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ES Bogdanova
Institute of Ecology of the Volga River Basin, Russian Academy of Sciences, 10 Komzina St., Togliatti, Russia

IG Zakhozhiy
Institute of Biology of the Komi Science Centre of the Ural Division Russian Academy of Sciences, 8 Kommunisticheskaya St., Syktyvkar, Komi Republic, Russia

GN Tabalenkova
Institute of Biology of the Komi Science Centre of the Ural Division Russian Academy of Sciences, 8 Kommunisticheskaya St., Syktyvkar, Komi Republic, Russia

VA Rozentsvet
Institute of Ecology of the Volga River Basin, Russian Academy of Sciences, 10 Komzina St., Togliatti, Russia

VN Nesterov
Institute of Ecology of the Volga River Basin, Russian Academy of Sciences, 10 Komzina St., Togliatti, Russia

OA Rozentsvet
Institute of Ecology of the Volga River Basin, Russian Academy of Sciences, 10 Komzina St., Togliatti, Russia

Correspondence
ES Bogdanova
Institute of Ecology of the Volga River Basin, Russian Academy of Sciences, 10 Komzina St., Togliatti, Russia

Bioaccumulation of nutrients, lipids, pigments, antioxidants and essential oils in plants of genus Artemisia growing in lake Elton region (South-East of the European part of Russia)

ES Bogdanova, IG Zakhozhiy, GN Tabalenkova, VA Rozentsvet, VN Nesterov and OA Rozentsvet

Abstract
The chemical composition of plants genus *Artemisia*, representatives of the natural flora of the Lake Elton region, was studied. The aboveground part of the studied species is characterized by a high accumulation of Na. The pool of free amino acids (AA) of the plants studied was characterized by an increased proline concentration. The fatty acids (FA) ratio ω3/ω6 of the lipid fraction was between 1.5 and 2.3. The highest content of phenolic compounds, flavonoids and carotenoids was found for *A. santonica*. The antiradical activity of alcoholic extracts decreased as follows: *A. santonica* > *A. pauciflora* > *A. lerchiana*. The yield of essential oil from *Artemisia* species was 0.18-0.33% of dry weight (DW). The main components of essential oils of *A. lerchiana* and *A. pauciflora* were camphor, isoborneol and 1,8-cineol terpinen-4-ol. The results of this study indicate that the three species examined: *A. santonica*, *A. pauciflora* and *A. lerchiana* – could be used as a raw material for the production of valuable biologically active substances.

Keywords: Amino acids, essential oil, fatty acids, lipids, phenolic compounds

1. Introduction
Artemisia is one of the largest genera including about 400 species. These perennial herbs or shrubs spread throughout the northern hemisphere, especially in the temperate zone of Europe and Asia [1]. Many species produce secondary metabolites of wide biological activity, such as essential oils, tannins, organic acids, carotene, ascorbic acid, glycosides, absinthin etc. [2]. Essential oils and alcohol extracts exhibit antioxidant, antiviral and bactericidal effect [3-6]. The biologically active compounds produced by the plants from genus Artemisia are used in pharmaceutical and cosmetic preparations [7].
Artemisia lerchiana, *A. pauciflora*, *A. santonica* are fairly abundant in the plant communities growing on salty soils in the areas adjoining Lake Elton (a large self-deposited salt lake) [8]. These plants are glycohalophytes and occupy the place between true halophytes (euhalophytes) and glycophytes [9]. Glycohalophytes are specific in their capability to limit salt intake thanks to low permeability of the root cells' membrane to inorganic ions [10]. It means that these plants can significantly differ from both halophytes and glycophytes in their components responsible for successful adaptation of the plants to different levels of salinity.
Among the evolutionally developed mechanisms allowing halophytes' life under saline conditions, secondary metabolites play an important role [9, 11, 12]. Certain amino acids (AA), phenol compounds and soluble sugars [13, 12] are universal components which allow the plants stabilize antioxidant and osmotic potential, tolerate water deficiency and toxic effect of excessive ions [14, 15]. Lipids of cell membranes play no less importance role in the ion homeostasis of cytoplasm and in the regulation of metabolic processes to neutralize the negative effects of salts [16].
Earlier we have shown the specificity of the composition of membrane-forming lipids in eu- and cryno- glycohalophytes [17]. Differences in the structure of the photosynthetic apparatus and the organization of antioxidant protection in different types of halophytes have been revealed [18, 19]. However, the chemical composition in the plants can vary considerably depending not only on the strategy of salt accumulation, but also on the growth conditions, the stage of the plant ontogenesis and the particularity of the species [20].

In the present work, three species of genus Artemisia growing in the South-East of the European part of Russia were investigated for the composition of the components facilitating halo-tolerance and having potential biology activity.

2. Materials and methods

2.1 Plant material

The Lake Elton region is a part of the Caspian depression (49°07'N, 46°50'E). Fresh aboveground parts of *A. lerchiana*, *A. pauciflora*, *A. santonica* L., (Asteraceae Dumort) were collected from 15–20 individuals of each species in the first half of the day in June of 2013 and 2014. For the purpose of this study allocative spectra were collected from fully developed plants and divided into the underground part, stems and stem leaves. Samples for chemical analysis (macro- and microelements, low-molecular carbohydrates, protein, free AA, phenolic compounds) were fixed with liquid nitrogen and freeze-dried. Samples for lipid analysis were fixed with hot isopropanol and kept in the dark at –20 ºC for one month.

2.2 Determination of metal content

The contents of metal elements in the aboveground plant parts were determined after their mineralisation according to the method [21]. The concentration of metal ions in the samples was measured using an optical emission spectrometer with an inductively coupled plasma instrument (Spectro Ciros-Poa1, Germany).

2.3 Determination of carbon and nitrogen content

The content of carbon and nitrogen in the dried and milled plant samples was determined using an EA 1110 elemental analyser (CHNS-O, Italy), after combustion of the samples in quartz tubes at 1000 ºC in the presence of oxygen.

2.4 Lipid extraction and analysis

Lipids were extracted by the method of Bligh and Dyer [22]. The quantification of phospholipids (PL) was performed by determining the content of inorganic phosphorus [23] followed by calculation of PL molar masses. The total amount of PL was approximately estimated by the method [24].

Galactolipids (GL) were pre-quantified using a Sorbfil densitometer (Russia) calibrated with galactose. A more accurate quantification was performed with the anthrone reagent [25], by measuring absorbance of samples at 620 nm using a Specol spectrophotometer (Germany). Monogalactosyldiacylglycerol (Laroden, Sweden) and galactose (Sigma, USA) were used as calibration standards. Neutral lipids (NL) were assayed densitometrically, with tripalmitate (Sigma, USA) as a calibration standard.

2.5 Fatty acid profiles

Fatty acids (FA) were analysed in the form of their methyl esters (FAMEs). The esters obtained were purified by preparative thin-layer chromatography and analysed using a gas–liquid chromatography system (Crystal 5000.1, Chromatek, Yoshkar-Ola, Russia). FAMEs were identified by comparing their retention times with FA standards (Supelco 37, Supelco, USA), and quantification was performed with heptadecanoate as the internal standard.

2.6 Pigment analysis

The content of pigments extracted from the plant material (0.2–0.5 g of fresh leaf mass) with acetone (80%) was determined by measuring absorbance of the samples at 470 nm using a Specol 1300 instrument (Analytik Jena, Jena, Germany). The content of carotenoids (Car) was assayed according to method [26].

2.7 Determination of the amino acids, phenolic compounds and isolation of essential oils

The content of total AA was calculated in the air-dried material after its hydrolysis with 6 N HCl at 105 °C for 24 h using a T-339 analyser (Czech Republic). Free AA were extracted from the freeze-dried material with 70% (v/v) ethanol and assayed n a lithium buffer system using an AAA-400 analyser (Czech Republic).

The content of total phenolic compounds in plant samples was determined by the Folin–Ciocalteu colorimetric method [27] using gallic acid as a standard. The content of phenols was expressed as gallic-acid mass (mg) equivalents (GAE)/g dry weight (DW) [28].

The antioxidant activity of plant phenolic extracts was estimated by their ability to scavenge 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals. The free-radical scavenging activity was calculated using the following equation: % inhibition = $(A_{ref}–A_{samp}) \times 100 / A_{ref}$,where A_{ref} is the reference (control) absorbance and A_{samp} is the absorbance of a sample. The IC_{50} values calculated were defined as the concentrations of phenolic extract samples required to decrease absorbance by 50%.

Essential oils were extracted from the milled aerial parts of the plants by water distillation using a Clevenger apparatus. The chemical composition of essential oils was examined by gas chromatography-mass spectrometry using a Finnigan Trace DSQ instrument (USA). Interpretation of mass spectra was performed using the Xcalibur Data System software (ver. 1.4 SR1) and the NIST–05 (ver. 2.0) library of mass spectra.

The results obtained are presented as means of three independent biological samples (n=3) ± standard errors. Statistical calculations were made using the Statistica 10 software (Statsoft Inc., USA).

3. Results discussion

Examination of phytomass allocation showed that the leaf mass amounted to 47.0±3.0% of the total plant mass for *A. santonica*, 20.0±2.5 for *A. lerchiana*, and 28.0±2.0 for *A. pauciflora*, with the stem mass percentages being 33.0±3.0, 47.0±2.0, and 50.0±2.0 respectively. The content of DW in the aboveground plant parts depended on the environmental conditions and varied between 45% in *A. lerchiana* and *A. pauciflora* and 25% in *A. santonica*.

The level of absorption and accumulation of mineral elements by plants is a function of their genotypic particularities and depends on their growing conditions (physical and chemical composition of the soil, presence of certain elements in the air). It was known that plants of the Asteraceae family (and the *Artemisia* genus in particular) are capable of accumulating considerable amounts of heavy metals from the soil substratum [29]. The content of macroelements also varies in species and depends on their habitat [4]. So, analysis of the mineral composition of *Artemisia* species showed that the content of N, P, K and Ca in those species did not vary significantly (Table 1). The aboveground part of *A. santonica* had higher concentrations of Mg and Na comparatively to other species. A high content of Na reflects an enhanced uptake of this element from the shallow-running, heavily mineralized groundwater and is associated with the regulation of water potential [19]. The amounts of Zn, Cu, Mn, Cr and Mo in *A. santonica* were within the range observed for other

Artemisia species (Table 1). At the same time, their quantities in the vegetative part of *A. santonica* were somewhat smaller than in the other two species studied. The content of the most toxic heavy metals, Pb, Cd, Hg, was relatively low and did not exceed the levels recommended by the World Health Organization (WHO) (according to those standards, Pb content may vary from 5 to 10 mg/kg; Cd, from 0.2 to 4 mg/kg; Hg, 0.1 mg/Kg DW).

Recently, biomedical science has become increasingly interested in lipids and FA [30]. Our analyses showed that in the plants studied, lipids totalled 5.2–6.5% of DW, which agrees with the data obtained by other authors [6]. The maximum accumulation of lipids was observed in the leaves of *A. santonica*. The contents of GL and PL, which form cell membranes, were 47.4–57.2% and 9–14% of the total lipid amount, respectively (Figure). The content of NL, which serves as a metabolic and energetic reserve, was 33–41%.

Depending on the number of double bonds they have, FA can be divided into saturated, monounsaturated and polyunsaturated acids, with unsaturated acids including the ω-3, ω-6 and ω-9 FA families. It is known that FA can inhibit the growth of various pathogens. For example, lipophilic extracts of palmitoleic (16:0) and linoleic (C18:2n6) acids are active against gram-positive bacteria, and oleic acid (C18:1n9) is known to resist a broad spectrum of saprophytic fungi and yeast. Furthermore, ω-3 can reduce the risk of diseases related to the cardiovascular system [31-34]. In the plants studied, C16 and C18 acids amounted to over 70% of total FA (Table 2). The pool of FA included C16:0, C16:1, hexadecadienic (C16:2), stearic (C18:0), oleic (C18:1n9), C18:2n6 and linolenic (C18:3n3) acids. The content of short-chain (below 16 C atoms) FA was between 3.5 and 12.5%. Long-chain (20 and more C atoms) FA amounted to less than 4% of total FA. The amount of unsaturated FA was 2–3 times higher than that of saturated FA; at the same time, the proportion of ω3 FA was larger than that of ω6 FA (Table 2). It should be noted that the species studied did not differ significantly in their FA composition; in general, it correlated with the FA composition of wild species (*A. leucodes*, *A. santonica*) [35] and with the composition of their close relatives cultivated artificially [5].

Another class of lipophilic compounds are Car. The yellow pigments Car, are widely used in traditional and folk medicine as dietary supplements. Car (lutein and zeaxanthin) are needed in the human diet to prevent eye diseases, and β-carotene is a metabolic precursor of vitamin A. The data obtained showed a high accumulation of carotenoids in the aboveground part of *A. santonica* (6.4 mg/g). The contents of yellow pigments in the other two *Artemisia* species were significantly lower (Table 3). The results of HPLC analysis showed that β-carotene, lutein and zeaxanthin amounted to 30, 32 and 5% of total *A. santonica* Car respectively.

Phenolic compounds are important components of plants because of their nutritional value. Phenolic compounds extracted from plants of the genus *Artemisia* possess antioxidant properties and manifest adaptogenic, immunomodulating and hepatoprotective activities [36]. Among the phenolic compounds identified in the above-ground parts of plants are catechin and epicatechin; flavonoids (quercetin, kampferol et al.); hydroxybenzoic and hydroxycinnamic acids and their derivatives [35, 37]. The data on the content and antiradical activity of phenols extracted from the aboveground parts of the *Artemisia* plants are shown in Table 5. The highest level of polyphenolic compounds, about 50 mg GAE/g DW, was a characteristic feature of *A. santonica*, with the content of phenols in the aboveground parts of *A. pauciflora* and *A. lerchiana* being 2.5–3.0 times lower. The same was true for the flavonoid content, which varied in the range of 12–33 mg/g DW. Plant phenols are known for their strong antioxidant properties. Evaluation of the antiradical activity of alcohol extracts from *Artemisia* by the DPPH test showed that the extract from *A. santonica* had the strongest activity. The IC_{50} values for the other two *Artemisia* species were significantly higher, indicating a much lower concentration of compounds capable to intercept free radicals.

Among the species examined in this work, *A. lerchiana* is particularly well studied in this respect. The leaves of *A. pauciflora* contain 1,8-cineol, α-pinene, camphor, artepaulin and α-santonin [20, 5, 38, 39]. The yield of essential oils isolated by hydrodistillation was 0.18–0.33% The main components of *A. lerchiana* essential oils were camphor (29.8%), isoborneol (8.0%) and 1,8-cineole (6.2%). The essential oil of *A. pauciflora* was dominated by 1,8-cineole (14.5%), camphor (5.7%), terpinene-4-ol (4.0%) and isoborneol (3.0%) (Table 3). All the essential oil samples contained the monocyclic sesquiterpene germakren D (0.3–2.9%).

Artemisia plants are glycohalophytes (facultative halophytes) and have a root system with low permeability to salt. In order to maintain the required water potential and to absorb water from saline soil, they accumulate compatible organic and inorganic osmolites in the roots and aboveground organs [40]. Free AA amounted to 5–8 mg/g of DW in those plants (Table 4). The 14 components were identified in the AA pool, including *β*-alanine, valine, *γ*-amino butyric acid, histidine, arginine. In Table 2 shows only the AA content of more than 1% total sum. Among those AA, proline was the most abundant (75–82% of the total AA content). The high level of proline accumulation reflects its significant role in plants growing in the saline environment. The high concentration of proline in the leaves of *A. santonica* correlates with the high content of Na (Table 1).

The AA content of the protein fraction varied between 66 and 113 mg/g DW. Dominating among the identified AA were mono amino carboxylic (glycine, alanine, valine, leucine, isoleucine) and dicarboxylic (glutamine and aspartic) acids (Table 5); sulfur-containing (cysteine and methionine) were present in trace amounts.

Table 1: Mineral composition of in the aboveground part of *Artemisia* species

Elements	*A. lerchiana*	*A. pauciflora*	*A. santonica*	*A.* spp*
Macroelements, mg/g DM				
N	19.0±3.0	23.0±4.0	28.0±1.9	no data
C	388.0±12.0	424.0±14.0	426.0±14.0	no data
P	1.9±0.6	1.9±0.6	2.4±0.7	no data
K	13.0±5.0	13.0±5.0	16.0±7.0	11.5–18.4
Ca	5.8±1.7	6.4±2.0	6.0±1.8	0.6–10.7
Mg	1.7±0.5	1.7±0.5	3.0±0.9	0.06–2.9
Fe	0.58±0.16	0.69±0.19	0.20±0.06	0.02–3.13
Na	4.2 ±1.7	4.7±1.9	30.0±12.0	1.0–2.4
Microelements and heavy metals mg/kg DW				
Cu	15.0±3.0	12.7±2.5	13.4±2.7	7.3–88.9
Zn	19.0±4.0	22.0±4.0	17.0±3.0	13.7–38.7
Mn	87.0±26.0	110.0±30.0	50.0±15.0	16.0–75.0
Cr	2.3±0.5	2.4±0.5	0.62±0.12	1.3–28.3
Mo	1.4±0.6	1.3±0.5	0.8±0.3	no data
Ni	2.5±0.9	2.6±0.9	2.3±0.8	0.1–11.3
Pb	0.93±0.23	0.75±0.19	< 0.5	0.5–25.8
Cd	0.11±0.06	0.10±0.05	0.24±0.12	0.1–5.3
Co	0.32±0.13	0.38±0.15	0.13±0.05	0.2–3.7
B	48.0±14.0	49.0±15.0	62.0±19.0	no data
Hg**	12.0±5.0	13.0±5.0	4.4±1.8	no data

Note: p = 0.95; * – According to [2]; **ng/g

Table 2: Fatty acid composition of in the aboveground part of *Artemisia* species (% of total FA)

Fatty acids	*A. lerchiana*	*A. pauciflora*	*A. santonica*
>16	12.5±1.2	7.4±0.7	3.5±0.4
16:0	13.6±1.3	15.4±1.5	17.1±1.7
16:1	0.8±0.1	0.8±0.1	1.7±0.1
16:2	–	1.2±0.1	–
18:0	1.2±0.1	1.5±0.1	2.8±0.3
18:1n9	4.6±0.5	5.1±0.5	6.9±0.7
18:2 n 6	19.4 ±1.9	24.9±2.5	19.7±1.9
18:3 n 3	37.9±3.7	37.8±3.8	44.6±4.4
SFA	28.1±2.8	25.6±2.5	23.4±2.3
USFA	64.7±6.5	72.0±7.2	76.6 ±7.8
SFA/USFA	2.3±0.2	2.8±0.3	3.2 ±0.3
ω3/ω6	1.5±0.1	1.9±0.2	2.3±0.3

Note: (mean±standard errors, n=3)

Table 3: Content of carotenoids, phenolic compounds, flavonoids and essential oils and antiradical activity of in the aboveground part of *Artemisia* species

Parameters	*A. lerchiana*	*A. pauciflora*	*A. santonica*
Total carotenoids, mg/g DM	0.8±0.1	0.7±0.1	0.9±0.1
Total phenols, mg GAE*/g DM	17.3±2.0	20.2±0.4	51.7±0.8
Flavonoids, mg GE**/g DM	12.1±0.4	13.7±0.4	32.7±0.3
IC_{50}, μg/cm^3	312.0±10.0	252.0±10.0	84.0±4.0
Yield of essential oil, mg/g DM	1.8±0.2	2.2±0.2	3.3±0.3

Note: *– Gallic acid equivalents; ** – Catechin equivalents

Table 4: Content of free amino acids) of in the aboveground part of *Artemisia* species (% of total AA)

Amino acid	*A. lerchiana*	*A. pauciflora*	*A. santonica*
Proline	74.8±11.2	78.1±11.7	81.7±12.2
Alanine	1.3±0.1	1.0±0.1	4.4±0.6
Valine	1.5±0.2	1.1±0.2	3.6±0.5
γ- aminobutyric acid	1.7±0.2	1.6±0.2	1.1±0.1
Histidine	1.3±0.1	1.2±0.1	0.6±0.1
Arginine	16.3±2.4	15.2±2.3	3.6±0.5
Total free AA	8.3±1.2	7.6±.1	4.8±0.7

Table 5: Amino acid composition of the protein fraction of in the aboveground part of *Artemisia* species (% of total AA)

Amino acid	*A. lerchiana*	*A. pauciflora*	*A. santonica*
Aliphatic	10.1±1.5	10.3±1.5	10.1±1.5
Alkaline	18.3 ±2.7	18.0±2.2	16.0±2.4
Aromatic	6.7±1.0	7.5±1.1	9.5±1.4
Dicarboxylic	23.2±3.4	21.9±3.3	23.0± 3.4
Heterocyclic	12.4±1.8	12.3±1.8	8.2±1.2
Mono-amino carboxylic	28.9±4.3	29.0±4.3	32.8±4.9
Sulfur-containing	0.4±0.1	1.0±0.1	0.4±0.1
Total AA, mg/g DW	73. ±10.5	66.0±10.0	113.0±17.0

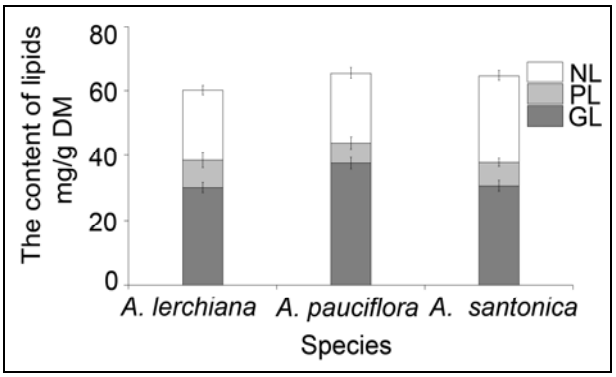

Fig 1: Content of lipids in the aboveground part of *Artemisia*; NL– neutral lipids, PL – phospholipids, GL – glycolipids

4. Conclusions

The chemical composition of plants of the genus *Artemisia*, representatives of the natural flora of the Lake Elton region (Prieltonie), was studied. The aboveground part of the studied species is characterized by a high accumulation of Na. The total content of Pb, Cd and Hg did not exceed concentrations established by WHO for medicinal plants. The pool of free AA of the plants studied was characterized by an increased proline concentration. The FA ratio ω3/ω6 of the lipid fraction was between 1.5 and 2.3. The highest content of phenolic compounds, flavonoids and carotenoids was found for *A. santonica*. The antiradical activity of alcoholic extracts decreased as follows: *A. santonica* > *A. pauciflora* > *A. lerchiana*. The yield of essential oil from *Artemisia* species was 0.18–0.33% of DW. The main components of essential oils of *A. lerchiana* and *A. pauciflora* were camphor, isoborneol and 1,8-cineol terpinen-4-ol. The results of this study indicate that the three species examined: *A. santonica*, *A. pauciflora* and *A. lerchiana* – could be used as a raw material for the production of valuable biologically active substances.

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