

Anatomical, morphological and phytochemical properties of *Aegopodium alpestre* Ledeb.: a case study of Kazakhstan

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Abstract

This article deals with the phytochemical, morphological and anatomical investigation of ethanol-based extracts derived from the leaves and stems of the *Aegopodium alpestre*. The vegetative organs of *A. alpestre* were conserved according to Strasburger-Fleeming method using a 1:1:1 mixture of alcohol-glycerin-water. A total of 1200 ethanol-based extracts (2 from leaves and 2 from stem tissues per plant) were prepared using the Soxhlet extractor. All extracts were used to identify organic and inorganic compounds in the leaves and stems of the studied plant. Contents of biologically active substances, microelements, vitamins and amino acids were determined. This article is the first paper to display very high concentration and diversity of vitamins (6 types), micronutrients (5 types), and aminoacids (13 types) in the leaves and stems of *A. alpestre*. Findings conclude that identification of biologically active substances in the above the ground vegetative organs of *A. alpestre* may be a common practice in the future. Considering the study results, *A. alpestre* may be used as a medicinal plant on a large scale. For this, the cultivation practice needs to be scaled up.

Keywords: *Aegopodium alpestre*; extraction; microelements; vitamins; amino acids; medicinal plant

Introduction

Why is it important to find new medicinal plants?

Medicinal plants have proven to be a valuable source of molecules with therapeutic potential. Today, they are crucial for the discovery of new drugs (Atanasov *et al.*, 2015). To create a stable supply of new herbal medicines, researchers should draw their attention to pharmacopoeial, vicarious and the promising species of plants (Grudzinskaya *et al.*, 2014). *Aegopodium alpestre* is one of those species.

General characteristics of the genus Aegopodium and the species A. alpestre

Aegopodium Linnaeus, 1753 is a perennial plant from the family *Apiaceae* growing in the mountainous regions of Europe, Siberia, the Caucasus, Kazakhstan and Central Asia. The genus *Aegopodium* includes 8 species: *Aegopodium tadshikorum* Schischk., *A. podagraria* L., *A. latifolium* Turcz., *A. kashmiricum* (R.R. Stewart ex Dunn) Pimenov, *A. henryi* Diels, *A. handelii* H. Wolff, *A. burttii* Nasir, *A. alpestre* Ledeb. (The Plant List, n.d.). However, only *A. podagraria* and *A. alpestre* are native to Kazakhstan.

A. alpestre creates a ground cover that is sparser as compared to that of *A. podagraria*, (Stukalyuk, 2016). It grows in conifer and mixed forests, mountain meadows, and shrub thickets. *A. alpestre* reproduces asexually from rhizomes, whilst its sexual mode of reproduction is suppressed. It is a melliferous plant, well eaten by the livestock animals.

Medical applications of ethanol-based extracts of A. alpestre

The medicinal properties of plants in the genus *A.* are well known. For instance, they have systemic, detoxification, and antihypoxic impact on the human body. Medicines that are made from these plants help improve metabolism and the overall health of the person taking it. Normally, this kind of medicine is a prescription for hypovitaminosis and iron deficiency anemia (Gridneva and Khanina, 2005). Galenicals from the aerial parts of *A. alpestre* are able to improve the kidney function and the renal blood flow. A plant containing such a variety of vitamins, amino acids, and other useful substances is a good candidate for medicinal plant research.

The liquid herbal extract of *A. alpestre* is applied in the treatment of rheumatism, whilst herbal infusions made from its parts are used as headache pain relievers. The available literature knows cases where *A. alpestre* was used in cancer medication (Grudzinskaya *et al.*, 2014).

Chemical composition of plants in the genus Aegopodium

The chemical composition of different parts of *A. podagraria* has been little studied. The plant was found to contain carbohydrates (i.e., umbelliferose, glucose, and fructose), cyclitols (e.g., glucinol), lectins, secondary metabolites (coumarins such as umbelliferone, bergapten, and methoxsalen; and steroids such as β -sitosterol), and choline. The aerial part of the plant contains vitamin C, carotene, and flavonoids (quercetin and kempferol). Amino acids found in *A. podagraria* include arginine, histidine, leucine, lysine, threonine, valine, and methionine. The plant accumulates a variety of micronutrients such as calcium, iron, silicon, phosphorus, magnesium, aluminum, molybdenum, vanadium, copper, gallium, boron, titanium, and zinc. The lipophilic essence of the plant contains 1.5% of chlorophyll. You may also find unsaturated and saturated fatty acids, such as palmitic, stearic, oleic, linoleic, and arachidic. Furthermore, *A. podagraria* comprises organic acids in its leaves and stem, such as malic and citric (Tovchiga *et al.*, 2006).

The state-of-the-art and relevance of the study

Issues addressed by different studies embrace the ecological characteristics, abundance, and role of *A. alpestre* in mountain ecosystems (Wu *et al.*, 2016; Yang *et al.*, 2016). Separate studies examine morphology of the fruits and seeds of the *A. alpestre* with the scanning electron microscopy technique (Ostroumova, 2018), and only one work explores the lipid and vegetable oil content in the seeds of plant specimens belonging to different families, including *Apiaceae* (Azimova *et al.*, 2012).

Although *A. alpestre* is widely used in folk medicine, the plant is very little studied. There are no works devoted to the chemical composition of different plant parts of *A. alpestre*. There are no studies examining the content of more complex compounds such as vitamins and amino acids. At the same time, there is a need to find new medicinal plants for the creation of new drugs. *A. alpestre* may be one of those plants. Although there are studies exploring the morphological and ecological characteristics of *A. alpestre*, phytochemical properties of this plant species remain understudied. If the content of inorganic compounds, vitamins and amino acids is high, *A. alpestre* can be recommended for pharmaceutical purposes. Thus, the aim of this work is to examine

the phytochemical characteristics of *A. alpestre* using a batch of plants collected in Kazakhstan. The objectives of the study are (1) to measure the concentration of micro- and macro-elements in the leaves and stems of *A. alpestre*, and (2) to establish the concentration of coumarins, flavonoids, vitamins and amino acids in the leaves and stems of *A. alpestre*.

Materials and Methods

Research object

The study examines the shoot system of *Aegopodium alpestre* Ledeb., plant in the genus *Aegopodium* (family Apiaceae). Samples of *A. alpestre* were collected during the period of flowering (early July) in 2018 in the Big Almaty Gorge (latitude/longitude: 43.136976, 76.903267; 1.500-2.500 m a.s.l.) and identified by scientists of the Institute of Botany and Phytointroduction, Ministry of Education and Science (Republic of Kazakhstan). Plant identification was performed through comparing visual characteristics with those indicated in (Baitenov, 2001).

Methods

For the morphological analysis, leaves and stems were collected, dried, and pressed. Leaf and stem samples were conserved according to Strasburger-Fleüming method using a 1:1:1 mixture of alcohol-glycerin-water.

Anatomical slides preparation of leaves and stems was performed using an electronic microtome MZP-01 Technom. A total of 106 temporary anatomical slides with 10-15 µm thickness were prepared and then placed into glycerol and balsam (Barykina *et al.*, 2004; Yeung *et al.*, 2015). Microphotographs were obtained with a video microscope MCX100 Trinocular MICROS (magnification x100, x400; Austria).

A total of 1200 samples were collected from 300 plants. One sample contained all the leaves or stems from one plant. Hence, there were four samples (2 with leaves and 2 with stems) per plant, which were tested for the presence of inorganic and organic compounds.

Methods of extraction and spectrophotometry

Moisture and ash content and extractivity of raw materials were estimated according to requirements of the State Pharmacopoeia of the Republic of Kazakhstan and the European Pharmacopoeia (Council of Europe, 2001; Tulegenova, 2008, 2009).

To determine the weight loss on drying, a certain amount of raw material was placed in an oven heated to 105 °C.

The content of inorganic compounds was determined by measuring inorganic mass that has remained after the incineration and calcination of pre-prepared raw material. For this, 1 g of biomass (either leaves or stems) was placed in a crucible for charring. The crucible with a charred sample was then placed into a laboratory muffle furnace. The furnace temperature was held at 550 °C until complete calcination. After calcination, the crucible was allowed to cool down for 2 hours and then placed in a flaw detector, which contained anhydrous calcium chloride at its bottom. Finally, sample was cooled down and weighed on a balance.

To determine the content of organic compounds, samples were crushed and passed through a sieve with an opening diameter of 1 mm. One gram of the resulting biomass was placed in a conical flask and added 50 cm³ of 80% ethanol. The flask was then closed with a lid. The sample was weighed on a balance with a weighing error of up to 0.01 g and kept intact for 1 hour. Afterwards, the collector was connected to a reverse condenser. The content of the flask was heated until boiling and maintained for 2 hours. When cooled down, the flask was again covered with a lid and weighed. The weight gap was closed with ethanol. The content of the flask was filtered through a paper filter and the filtrate volume was 25 cm³. The sample was heated in a water bath. After

evaporation, the content of the flask was dried at 105 °C for 3 hours and then cooled down for 30 minutes in a desiccator with dehydrated calcium chloride at the bottom. The final weigh was taken afterwards.

All tests were conducted in triplicate.

The content of flavonoids, secondary metabolites (coumarins), vitamins, macro- and microelements and amino acids was determined by spectrophotometry, high-performance liquid chromatography (HPLC), and gas chromatography/mass spectrometry (GC/MS).

Data analysis

Normally distributed data were analyzed in Past v. 3.0. Differences were determined using a two-sample t-test with a significance level of 0.05. Tables provide information about the mean values and standard errors (mean \pm SEM).

Results

Morphological properties

Aegopodium alpestre Ledeb. is a perennial grassy plant, growing to a height of 69.13 ± 14.8 cm with rhizomes. The stem is hollow, furrowed, simple or slightly branching at the top. Both sides of the leaves are glabrous. Lower leaves are petiolate (attached to the stem by a petiole), and upper leaves are sessile (attached directly to the stem, lacking a petiole). The plates of lower leaves in the outline are broadly triangular, almost threefold; segments of its pinnate are dissected into ovate, pointed, incised-dentate lobes, about 3 cm long. The upper leaves have the lobes lanceolate and sharp. A compound umbel has 15 to 20 rays (floral branches), without involucre and involucels. These rays are angular, and the outer rays are much longer than the inner ones. The flower petals are 1 mm long, without clear tubules (Figure 1).



Figure 1. *Aegopodium alpestre* Ledeb

Anatomical properties

The cross-section of the stem is ribbed; it consists of primary cortex and central cylinder. Stem thickness is $5630.77 \pm 0.31 \mu\text{m}$, and epidermal thickness is $37.61 \pm 0.70 \mu\text{m}$. Under the epidermis, parenchyma cells with thickened walls are located. In the ribs of the stem, collenchyma cells can be found, arranged in eight rows (Figure 2).

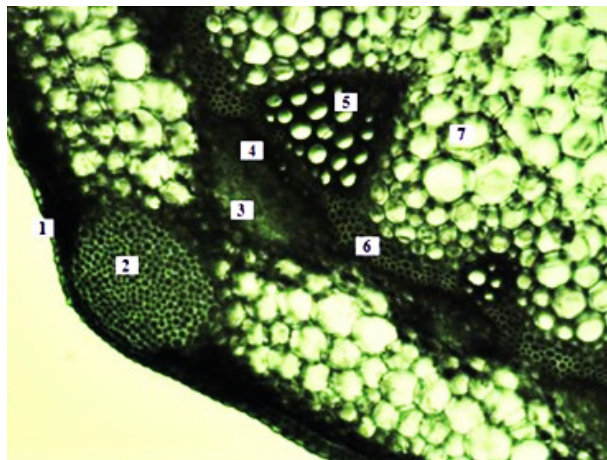


Figure 2. Internal structure of plant *Aegopodium alpestre* Ledeb.

*Note: 1 - epidermis, 2 - collenchyma, 3 - bast fibers, 4 - phloem, 5 - xylem, 6 interfascicular vascular cambium, 7 – parenchyma

As a characteristic of dicotyledonous plants, the intercellular cambium forms a ring. In the center of the stem, an air cavity is located. In seed plants, conductive bundles are typically collateral, the xylem is located adaxially, and the phloem is positioned abaxially. The number of conductive bundles in the stem is 28; they form in different sizes, with the maximum phloem thickness of $52.41 \pm 0.40 \mu\text{m}$, and the maximum xylem thickness $335.75 \pm 0.37 \mu\text{m}$ (Table 1). On the outside, the conductive bundles are surrounded by sclerenchyma.

Table 1. The *A. alpestre* stem parameters

Stem thickness, μm	Collenchyma, μm	Bast fibre, μm	Epidermis, Mm	Conductive bundle thickness (max), μm	
				Xylem	Phloem
5630.77 ± 0.31	560.83 ± 0.38	142.35 ± 0.36	37.61 ± 0.70	335.75 ± 0.37	52.41 ± 0.40

Leaves of *A. alpestre* in a cross-section consist of the upper and lower epidermis, mesophyll, conductive bundles, and cells of mechanical tissue. Cells of the upper and lower epidermal layers come in different quantities, shapes, and sizes. Hence, the upper-layer cells are larger ($97.08 \pm 1.4 \mu\text{m}$) compared to cells of the lower epidermis ($52.41 \pm 0.70 \mu\text{m}$). The palisade cell layer of mesophyll is thin, which is characteristic of shade plants. The thickness of the central vein is $2493.38 \pm 1.37 \mu\text{m}$. The central conductive bundle is large, with the length of $471.20 \pm 3.0 \mu\text{m}$, collateral, surrounded by sclerenchyma cells. The xylem is located on the upper side of the leaf plate and the phloem is located on the bottom side, both are well developed (Figure 3).

Phytochemical analysis

Data in Table 2 show high extractivity of leaves and stems (28.77% and 25.44%, respectively). Perhaps, this is due to a high concentration of organic compounds. The quantitative content of basic biologically active substances in the above the ground vegetative organs of *A. alpestre* is depicted in Table 3.

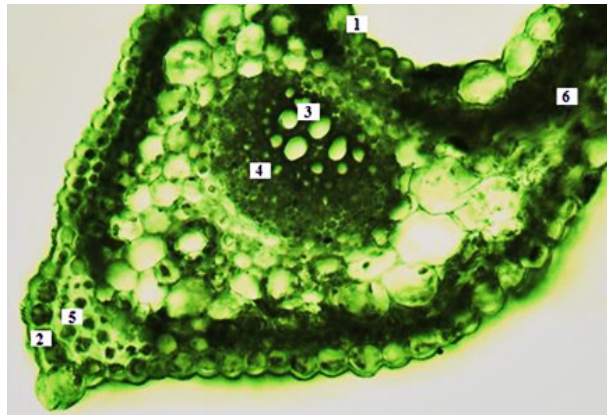


Figure 3. Internal structure of the *Aegopodium alpestre* leaves.

*Note: 1 - upper epidermis, 2 - lower epidermis, 3 - xylem, 4 - phloem, 5 - collenchyma, 6 - spongy mesophyll

Table 2. Parameters of good-quality raw materials from *Aegopodium alpestre*

Indicator	Item	
	Leaf	Stem
Moisture content	6.44% ¹	7.33% ¹
Ash content	16.15%	27.9%
Extractivity	28.77%	25.44%

¹ - Differences were considered significant at $p \leq 0.05$.

Table 3. Content of biological active substances in *Aegopodium alpestre*

Indicator	Item	
	Leaf	Stem
Coumarins	0.32%	0.25%
Flavonoids	0.77%	0.34%
Mass fraction of carbohydrates	6.99%	9.7%
Mass fraction of protein	3.5%	2.84%
Tannin	4.25%	0.86%

Data in Table 3 show that the content of coumarins in the leaves of *A. alpestre* (0.32%) is higher by 0.25% than that in the stems ($p \leq 0.05$). There are two times as many flavanoids in leaves as in stems (0.77% vs 0.34% $p \leq 0.01$). The content of carbohydrates is also high. Leaves contain 6.99% of carbohydrates and stems contain 9.7% of carbohydrates ($p \leq 0.05$). Protein content in leaves and stems is 3.5% and 2.84% ($p \leq 0.05$), respectively. Tannin content in leaves is almost five times higher than that in stems (4.25% vs 0.86%, $p \leq 0.01$). The content of heavy metal Cd is within the range permissible for medicinal plants (Table 4).

Table 4. Findings from the elemental composition analysis

Element	Concentration, mg per 100 g		Value
	Leaf	Stem	
Iron, Fe	12.973	3.144	Microelement
Copper, Cu	Not detected	Not detected	Microelement
Zinc, Zn	7.138	3.619	Microelement
Cadmium, Cd	0.00004	0.00024	Heavy metal
Calcium, Ca	Not detected	Not detected	Microelement
Potassium, K	0.0387	0.0110	Microelement
Phosphorus, P	87.63	67.37	Microelement
Selenium, Se	0.010	0.019	Microelement

From the data presented in Table 4, it can be seen that vegetative organs of *A. alpestre* have a sufficient quantity of phosphorus, iron and zinc. The phosphorus content in leaves (87.63 mg per 100 g) is 1.3 times higher than that in stems (67.37 mg per 100 g, $p \leq 0.05$). Iron content is four times higher (12.973 vs 3,144 mg per 100 g, $p \leq 0.01$) and zinc content is two times higher (7.138 vs 3,619 mg per 100 g, $p \leq 0.05$). Leaves and stems have a similar quantity of selenium, which belongs to a group of essential trace elements (0.010 vs 0.019 mg per 100 g). Here, the following five vitamins were detected: B6, C, B3, B5, and Bc. Ascorbic acid (vitamin C) and the vitamin B3 (pantothenic xylitol) were found in large quantities: 79.0 and 32.0 mg per 100 g, respectively (Table 5).

Table 5. Vitamins and their concentration in the *Aegopodium alpestre* Ledeb. plant samples

Vitamins, mg per 100 g	Leaf	Stem
B ₆ (pyridoxine)	18.0	14.0
C (ascorbic acid)	79.0	31.0
B ₃ (pantothenic xylitol)	32.0	4.00
B ₅ (nicotinic xylitol)	53.0	11.0
B _c (folic acid xylitol)	24.0	11.0

Table 5 shows that leaves pack more vitamins than stems. For instance, leaves contain 1.3 times more pyridoxine, 2.5 times more ascorbic acid ($p \leq 0.05$), 8 times more pantothenic acid, 4.8 times more nicotinic acid ($p \leq 0.01$), and almost 2 times more folic acid ($p \leq 0.05$).

A. alpestre is comprised of 13 amino acids, which may be found in its leaves and stem. Of those, only eight are indispensable. The percentage of essential amino acids from the total amino acids is 59.29% in leaves and 54.11% in stems (Table 6).

Table 6. Amino acids and their proportion in the *Aegopodium alpestre* Ledeb. plant samples

Amino acids	Mass fraction (mg %)	
	Leaf	Stem
Arginine *	1.14±0.46	0.26±0.10
Lysine*	0.66±0.22	0.14±0.05
Tyrosine	0.37±0.11	0.05±0.01
Phenylalanine *	0.66±0.20	0.14±0.04
Histidine *	0.23±0.12	0.04±0.02
Leucine + isoleucine *	1.05±0.27	0.22±0.06
Valine *	0.77±0.31	0.19±0.08
Proline	1.16±0.30	0.56±0.15
Threonine *	0.85±0.34	0.26±0.10
Serin	0.64±0.17	0.15±0.04
Alanin	0.74±0.19	0.16±0.04
Glycine	0.77±0.26	0.14±0.05
Total amino acids	9.04	2.31
Essential amino acids	5.36	1.25
The proportion of essential amino acids in total amino acids, %	59.29	54.11

*Essential amino acids

Table 6 demonstrates that the content of amino acids is higher in leaves than in stems. Thus, arginine content in leaves is 4.4 times higher than that in stems; lysine content is 4.7 times higher; and tyrosine content is 7.4 times higher than that in stems ($p \leq 0.01$). Leaves contain 4.7 times more phenylalanine, 5.7 times more histidine; and 4.7 times more leucine + isoleucine ($p \leq 0.01$). The content of valine in leaves is 4 times higher than that in stems. Leaves comprise two times more proline and 3.3 times more threonine than stems ($p \leq$

0.01). The serine content in leaves is 4.3 times higher than that in stems. The alanine content is 4.6 times higher than that in stems. Finally, glycine content is 5.5 times higher than that in stems ($p \leq 0.01$).

Discussion

A. alpestre does not cover large areas with dense stands. On the contrary, one can find *A. alpestre* growing either singly or in small groups (Ilyas *et al.*, 2018). The habitat of this particular plant species is hardly accessible and is located high in the mountains such as the Tien Shan Mountains and the Alatau Mountains (Yang *et al.*, 2016; Nesterova *et al.*, 2018). Hence, plant pickers will be not able to gather *A. alpestre* in large quantities. Thus, due to inaccessibility of habitats and low plant density per unit area, the economic feasibility of collecting *A. alpestre* in the wild is rather low compared to industrial-scale cultivation.

There are no studies on medicinal properties of *A. alpestre*. Yet, there are three research papers devoted to the chemical composition of this plant's roots and seeds (Sun, 2009; Zhu *et al.*, 2010; Azimova *et al.*, 2012). Authors of the first study found a higher concentration of volatile oils and related compounds in the *A. alpestre* than in *A. podagraria* (i.e., 3.12% vs 0.04% and 0.14% in seeds and fruits, respectively) (Azimova *et al.*, 2012). This indicates that *A. alpestre* is of great value as a medicinal plant. Other studies focus more on a detailed analysis of seeds and roots in terms of composition (Sun, 2009; Zhu *et al.*, 2010). In particular, it was found that seeds of the *A. alpestre* contain 18 substances (in the essential oil), among which apiol with the proportion of 59% as well as undecane (19%) and limonene (5%). While these results were obtained by supercritical extraction, the second method (i.e., steam distillation) revealed 14 substances, among which apiol (21%), undecane (41%), β -felandren (7%), and lemonene (13%). These two approaches have provided slightly different results, although the overall composition of the substances is approximately the same (Sun, 2009). Another, this time Chinese study made it possible to establish the most optimal parameters for the extraction of oils from raw materials (water-to-raw materials ratio, 2.75 to 1.0; temperature, 71 °C; extraction duration, 2.0 hours) (Zhu *et al.*, 2010). The calculated extraction rate was 19.2%.

Note that these few results relate to the seeds but not to other parts of the plant like stems and leaves. This article is first to display the high concentration and diversity of vitamins (6 types), micronutrients (5 types), and aminoacids (13 types) in the leaves and stems of *A. alpestre*.

In this study, the height of collected plants was 69.13 ± 14.8 cm. Since the layer of palisade cells in these plants is thin, they can be argued shade-tolerant. By contrast, the stem contains 28 well-developed conductive bundles.

The chemical analysis revealed high extractivity of *A. alpestre* and higher concentration of certain biologically active substances (e.g., flavanoids, coumarins, tannins, carbohydrates, and proteins) in leaves compared to stems. The plant produces 8 essential amino acids and a large quantity of vitamins. The concentration of heavy metals falls within the normal range.

Conclusions

It was established that *A. alpestre* has a significant concentration of vitamins, microelements and other substances. Hence, it can be recommended for the production of vitamin and mineral complexes. The medicinal effect of *A. alpestre* requires further investigation. The present findings conclude that identification of biologically active substances in the above the ground vegetative organs of *A. alpestre* may be a common practice in the future. Considering the study results, *A. alpestre* may be used as a medicinal plant on a large scale. For this, the cultivation practice needs to be scaled up.

Authors' Contributions

Conceptualization: AS and SS; Data curation: NS and KA; Formal analysis: EK and GZ; Funding acquisition: EI; Investigation: EI and SS; Methodology: AS and KA; Project administration: NS and SS; Resources: GZ and KA; Software: AS; Supervision: EK; Validation: GZ; Visualization: EK; Writing - original draft: EI; Writing - review and editing: NS.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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