromatographic Mass Spectrometric Analysis

Gas chromatographic mass spectrometric analysis of ethanol extract of S. europaea was performed on an Agilent 7890A gas chromatograph with an Agilent 5975C mass-selective detector. The components were separated on an Rtx-100DHA capillary column (30 m x 0.25 mm x 0.5 μm) at a helium flow rate of 1.0 ml/min.

Analysis conditions: injector temperature – 280 °C; thermostat mode: from 60 °C to 300 °C at a speed of 8 °C/min; ion source temperature - 230 °C.

The mass spectra were recorded in full scan mode. The compounds were identified by comparing the mass spectra with the NIST 08 database. The quantitative analysis was performed using the peak area normalization method using the GS-MSD Data Analysis software.

The above-ground part of *S. europaea* was extracted in a Soxlet apparatus using 90% C_2H_5OH (raw material: solvent ratio 1:20) at 80 °C for 6 hours. The resulting extract was filtered, cooled, and then the solvent was removed on an IKA RV 10 rotary evaporator (China) under vacuum.

4.4. IR- Fourier Spectroscopy

The IR spectra of the feedstock and the ash fraction were recorded in the mode of disturbed total internal reflection (NPVO/ATR) on a Bruker Alpha II spectrometer with a diamond module. The measurements were carried out in the range of 4000-400 $cm^{-}1$ with a resolution of 4 $cm^{-}1$ (24 scans).

To ensure statistical reliability, each sample was analyzed nine times. Before each measurement, the background spectrum of the air was recorded and purified with isopropanol. Data processing, including baseline correction and spectrum normalization, was performed using OPUS (Bruker) software.

The preparation of plant raw materials for IR spectroscopic analysis included freeze-drying (Alpha 1-2 LD Plus, Martin Christ GmbH, Germany) for 24 hours and grinding in an RM 200 mill (Retsch, Germany). The resulting powder was divided into two parts: the first fraction (the initial sample) was used for IR analysis, the second fraction (ash) was mineralized in a Phoenix microwave muffle system (CEM, USA).

The samples were ashed by stepwise heating: exposure at 250 °C (30 minutes), followed by an increase in temperature to 450 °C (50 °C step every 30 minutes). The total duration of the process was 5 hours.

4.5. Liquid Chromatography – Mass Spectrometry LC-MS–TOF

 doi:10.20944/preprints202605.1100.v1

0.5 g of the crushed bark of the plant was poured into 10 ml of 70% methanol, extracted for 30 minutes at a temperature of 400° C in an ultrasonic bath and filtered through a filter with a thickness of 0.22 microns. The extracts were analyzed using an ultra-efficient liquid chromatography system (UPLC, Acquity, Waters) and high-resolution tandem mass spectrometry (HRMS/MS, Q-Eexactive, Thermo). The samples (5 μl each) were separated using an Acquity BEH Shield column (2.1 × 150 mm, grain size 1.7 μm, water) at a temperature of 40 °C. Water was mixed with formic acid (A, LC-MS class, Merck) and acetonitrile (B, LC-MS class, Merck) at a concentration of 5-75%. The compounds were identified based on their m/z values, retention time, and fragmentation spectra. The identification of the compounds was confirmed based on their exact masses using the MSDIAL database (version 4.92).

4.6. Determination of Total Phenolic Content (TPC)

The total content of polyphenols was determined by the colorimetric method with the Folin-Chocalteu reagent (a mixture of phosphomolybdenum and phosphovolframic heteropoly acids). Dilute Folin-Chocalteu reagent and 0.5 ml of 20% Na_2CO_3 solution were added to 100 ml of methanol solution. After incubation in the dark at room temperature for 2 hours, the optical density was measured at 760 nm (U-2810, Hitachi, Japan). The calculation was carried out using the gallic acid calibration curve (0,20; 0,16; 0,12; 0,08; 0,04 and 0.02 mg/ml). The results were expressed in milligrams of gallic acid equivalents per gram of dry feed (mg GAE/g). All measurements were performed three times.

4.7. Determination of Total Flavonoid Content (TFC)

The total flavonoid content was determined by spectrophotometric method using aluminum nitrate. 1 ml of $Al(NO_3)_3 \cdot 9H_2O$ solution was added to the methanol solution of the extract (10 mg/ml). The reaction mixture was incubated for 5 minutes at room temperature. Optical density was measured at 420 nm on a spectrophometer (U-2810, Hitachi, Japan), taking into account background correction for colored extracts. The calculation was performed using the calibration curve of quercetin in the concentration range of 0.015-0.150 mg/ml. The results were expressed in milligrams of quercetin equivalents per gram of dry vegetable raw materials (mg QE/g). All measurements were performed three times.

4.8. Determination of Antioxidant Activity (ABTS Method)

The antioxidant activity was determined by the method of bleaching the radical cation $ABTS^{\bullet+}$. The radical was obtained by mixing 14 mM solutions of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and 7 mM $K_2S_2O_8$ in equal volumes, followed by incubation in a dark place at room temperature for 12-20 hours. The working solution of $ABTS^{\bullet+}$ was diluted with deionized water to an optical density of 0.70-0.80 at 734 nm. 100 ml of dilute methanol solution of the extract was added to 3.0 ml of the $ABTS^{\bullet+}$ working solution. Optical density was measured on a spectrophotometer (U-2810, Hitachi, Japan) at 734 nm after 6 minutes of incubation. The background correction for the stained samples was taken into account. The degree of inhibition of the ABTS radical was calculated by the formula (1):

$$I, \% = \frac{A_0 - A_p}{A_0} \times 100 \quad (1)$$

where I - percentage of inhibition, A_0 - optical density of the control sample, and a_p - optical density of the analyzed sample. The concentration of antioxidants was determined by the trolox calibration curve (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) in the concentration range of 0.02-0.20 mg/ml. Antioxidant activity was expressed in trolox equivalents per gram of dry raw materials (mg TE/g). All measurements were performed three times.

4.9. Determination of Vitamin Content

 doi:10.20944/preprints202605.1100.v1

The vitamin C content was determined by HPLC on an Agilent 1200 Series system (USA). Extraction was performed with a solution of metaphosphoric acid, and the total vitamin C content was determined after reduction of dehydroascorbic acid with L-cysteine. Separation was performed on a reversed-phase C18 column (250 × 4.0 mm, 5 μm) using a mixture of a solution of KH_2PO_4 and a solution of cetrimide in methanol (900:100, v/v) as the mobile phase. The flow rate was 0.7 ml/min, the injection volume was 30 μl, and detection was performed at 265 nm. Quantitative determination was performed using the external standard method.

The vitamin A content was determined after alkaline saponification of the sample with ethanol KOH solution in the presence of an antioxidant, followed by extraction of the unsaponifiable fraction with an organic solvent. Chromatographic separation was performed on a normal phase column of LiChrosorb Si 60 (250 × 4 mm, 5 μm) using a mixture of n-hexane and n-butanol (98:2, v/v) as the mobile phase. Detection was performed at 325 nm. The quantitative content was determined according to the calibration schedule of standard all-trans-retinol solutions.

The vitamin E (α-tocopherol) content was determined by HPLC after preliminary alkaline saponification of the sample in the presence of an antioxidant and extraction with an organic solvent. The analysis was performed on a normal-phase silica gel column (250 × 4.6 mm, 5 microns) using a mixture of n-hexane and 2-propanol (99.5:0.5, v/v) as the mobile phase. Detection was carried out fluorimetrically at λex 295 nm and λem 330 nm. All measurements were carried out in three repetitions, the results were expressed in mg/100 g of dry vegetable raw materials.

5. Conclusions

The complex application of complementary analytical methods (HPLC, GC-MS, IR-Fourier spectroscopy, elemental analysis) allowed an integrated assessment of its phytochemical profile for the population of *Salicornia europaea* L, native to Eastern Kazakhstan.

Flavonoid derivatives, phenolic acids, lipophilic compounds, and low molecular weight components forming the bioactive potential of the extract were identified using liquid chromatography with mass spectrometric detection (LC-MS-TOF) and gas chromatography with mass spectrometry (GC-MS).

IR spectroscopy confirmed the presence of hydroxyl, carbonyl, and aliphatic groups characteristic of phenolic compounds, organic acids, and lipids.

The elemental composition of the aboveground organs reflects the biogenic nature of the plant. Quantitative indicators of the total content of polyphenols and flavonoids, antioxidant activity, as well as established concentrations of vitamins A, C and E indicate the bioactive potential of the studied sample.

The data obtained allow us to consider *S. europaea* L from East Kazakhstan as a promising plant raw material for further applied research aimed at the development of cosmetic and cosmeceutical products.

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