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Biodiversity and Phytochemical Characterization of *Adonis volgensis* Populations from Central and Northern Kazakhstan: Insights into Bioactivity and Toxicity

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Academic Editors: Luca Rastrelli, Ina Yosifova Aneva and Ekaterina Kozuharova

Received: 6 April 2025
Revised: 8 May 2025
Accepted: 9 May 2025
Published: 16 May 2025

Citation: Zhumagul, M.; Rašeta, M.; Iskakova, Z.; Kubentayev, S.; Myrzagaliyeva, A.; Tleubergenova, G.; Mukhtubayeva, S.; Mišković, J.; Gafforov, Y. Biodiversity and Phytochemical Characterization of *Adonis volgensis* Populations from Central and Northern Kazakhstan: Insights into Bioactivity and Toxicity. *Diversity* **2025**, *17*, 352. https://doi.org/10.3390/d17050352

Abstract: This study examines the phytocenotic, phenotypic, phytochemical, antioxidant, and toxic effects of four geographically distinct populations of the traditionally used plant species *Adonis volgensis* Steven ex DC. from Central and Northern Kazakhstan. These populations, found in diverse habitats such as steppe-like forest edges and moist plains, coexist with species like *Achillea nobilis* L. and *Artemisia absinthium* L. Significant variations were observed in plant community composition and environmental stressors, including grazing and habitat degradation. Morphological analysis revealed that Population 2 exhibited greater vigor, while Population 3 was more constrained by local conditions, highlighting adaptive strategies influenced by both genetic and environmental factors. FTIR analysis of *A. volgensis* extracts revealed distinct solvent-specific profiles of bioactive compounds. Ethanol (EtOH) and ethyl acetate extracts were rich in phenolic and flavonoid compounds, whereas the chloroform ($CHCl_3$) extract was less effective in extracting phenolics, displaying weaker O–H bands. Phytochemical analysis showed notable variations in total phenolic content (TPC) and total flavonoid content (TFC). The highest TPC (89.351 ± 4.45 mg GAE/g d.w.) was found in the ethyl acetate extract from the Akmola region, while the highest TFC (33.811 ± 0.170 mg QE/g d.w.) was observed in the $CHCl_3$ extract from Kostanay region. Toxicity assessment using the *Artemia salina* lethality assay revealed significant mortality rates (88–96%) in $CHCl_3$ extracts of aerial parts, demonstrating a dose-dependent effect. These findings highlight the antioxidant and potential toxic properties of *A. volgensis*, emphasizing the importance of solvent selection in bioactive compound extraction for nutraceutical and pharmaceutical applications.

Keywords: antioxidants; aromatic plants; *Artemia salina*; FTIR; geobotanical survey; GC–MS; Kazakhstan ecosystem

1. Introduction

Diets that focus on the sustainable use of local biodiversity support both human health and ecological balance while conserving natural resources [1]. However, biodiversity loss is one of the most pressing environmental challenges, primarily driven by habitat destruction and degradation, leading to plant and animal extinctions [2–4]. This decline underscores the necessity of studying rare and understudied plant species such as *Adonis volgensis* Steven ex DC. Research on these species not only enhances our understanding of their ecological roles but also helps identify their medicinal properties, sustainable use, and conservation strategies. Understanding such plants is critical for addressing global health threats and environmental changes, facilitating the development of novel treatments while safeguarding biodiversity [2].

The genus *Adonis* belongs to the Ranunculaceae family and comprises approximately 40 to 50 species distributed across temperate Asia, Europe, North America, and occasionally as far south as North Africa [5–11]. These plants typically thrive in grasslands, meadows, and forest edges, favoring well-drained soils. According to the Plants of the World Online database (2024), 35 species are currently recognized within the *Adonis* genus, with eight species documented in Kazakhstan: *Adonis apennina* L., *A. villosa* Ledeb, *A. tianschanica* (Adolf) Lipsch., *A. chrysocyathus* Hook.f. & Thomson, *A. vernalis* L., *A. volgensis* Steven ex DC., *A. scrobiculata* Boiss., and *A. aestivalis* L. [12–14]. Of these, *A. vernalis*, *A. tianschanica*, *A. volgensis*, *A. villosa*, and *A. chrysocyathus* are listed as endangered in the Red Book of Kazakhstan [15].

Adonis volgensis thrives in steppe-like forest edges and transitional zones, playing a role in maintaining herbaceous plant biodiversity in regions facing habitat degradation and overgrazing [16–18]. Due to their limited distribution and population size, *A. volgensis* and other *Adonis* species face conservation challenges. This underscores the importance of studying and protecting these plants while also exploring alternative sources for medicinal use.

Adonis species are not only valued for their ornamental qualities but also for their traditional medicinal uses. Historically, *Adonis* species have been valued for their medicinal properties, particularly for treating cardiovascular diseases, respiratory issues, and inflammatory disorders. *A. volgensis* has been recognized for its cardiotonic effects, suggesting it may serve as an alternative to *A. vernalis*, a well-documented medicinal species [19]. In addition, *A. volgensis* demonstrates antioxidant and antibacterial properties, which may be attributed to its diverse phytochemical profile, including carbohydrates, coumarins (mainly in their aglycone form), phenolic glycosides (such as flavonoids and cardenolides), and other glycosides [20–23]. Traditional medicine has also utilized infusions of *A. volgensis* combined with bromine to treat nervous excitability and insomnia [20].

More than 120 chemical compounds have been identified in various *Adonis* species, including cardenolides, flavonoids, and phenolic glycosides. Mohadjerani et al. [20] analyzed the fatty acid composition of *A. volgensis*, identifying α-linolenic acid (45.83%) as the predominant fatty acid in the leaves and oleic acid (47.54%) as the primary fatty acid in the stems. Other key fatty acids include palmitic acid (28.25% in leaves and 33.10% in stems) and linoleic acid (12.55% in leaves, 11.26% in stems). In addition to cardiac glycosides, the hydroxycoumarins scopoletin and umbelliferone have been isolated and characterized from this genus [21]. Given their diverse pharmacological activities—including

anti-allergic, anti-inflammatory, antimicrobial, antioxidant, cardioprotective, and neuroprotective properties—these compounds warrant further investigation [24–27].

Advancing phytochemical research has identified additional bioactive compounds within *Adonis* species, particularly cardiac glycosides, which have long been used in traditional medicine for their cardioprotective effects [28]. Various flavones, carotenoids, coumarins, and other bioactive constituents have also demonstrated anti-angiogenic, antibacterial, anti-inflammatory, diuretic, and acaricidal activities [20,29]. For example, the flavonoid orientin was isolated from *A. volgensis*, while orientin and adonivernitol were identified in *A. tianschanicus* and *A. turkestanicus* [30]. Furthermore, umbelliferone and scopoletin have been isolated from the roots of *A. amurensis*, *A. volgensis*, *A. leucocephala*, and *A. mongolica* [29]. However, the genus also poses toxicity risks, necessitating further study into its safe medicinal applications.

Beyond its medicinal importance, *A. volgensis* faces increasing threats from habitat loss and anthropogenic activities. Conservation efforts should focus not only on habitat protection but also on exploring sustainable medicinal applications that balance biodiversity preservation with human benefit. The species' natural occurrence in Akmola and Kostanay makes these regions particularly significant for conservation strategies.

Given the ecological significance, pharmacological potential, and conservation needs of *A. volgensis*, this study aimed to carry out the following:

- Assess the current ecological status of *A. volgensis* populations in Akmola and Kostanay regions of Kazakhstan.
- Conduct a comprehensive phytochemical analysis of extracts (chloroform, ethyl acetate, and ethanolic) from the aerial parts of the plant.
- Evaluate the biological activities of these extracts to determine their therapeutic potential.
- Compare the phytochemical profiles and biological activities between regional populations to identify geographical variations.

2. Materials and Methods

2.1. Study Area

The studies were conducted from 2021 to 2024 during the flowering season (April to June) in the territory of Central and Northern Kazakhstan. This region features a combination high (over 1000 m above sea level) and low hillocks, hilly and rocky landscapes, and narrow gorges and wide plains. Most of the area belongs to the steppe zone, with northern sections transitioning into forest-steppe vegetation. In the south, a broad ecotone separates the steppe and desert zones.

The region is characterized by a sharply continental climate, with large diurnal and seasonal temperature fluctuations, low air humidity, and high evaporation rates that exceed precipitation. January is the coldest month, with average temperatures ranging from −16 to −19 °C and absolute minimums reaching −45 °C. July is the warmest month, with average temperatures around +22 °C and maximums up to +45 °C. The annual precipitation averages about 250 mm, with the highest rainfall occurring in summer.

Soils are predominantly semi-desert types, including light brown and brown soils [31].

2.2. Plant Material

Four populations of *A. volgensis* were examined as presented in Figure 1.

Surveys were conducted during flowering (10.04–30.04) and fruiting (25.05–05.06) periods. Plant nomenclature follows the Plants of the World Online database (2024) [12]. Herbarium specimens (NUR 005786—NUR 005821) were deposited in the Astana Botanical Garden (NUR).

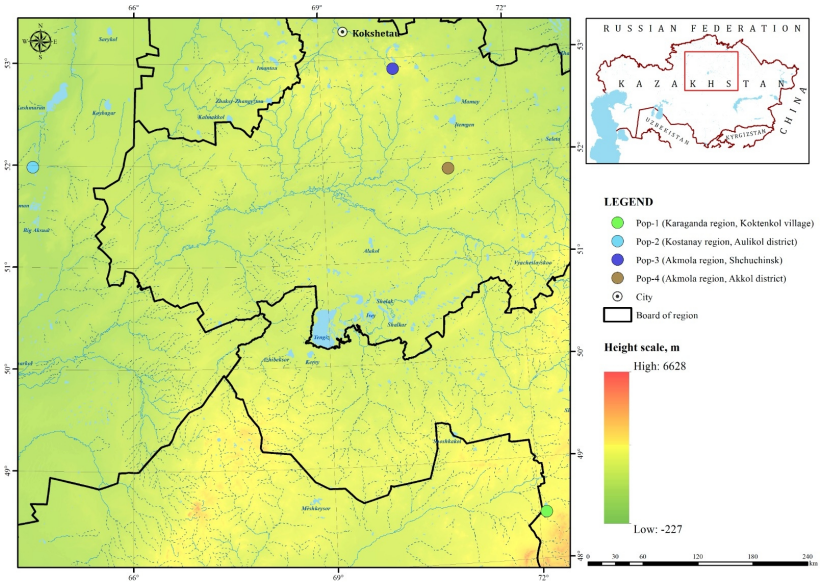


Figure 1. Geographic locations of the natural populations of *Adonis volgensis* analyzed in this study. The following are the abbreviations of the examined parameters: Pop-1—Population 1 (Karaganda region, Koktenkol village); Pop-2—Population 2 (Kostanay region, Aulikol district); Pop-3—Population 3 (Akmola region, Shchuchinsk); Pop-4—Population 4 (Akmola region, Akkol district).

2.3. Geobotanical and Phytocenotic Surveys

Geobotanical surveys were conducted in 15 plots (10 × 10 m) per population site, following standard field approaches [32]. For each plot, general environmental and vegetation characteristics were recorded, including GPS coordinates, elevation, dominant vegetation type, and species composition. The floristic composition of the communities was described qualitatively, based on field observations of dominant and accompanying species. No quantitative abundance–dominance classification was applied. Community structure and population parameters of *A. volgensis* were assessed according to Rabotnov [33] and Kubentayev et al. [34].

2.4. Extract Preparation

Soxhlet extraction was used to prepare extracts from *A. volgensis* samples collected from the Akmola and Kostanay regions of Kazakhstan. The aerial parts (stems, leaves, and flowers) were shade-dried at room temperature for 20 days, then ground into a uniform powder using a plant grinder (Retsch ZM 200 Ultra Centrifugal Mill, Retsch GmbH, Haan, North Rhine-Westphalia, Germany). Soxhlet extraction was performed on approximately 10 g of aerial parts using 150 mL of solvent (1:10 w/v ratio) for eight hours with a Soxhlet Extractor (Büchi Extraction System B-811, Büchi Labortechnik AG, Flawil, Switzerland). All solvents and chemical reagents were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

The following extracts were obtained:

From the Akmola Region:

- Chloroform ($CHCl_3$) (Ad.1), ethyl acetate (Ad.2), and ethanolic (EtOH) (Ad.3) extracts from the aerial parts.

From the Kostanay Region:

- Chloroform ($CHCl_3$) (Ad.Q1), ethyl acetate (Ad.Q2), and ethanolic (EtOH) (Ad.Q3) extracts from the aerial parts.

2.5. Phytochemical Composition

2.5.1. Gas Chromatography Coupled with Mass Spectrometry, GC–MS

Samples were analyzed by gas chromatography with mass spectrometry detection (7890A/5975C), as outlined by Zhumagul et al. [35].

Analytical conditions: Sample volume 0.5 µL, sample injection temperature 280 °C, flow division 10:1, solvent retention time 10 min. Separation was carried out using a DB-WAXetr chromatographic capillary column (Agilent Technologies, Santa Clara, CA, USA) with a length of 30 m, an inner diameter of 0.25 mm, and a film thickness of 0.25 µm, at a constant carrier gas (helium) flow rate of 1 mL/min. The chromatographic temperature was programmed from 40 °C (held for 5 min) to 260 °C (held for 5 min) at a heating rate of 5 °C/min. The analysis time was 54 min. Detection was performed in SCAN m/z mode 34-850. Agilent MSD ChemStation software (version 1701EA) was used to control the gas chromatography system and to record and process the results and data obtained. The data processing included determination of retention times, peak areas, as well as the processing of spectral information obtained using a mass spectrometric detector (Agilent Technologies, Santa Clara, CA, USA). The Wiley 7th edition and NIST'02 libraries (containing over 550,000 spectra) were used for mass spectra interpretation.

2.5.2. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

IR spectra were recorded using an FTIR InfraLUM FT-08 spectrophotometer (Lumex, St. Petersburg, Russia), which used an impaired total internal reflection (ATR) element made of ZnSe with an angle of 45° [36]. The device has a resolution of 1 cm^{-1}; at least 20 scans were performed, while the frequency range of 400–4000 cm^{-1} was used.

2.5.3. Determination of Total Phenolic and Flavonoid Contents

The total phenolic content (TPC) was measured using the Folin–Ciocalteu (FC) method as described by Singleton et al. [37]. A volume of 25 µL of each extract was mixed with 125 µL of 0.1 mol/L Folin–Ciocâlteu (FC) reagent and 100 µL of sodium carbonate (7.5%). After a 2 h incubation period, absorbance was measured at 760 nm. This method relies on the spectrophotometric detection of phenolic compounds, which form a colored complex with the FC reagent, with absorbance readings taken at 760 nm (Thermo Scientific™ Multiskan™ Sky Microplate Spectrophotometer, Waltham, MA, USA). TPC is expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g d.w.), calculated based on a standard calibration curve. The TPC was determined using the following equation:

$$TPC = (x \times m)/V, \quad (1)$$

where m is the weight of the extract (mg), V is the volume of the extract (mL), and x represents the curve value (mg GAE/g d.w.).

The total flavonoid content (TFC) was determined spectrophotometrically using aluminum chloride ($AlCl_3$) as the complexing reagent for flavonoids. A volume of 30 µL from each extract was combined with 90 µL of absolute methanol, 6 µL of 0.75 mol/L $AlCl_3$, 6 µL of 1 mol/L CH_3COONa, and 170 µL of distilled water. After a 30-min incubation, absorbance was recorded at 415 nm (Thermo Scientific™ Multiskan™ Sky Microplate Spectrophotometer, Waltham, MA, USA), using quercetin as the calibration standard. The TFC value of the extract is reported as milligrams of quercetin equivalents (QE) per gram of dry weight (mg QE/g d.w.) [38].

The TFC was determined using the following equation:

$$TFC = (x \times m)/V, \quad (2)$$

where m is the weight of the extract (mg), V is the volume of the extract (mL), and x represents the curve value (mg QE/g d.w.).

2.6. Biological Activity

2.6.1. Antioxidant Potential

The antioxidant activity was determined using two methods: the FRAP (Ferric Reducing Antioxidant Power) assay and the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The FRAP assay evaluates the reducing potential of antioxidants in a sample by their ability to reduce Fe^{3+}-2,4,6-tripyridyl-*S*-triazine (TPTZ) to Fe^{2+}-TPTZ, forming a colored complex measurable at 593 nm. Conducted at a low pH, the assay employs reagents such as acetate buffer, TPTZ solution (10 mmol/L in 40 mmol/L HCl), and $FeCl_3 \cdot 6H_2O$. In the procedure, 10 µL of the extract is mixed with 225 µL of FRAP reagent and 22.5 µL of distilled water. After six minutes of reaction, the sample's absorbance is measured at 593 nm (Thermo Scientific™ Multiskan™ Sky Microplate Spectrophotometer, Waltham, MA, USA). The reducing potential of various extracts of *A. volgensis* from two different regions were evaluated using the FRAP assay, whereas gallic acid served as the standard reference, and the optical densities (OD) were recorded at four different extract concentrations (10, 20, 30, and 40 µg/mL) [39]. The ability to neutralize the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was assessed using the DPPH radical scavenging assay described by Espín et al. In this method, 10 µL of the extract (9.71, 19.42, 29.13, and 38.83 µg/mL) was combined with 180 µL of absolute methanol and 60 µL of a 90 µmol/L DPPH working solution. The mixture was incubated for one hour, after which the absorbance was measured spectrophotometrically at 515 nm (Thermo Scientific™ Multiskan™ Sky Microplate Spectrophotometer, Waltham, MA, USA) [40].

2.6.2. *Artemia salina* Toxicity Assay

Toxicity was assessed using the *Artemia salina* brine shrimp lethality bioassay, as previously described in Parra et al. [41], and published in Yerezhepova et al. [42]. This method involves comparing the mortality rate of *A. salina* larvae in the presence of the tested sample (experimental group) with that in seawater devoid of toxic substances (control group). Acute toxicity is commonly evaluated by estimating the LC_{50}, which represents the concentration causing 50% mortality. However, even lower mortality rates, if statistically significant compared to the control can indicate toxicity and should not be disregarded.

In this assay, 200 mg of *A. salina* eggs were incubated in a dividing funnel containing 55 mL of artificial seawater. The funnel was exposed to a gentle airflow for three days to allow the larvae to hatch. After this period, the larvae congregated on the illuminated side of the funnel, with the opposite side shielded with aluminum foil. Larvae were collected using a Pasteur pipette, and approximately 20–40 larvae were placed in each well of a 24-well plate, each containing 990 µL of seawater. A 10 µL solution of DMSO was used to dissolve the tested samples to achieve a final concentration of 0.10 mg/mL.

The reference toxic agent used in this assay was Actinomycin D, while the negative control received 10 µL of DMSO alone. After a 24-h incubation period followed by an additional 24 h of observation to ensure immobility, dead larvae were counted under a microscope. Additionally, we observed nauplii for typical indicators of neurotoxicity, such as reduced mobility, tremors, paralysis, loss of coordination, or abnormal behavior (e.g., floating on the surface or immobility).

2.7. Statistical Analysis

Statistical assessments were carried out utilizing IBM SPSS statistical software, version 19.0 (IBM Corp., Armonk, NY, USA). Each experiment was replicated three times, and the results are presented as the mean ± standard deviation (SD). Microsoft Excel was

employed for data manipulation and analysis. Statistical differences between the extracts were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) test, with statistical significance set at $p < 0.05$ (STATISTICA software, version 10.0 (StatSoft, Inc., Tulsa, OK, USA)). To identify potential outliers, we applied Tukey's method for detecting outliers, which uses interquartile range (IQR) criteria. Data points falling beyond 1.5 times the IQR were considered potential outliers. However, no outliers were removed from the dataset, ensuring that all collected data points were included in the analysis.

3. Results and Discussion

3.1. Phytocenotic Characteristics of A. volgensis

In Central and Northern Kazakhstan, we examined four populations of *Adonis volgensis*, focusing on their habitat preferences and phenological development. In this region, *A. volgensis* begins vegetative growth shortly after snowmelt in early April, blooms from mid-April to early May, and completes fruiting by early June. Leaves remain photosynthetically active until mid-August, with complete senescence occurring by late September. This species typically inhabits steppe-like forest edges, northern hill slopes, and moist plains, often coexisting with species such as *Achillea nobilis* L., *Artemisia absinthium* L., *A. austriaca* Jacq., *Festuca valesiaca* Schleich. ex Gaudin, *Galatella villosa* (L.) Rchb.fil., *Phleum phleoides* (L.) H.Karst., *Phlomoides tuberosa* (L.) Moench, *Potentilla bifurca* L., *P. chrysantha* Trevir., *Ranunculus polyrhizos* Stephan ex Willd., *Spiraea hypericifolia* L., and *Stipa capillata* L., including grazing and flower picking as well as harsh environmental conditions such as strong winds, droughts, and late frosts. The studied populations were situated at considerable distances from one another and exhibited pronounced differences in habitat characteristics, plant community composition, and environmental pressures—highlighting the species' ecological adaptability. Although *A. volgensis* was relatively widespread in the study area, population densities were low, ranging from 5 to 8 individuals per 100 m^2.

Population 1 is located in the Karaganda region (Shet district), near the village of Koktenkol (48.457299° E, 72.111978° N). This population inhabits a plain between low hills with a gentle northeastern slope and integrates into a *Stipa* steppe community dominated by *S. capillata* and *S. zalesskii* Wilensky. The light loamy chestnut soil supports a relatively open plant cover (35% total projective cover), with a sparse shrub layer composed mainly of scattered *Spiraea hypericifolia* (5–6%, 40–50 cm in height). No trees were present in this community. Associated flora includes *Gagea fedtschenkoana* Pascher, *F. valesiaca* Schleich. ex Gaudin, *Tulipa patens* C.Agardh, *Ranunculus polyrhizos*, *Achillea nobilis*, *Artemisia austriaca*, *Potentilla bifurca*, *Ferula tatarica* Fisch. ex Spreng., *Taraxacum* spp., *Phlomoides tuberosa*, *Galatella villosa*, and *Eryngium planum* L. The population covers approximately 0.7 hectares. Grazing and unpaved roads are the main limiting factors at this site.

Population 2 is located in the Kostanay region (Aulikol district), approximately 10 km from the Karamendy-Auliekol road (51.983026° E, 64.395259° N). Situated on gently sloping terrain (<5°), this population grows on loamy chestnut soil, with a total projective cover of about 50%. The vegetation belongs to a *Festuca-Stipa* community, mainly comprising *Festuca valesiaca* and *Stipa capillata*. Although subjected to light grazing, the population is in good condition. The shrub layer includes *Spiraea hypericifolia* (50–70 cm in height), and associated species include *Tulipa patens*, *Artemisia austriaca*, *Phlomoides tuberosa*, *Carum carvi* L., and *Peucedanum morisonii* Besser ex Schult. This population covers approximately 1.5 hectares.

Population 3 is located in the Akmola region, near the city of Shchuchinsk (52.891447° N, 70.188515° E), on a northwest-facing slope with a 15° incline at the edge of a pine–birch forest. It is part of a *Spiraea–Phleum* community dominated by *S. hypericifolia* and *P. phleoides*.

The acidic, loamy soil supports a moderately covered site (45% total projective cover). This population is under significant environmental stress due to overgrazing, trampling, and nearby unpaved roads, all of which contribute to habitat degradation. Associated species include *Artemisia absinthium*, *Carex supina* Willd. ex Wahlenb., *Galatella villosa*, *Potentilla chrysantha*, *Stipa capillata*, and *Veronica spicata* L. The population spans approximately 0.6 hectares and is in urgent need of protective measures.

Population 4 is situated in the Akkol district (Akmola region), near the village of Enbek (51.885605° E, 70.982453° N). The site lies in on a slightly inclined plain with brown, slightly saline soils. *A. volgensis* occurs here within a *Stipa-Festuca* steppe community composed of *Stipa capillata*, *S. lessingiana* Trin. & Rupr., and *Festuca valesiaca*. The total projective cover ranges from 50 to 60%. Associated species include *Thymus pannonicus* All., *Plantago media* L., *Artemisia frigida* Willd., *A. sericea* (Besser) Weber ex Stechm., *Pulsatilla patens* (L.) Mill., *Calamagrostis epigejos* (L.) Roth, *Agropyron cristatum* (L.) Gaertn., *Galium verum* L., *Dracocephalum nutans* L., *Achillea millefolium* L., and halophytes such as *Limonium gmelini* (Willd.) Kuntze and *Glycyrrhiza glabra* L. The population occupies an area of about 1.1 hectares.

The findings indicate a marked decline in *A. volgensis* populations across its range, primarily due to the destruction of suitable habitats. Extensive plowing during the 20th century [43], combined with unregulated grazing and haymaking [44], have significantly reduced viable habitats. Given the species' specific life history traits and ontomorphogenesis, these factors likely contributed to a reduction in the soil seed bank and recruitment of new individuals. Low reproductive success and high mortality from mechanical damage, compounded by increasing economic pressures, may explain the species' disappearance from some phytocenoses.

Despite the dry steppe environment and low total projective cover, Population 1 appears stable. However, its lack of trees and sparse shrub layer render it vulnerable to increased grazing or other disturbances. Population 2, by contrast, is in relatively good condition due to favorable terrain and moderate human impact. The presence of indicator species such as *Carum carvi* and *Peucedanum morisonii* suggests ecosystem integrity.

Population 3 is under considerable environmental stress, evidenced by habitat degradation and the dominance of stress-tolerant species like *Carex supina* and *Artemisia absinthium*. The steep slope, combined with reduced vegetation cover, may promote erosion and further habitat loss. Immediate conservation action is warranted.

Population 4 shows adaptation to slightly saline conditions and benefits from a diverse plant community and higher vegetation cover. However, the presence of halophytes points to a risk of soil salinization, which could threaten long-term viability.

Several prior studies in Northern Kazakhstan have assessed the age structure, genetic diversity, and coenoflora of *A. volgensis* populations [45–49]. Our findings expand the known distribution range and contribute updated information from Central Kazakhstan. The observed variation in community composition and soil types across sites underscores the ecological plasticity of *A. volgensis*, which thrives in steppe margins, northern slopes, slightly saline soils, and moist depressions.

Anthropogenic pressures such as intensive grazing contribute to coenopopulation aging by increasing juvenile mortality while favoring the persistence of older individuals [50,51]. Interestingly, such disturbances may also promote recruitment and population recovery under certain conditions. Overall, the contrasting conditions observed across the four populations suggest that *A. volgensis* can persist under varying ecological regimes, but degraded populations (e.g., Population 3) require targeted conservation strategies, including grazing control and habitat restoration. Long-term monitoring will be essential for evaluating the species' resilience in the face of ongoing environmental change.

3.2. Morphological and Quantitative Variation Across Populations of *A. volgensis*

The morphological and quantitative traits of *A. volgensis* varied significantly among the four studied populations (Table 1).

Table 1. Morphological and quantitative characteristics of *Adonis volgensis* individuals from the study populations (Pop1–Pop4).

Characteristic	Pop-1 (Karaganda, Koktenkol)	Pop-2 (Kostanay, Aulikol)	Pop-3 (Akmola, Shchuchinsk)	Pop-4 (Akmola, Akkol)
	Average Scores			
Plant height (cm)	14.36 ± 0.58 ^{b}	17.23 ± 1.04 ^{a}	9.07 ± 0.37 ^{d}	12.87 ± 0.95 ^{c}
Bush diameter (cm)	22.40 ± 1.60 ^{b}	24.87 ± 1.55 ^{a}	9.70 ± 0.42 ^{d}	12.60 ± 1.24 ^{c}
No. of generative shoots per plant	12.80 ± 1.68 ^{b}	19.93 ± 2.69 ^{a}	2.70 ± 0.37 ^{d}	4.60 ± 0.46 ^{c}
No. of vegetative shoots per plant	9.20 ± 1.41 ^{a}	9.00 ± 1.70 ^{a}	3.13 ± 0.50 ^{b}	1.93 ± 0.32 ^{c}
Leaf blade length (cm)	3.78 ± 0.20 ^{a}	3.47 ± 0.17 ^{b}	2.63 ± 0.23 ^{c}	3.44 ± 0.19 ^{b}
Leaf blade width (cm)	3.49 ± 0.22 ^{b}	3.35 ± 0.17 ^{b}	2.55 ± 0.15 ^{c}	3.78 ± 0.24 ^{a}
Flower diameter (cm)	2.38 ± 0.15 ^{b}	3.27 ± 0.18 ^{a}	2.60 ± 0.12 ^{b}	3.40 ± 0.23 ^{a}
Stem thickness (cm)	0.27 ± 0.02 ^{a}	0.29 ± 0.02 ^{a}	0.27 ± 0.07 ^{a}	0.33 ± 0.03 ^{a}
No. of generative individuals per 100 m^2	12.07 ± 5.34 ^{a}	8.27 ± 1.10 ^{b}	4.67 ± 0.43 ^{d}	6.00 ± 0.75 ^{c}

Each value is expressed as mean ± SD. Different superscript letters (a–d) indicate significant differences among populations (Tukey's HSD test, $p < 0.05$).

Population 2 exhibited the most robust growth, characterized by the tallest plants, the largest bush diameter, and the highest number of generative shoots (Table 1), suggesting superior adaptation to local environmental conditions. In contrast, Population 3 had the smallest plant size, fewer vegetative and generative shoots, and narrower leaves, indicating possible environmental stress or a more compact growth strategy.

Significant variation was observed in key morphological traits. Population 1 had the longest leaf blades, while Population 4 exhibited the widest (Table 1). Flower diameter was largest in Population 4, followed by Population 2, while Populations 1 and 3 had smaller flowers (Table 1), which could indicate differences in reproductive strategies or pollination efficiency. Additionally, stem thickness showed minor variation, with Population 4 displaying the thickest stems. The density of generative individuals per 100 m^2 was highest in Population 1, suggesting a higher reproductive output.

The observed phenotypic variation in *A. volgensis* across the four studied populations suggests a significant influence of genetic diversity and local environmental adaptation. Population 2 consistently exhibited the most robust vegetative and reproductive growth traits, including the tallest plants, largest bush diameter, and highest number of generative shoots, indicating its superior adaptation or favorable genetic traits. These characteristics suggest a higher reproductive potential, possibly enabling more effective colonization and population establishment under specific ecological conditions. Such traits may confer a competitive advantage through denser growth and better resource acquisition, pointing to evolutionary strategies that optimize fitness. In contrast, Population 3 had markedly smaller plants with fewer vegetative and generative shoots, narrower leaf blades, and reduced overall biomass. This pattern may reflect environmental stress or an intrinsic genetic disposition for a compact growth form. The trade-off between size and survival may be a response to resource limitations or environmental pressures that constrain both vertical and horizontal development [52]. Furthermore, such growth suppression may be associated with epigenetic mechanisms, where environmental cues trigger reversible changes in gene expression, affecting morphology and reproduction [53].

Populations 1 and 4 displayed intermediate traits, reflecting varying investment in vegetative vs. reproductive growth. Population 4's large flower diameter may enhance reproductive success [54], while its lower number of vegetative shoots suggests a shift in resource allocation toward reproduction [55,56].

These findings highlight the role of genetic diversity and environmental pressures in shaping phenotypic variation in *A. volgensis*. Further research should explore the underlying ecological and evolutionary mechanisms driving these differences.

3.3. Phytochemical Analysis

3.3.1. GC–MS Analysis

The phytochemical GC–MS analysis of the EtOH extract obtained from the aerial parts of *A. volgensis* sampled from the Kostanay region (Ad.Q3) identified 52 compounds (Figure S1, Table 2). The GC–MS analysis revealed that the sample predominantly consists of sugar alcohols (39.0%), followed by fatty acids and esters (18.5%), sterols (8.6%), and phenolic compounds (3.2%). Additionally, other minor compounds were identified, accounting for 30.7% of the total composition.

Table 2. Chemical composition of the ethanol extract (Ad.Q3) from aerial parts of *Adonis volgensis* collected in the Kostanay region, as identified by GC–MS analysis.

No.	Retention Time (min)	Compound Name	Identification Probability (%) [1]	Composition (%)
1	12.59	Acetic acid	80	0.15
2	12.81	Propanoic acid, 2-oxo-, methyl ester	82	0.37
3	20.04	2-Cyclopenten-1-one, 2-hydroxy-	80	0.13
4	20.15	4-Pyranone, 2,3-dihydro-	71	0.18
5	20.95	2-Furanmethanol, tetrahydro-, acetate	76	0.10
6	21.84	Phenol, 2-methoxy-	88	0.30
7	24.07	3-Buten-1-ol, 2-methyl-	70	0.06
8	24.62	Phenol	87	0.15
9	25.11	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	88	0.17
10	25.30	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	86	0.29
11	25.56	Phytol, acetate	87	0.16
12	26.84	Tetradecanoic acid, ethyl ester	88	0.21
13	27.19	4-Chloro-3-methylbut-2-en-1-ol	79	0.75
14	27.38	2-Hydroxy-gamma-butyrolactone	88	0.22
15	27.94	Nonanoic acid	66	0.07
16	28.28	2-Methoxy-4-vinylphenol	91	0.51
17	28.75	Pentadecanoic acid, ethyl ester	65	0.13
18	28.96	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	82	0.23
19	29.53	Phenol, 2,6-dimethoxy-	89	0.27
20	29.85	Glycerin	94	1.78
21	30.61	Hexadecanoic acid, ethyl ester	91	2.29
22	30.90	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	80	0.09
23	31.21	Ethyl 9-hexadecenoate	78	0.17
24	31.60	Benzofuran, 2,3-dihydro-	81	0.08
25	31.97	Benzoic acid	71	0.15

Table 2. *Cont.*

No.	Retention Time (min)	Compound Name	Identification Probability (%) [1]	Composition (%)
26	32.19	Methyl 8,11,14-heptadecatrienoate	84	0.36
27	32.37	Heptadecanoic acid, ethyl ester	72	0.12
28	33.20	5-Hydroxymethylfurfural	81	0.12
29	34.15	Ethyl oleate	86	0.87
30	**34.61**	**2-Propenoic acid, 2-methyl-, pentyl ester**	**74**	**5.96**
31	34.73	9,12-Octadecadienoic acid, ethyl ester	92	2.38
32	35.40	Apocynin	85	0.12
33	**35.62**	**9,12,15-Octadecatrienoic acid, ethyl ester, (*Z*,*Z*,*Z*)-**	**96**	**4.34**
34	36.00	Phytol	93	1.69
35	38.04	3-(1-Methylhept-1-enyl)-5-methyl-2,5-dihydrofuran-2-one	71	0.09
36	38.78	2,5-Monomethylene-l-rhamnitol	78	1.20
37	39.54	Sorbitol	71	0.22
38	**39.74**	**Hexadecanoic acid**	**93**	**4.31**
39	40.19	2,6,8-Trimethylbicyclo[4,2,0]oct-2-ene-1,8-diol	68	0.60
40	41.18	Heptadecanoic acid	77	0.18
41	42.59	Octadecanoic acid	82	0.36
42	42.86	Oleic acid	87	0.78
43	43.48	9,12-Octadecadienoic acid (*Z*,*Z*)-	93	2.97
44	43.84	4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol	86	0.33
45	**44.30**	**9,12,15-Octadecatrienoic acid, (*Z*,*Z*,*Z*)-**	**93**	**13.51**
46	44.43	D-Allose	77	0.58
47	45.26	Eicosanoic acid	72	0.74
48	47.78	Docosanoic acid	79	1.45
49	**48.57**	**Xylitol**	**91**	**37.81**
50	**49.41**	**Stigmasterol**	**77**	**5.86**
51	51.43	γ-Sitosterol	86	2.72
52	52.79	Phytol, acetate	72	1.34

[1] Identification based on NIST database comparison. Compounds with probability > 90% are considered highly reliable. Bolded values indicate the most abundant compounds.

Our conducted GC–MS analysis identified several key compounds, with the six most abundant being xylitol (37.81%), 9,12,15-octadecatrienoic acid (*Z*,*Z*,*Z*)-(13.51%), 2-propenoic acid, 2-methyl-, pentyl ester (5.96%), stigmasterol (5.86%), 9,12,15-octadecatrienoic acid, ethyl ester (*Z*,*Z*,*Z*)-(4.34%), and hexadecanoic acid (4.31%) (Table 2). These compounds represent a diverse array of sugar alcohols, fatty acids, fatty acid esters, and phytosterols, many of which have been linked to antioxidant, anti-inflammatory, and lipid-lowering effects [57–59]. While xylitol is identified here as the most abundant compound (37.81%), we acknowledge that its prominence may seem unusual given its presence in a variety of plant species. However, xylitol is a known sugar alcohol with well-documented biological activity, including antioxidant and anti-inflammatory properties, which likely contributes to the observed bioactivity in *A. volgensis* extracts [60]. Its isolation without derivatization is feasible due to its volatility and its ability to be detected via GC–MS under the appropriate conditions. Additionally, xylitol exhibits potential antioxidant and

anti-inflammatory activities, which may contribute to the bioactive properties observed in *A. volgensis* extracts [60].

Regarding the presence of 9,12,15-octadecatrienoic acid (*Z*,*Z*,*Z*)-(α-linolenic acid), this essential omega-3 fatty acid is widely recognized for its health benefits, including anti-inflammatory, antioxidant, and cardioprotective effects [20]. The significant presence of this polyunsaturated fatty acid aligns with the traditional use of *A. volgensis* for promoting cardiovascular health and reducing inflammation [20,28]. Also, 9,12,15-octadecatrienoic acid, ethyl ester (*Z*,*Z*,*Z*)-(palmitic acid), was detected as well (4.34%). Palmitic acid is associated with lipid metabolism and structural roles in cellular membranes and exhibits antimicrobial and anti-inflammatory effects [20]. The identification of these fatty acids and esters (Figure S1, Table 2) further underscores the diverse bioactive potential of the analyzed plant extract.

Namely, Mohadjerani et al. [20] studied the fatty acid composition of *A. volgensis* (syn. *A. wolgensis*) and identified linolenic acid (45.83%) and oleic acid (47.54%) as the most abundant in the leaves and stems, respectively. Another key component, 2-propenoic acid, 2-methyl-, pentyl ester (5.96%), is an ester compound often associated with antimicrobial and anti-inflammatory effects, further supporting the pharmacological potential of this plant. Additionally, stigmasterol (5.86%), a well-known phytosterol, is linked to cholesterol-lowering and anticancer properties [61]. Phytosterols like stigmasterol can inhibit cholesterol absorption in the intestine and exhibit anti-inflammatory effects, making them important bioactive agents in both traditional and modern medicine [62].

The identification of compounds such as xylitol and unsaturated fatty acids strengthens the hypothesis that *A. volgensis* may provide substantial health benefits, particularly regarding its antioxidant and anti-inflammatory properties. These findings align with the plant's traditional applications in folk medicine, offering a biochemical foundation for further investigation into its pharmacological potential.

3.3.2. FTIR Analysis

The prepared extracts were analyzed using FTIR spectroscopy (4000–400 cm^{-1}) to identify the functional groups present in each extract based on the observed absorption bands. A broad band at 3400–3200 cm^{-1} in the EtOH extracts indicates O–H stretching vibrations, characteristic of alcohols and phenolic compounds (Figure 2c,f).

Peaks at 2919.88–2902.07 cm^{-1} correspond to C–H stretching in alkanes, while bands around 1740 cm^{-1} (C=O stretching in esters) suggest the presence of flavonoids or other ester-containing compounds. The band in the 1610–1500 cm^{-1} range indicates C=C stretching in aromatic rings, typically found in phenolic compounds. Ethyl acetate extracts (Figure 2b,e) also exhibit O–H stretching, though with less intensity than EtOH, suggesting a lower concentration of hydroxyl-containing compounds.

A peak at 1711.83 cm^{-1} (Figure 2e) indicates C=O stretching in esters, suggesting the presence of esterified flavonoids or terpenoids. Bands between 1230 and 1260 cm^{-1} correspond to C–O stretching, further supporting the presence of esters or carboxylic acids. $CHCl_3$ extracts (Figure 2a,d) show C–H stretching at 2917–2919 cm^{-1}, with weaker O–H bands, indicating a lower concentration of polar compounds. A band around 1740 cm^{-1} corresponds to C=O stretching vibrations in esters, suggesting the presence of lipid-like compounds or esterified flavonoids.

A broad band at 3354.76 cm^{-1} in EtOH extract suggests strong O–H stretching vibrations, indicating the presence of hydroxyl groups, which is typically associated with alcohols, phenolics, or carboxylic acids. The band at 2916.90 cm^{-1} indicates C–H stretching vibrations in aliphatic hydrocarbons such as fatty acids or terpenoids. A peak at

1029.70 cm^{-1} in the EtOH extract suggests C–OH bending or C–O stretching, indicative of polysaccharides or carbohydrates.

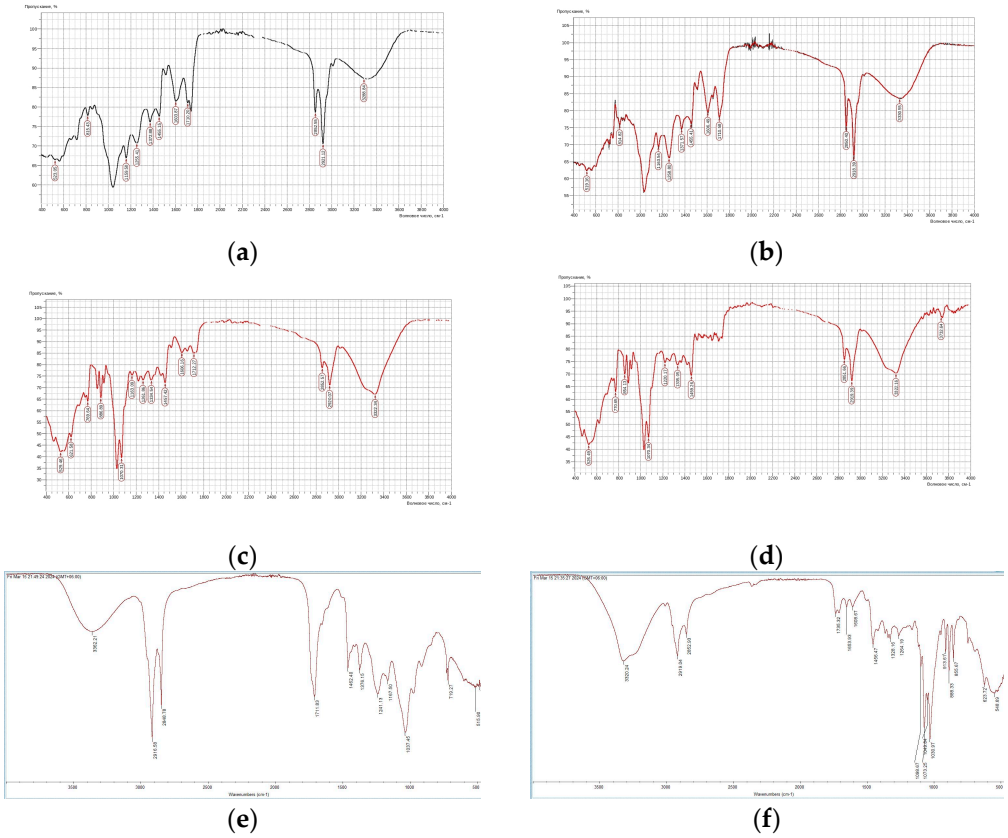


Figure 2. FTIR analysis of three different extracts from *Adonis volgensis*. (**a**–**c**) Aerial samples from the Akmola region: (**a**) Ad.1—chloroform extract; (**b**) Ad.2—ethyl acetate extract; (**c**) Ad.3—ethanolic extract. (**d**–**f**) Aerial samples from the Kostanay region: (**d**) Ad.Q1—chloroform extract; (**e**) Ad.Q2—ethyl acetate extract; (**f**) Ad.Q3—ethanolic extract.

The EtOH extract prominently exhibits O–H stretching at 3400 cm^{-1}, indicating the effective extraction of polar compounds such as flavonoids and phenolics. The ethyl acetate extract displays a similar but diminished O–H stretching signal, suggesting a lower concentration of hydroxyl groups and a selective extraction of esterified compounds. In contrast, the $CHCl_3$ extract features weaker O–H and C=C bands, indicating a reduced presence of polar compounds, yet it displays pronounced C–H and C=O bands, which point to the presence of non-polar compounds like lipids. All extracts demonstrate C=O stretching, indicative of ester compounds. The EtOH and ethyl acetate extracts are associated with flavonoid glycosides, whereas the $CHCl_3$ extracts suggest the presence of fatty acids or terpenoid esters.

In conclusion, FTIR analysis reveals solvent-dependent extraction profiles. EtOH and ethyl acetate extracts are rich in phenolic and flavonoid compounds, as evidenced by strong O–H and C=C stretching bands, while $CHCl_3$ is less effective for phenolics, displaying weaker O–H bands [63]. Conversely, $CHCl_3$ extracts exhibit strong C–H and C=O stretching, indicating the presence of terpenoids, sterols, and fatty acids, which are less abundant in EtOH extracts. This emphasizes the importance of solvent choice in targeting specific bioactive compounds (Figure S1).

3.3.3. Total Phenolic and Flavonoid Contents of *A. volgensis*

The total phenolic content (TPC) and total flavonoid content (TFC) of various extracts of *A. volgensis* are presented in Figure 3.

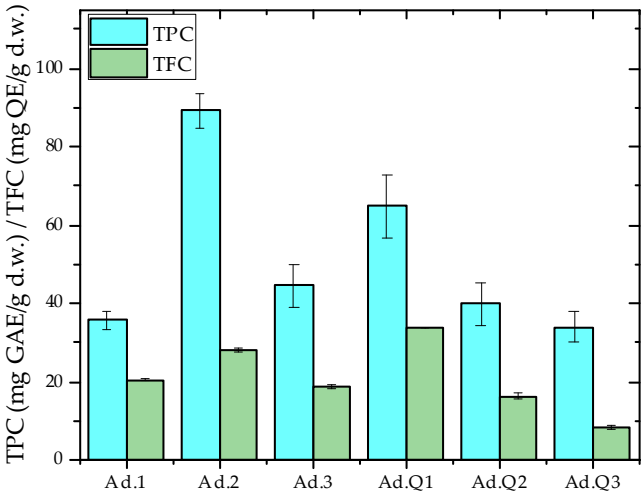


Figure 3. Total phenolic content (TPC, mg GAE/g d.w.) and total flavonoid contents (TFC, mg QE/g d.w.) in *Adonis volgensis* extracts from Akmola and Kostanay regions. Values are expressed as mean ± SD. Significant differences were determined by one-way ANOVA with the Fisher (F) test, and results are presented as *p*-values. The following are the abbreviations of the examined parameters: TPC—total phenolic content; TFC—total flavonoid content; GAE—gallic acid equivalents (unit for presentation results for quantification of TPC); QE—quercetin equivalents (unit for presentation results for quantification of TFC). Aerial samples from the Akmola region: Ad.1—$CHCl_3$ extract; Ad.2—ethyl acetate extract; Ad.3—EtOH extract. Aerial samples from the Kostanay region: Ad.Q1—$CHCl_3$ extract; Ad.Q2—ethyl acetate extract; Ad.Q3—EtOH extract; EtOH—ethanol extract; $CHCl_3$—chloroform extract.

The TPC exhibited significant variation across different solvent extractions, ranging from 33.92 ± 3.96 mg GAE/g d.w. for extract Ad.Q3 to 89.35 ± 4.45 mg GAE/g d.w. for extract Ad.2. Notably, the ethyl acetate extract of Ad.2 yielded the highest TPC, indicating that this solvent is particularly effective in extracting phenolic compounds. In contrast, the EtOH extract of Ad.Q3 demonstrated the lowest TPC among all samples, which may indicate lower solubility of phenolic compounds in EtOH for this specific sample or reflect regional variations in phenolic content influenced by environmental factors. Similarly, the TFC displayed considerable variation, ranging from 8.16 ± 0.48 mg QE/g d.w. for the EtOH extract Ad.Q3 to 33.81 ± 0.17 mg QE/g d.w. for the $CHCl_3$ extract Ad.Q1. The elevated TFC observed in the $CHCl_3$ extract Ad.Q1 suggests that flavonoids are more readily extracted in non-polar solvents like $CHCl_3$. These findings underscore the significance of solvent choice in maximizing the extraction of bioactive compounds from *A. volgensis*.

Notably, *A. volgensis* contains flavonoid compounds like orientin, as previously reported by Komissarenko et al. [21]. Additionally, coumarins, a related class of compounds, were identified in other species of the genus *Adonis*, including umbelliferone and scopoletin [21,64–67]. This suggests that the presence of flavonoids and coumarins could contribute to the antioxidant potential of the extracts. A previous study by Mohadjerani et al. [20] reported that the TPC in the hydromethanolic extract of *A. volgensis* species was 9.20 mg GAE/g d.w., which is lower than the values observed in the present study. This difference could be due to the use of different solvents and extraction methods, or variations in phenolic content among species or regions. Based on Figure 3, it can be concluded that the ethyl acetate extract (Ad.2) of *A. volgensis* sampled from the Akmola region had the highest amount of TPC (89.35 ± 4.45 mg GAE/g d.w.), while the highest amount of TFC

was measured in the $CHCl_3$ extract (Ad.Q1) of *A. volgensis* sampled from the Kostanay region, with a value of 33.81 ± 0.17 mg QE/g d.w.

In conclusion, the variations in TPC and TFC across different extracts of *A. volgensis* from two regions in Kazakhstan indicate that the solvent used for extraction plays a crucial role in determining the concentration of these bioactive compounds.

3.4. Biological Activity

3.4.1. Antioxidant Activity

FRAP (Ferric Reducing Antioxidant Power) assay

The antioxidant capacity of each extract was analyzed by comparing it to the antioxidant activity of gallic acid (Figure 4).

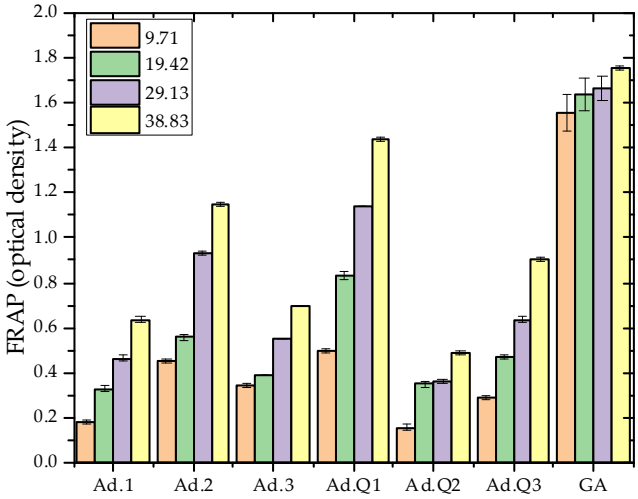


Figure 4. FRAP activity in *Adonis volgensis* extracts at four concentrations (9.71–38.83 µg/mL), and the effect of extract concentration on reduction potential from Akmola and Kostanay regions. Values are expressed as mean ± SD. Significant differences were determined by one-way ANOVA with the Fisher (F) test, and results are presented as *p*-values. Aerial samples from the Akmola region: Ad.1—$CHCl_3$ extract; Ad.2—ethyl acetate extract; Ad.3—EtOH extract. Aerial samples from the Kostanay region: Ad.Q1—$CHCl_3$ extract; Ad.Q2—ethyl acetate extract; Ad.Q3—EtOH extract. The following are the abbreviations of the examined parameters: FRAP—Ferric Reducing Antioxidant Power; EtOH—ethanol extract; $CHCl_3$—chloroform extract; GA—gallic acid.

The ethyl acetate extract from the aerial part of *A. volgensis* collected in the Akmola region (Ad.2) exhibited the highest ferric reduction antioxidant power (FRAP) among the extracts tested. Its antioxidant potential increased with concentration, reaching an optical density (OD) of 1.15 at 38.83 µg/mL, comparable to gallic acid (GA), suggesting its superior antioxidant activity. In contrast, the $CHCl_3$ extract (Ad.1) demonstrated lower antioxidant activity, with OD values ranging from 0.18 to 0.63 across the four tested concentrations. Similarly, the $CHCl_3$ extract from the Kostanay region (Ad.Q1) showed low activity at 9.71 µg/mL but increased significantly at higher concentrations, reaching an OD of 1.44 at 38.83 µg/mL. The EtOH extract (Ad.Q3) also exhibited a notable rise in antioxidant activity with increasing concentration, reaching an OD of 0.90 at 38.81 µg/mL. These findings highlight the variability in antioxidant capacity among the extracts, with ethyl acetate extracts demonstrating the strongest activity, followed by EtOH and $CHCl_3$ extracts. This trend aligns with previously published research [7,20].

DPPH anti-radical scavenging activity

The anti-radical activity of these extracts measured via DPPH assay (% inhibition), followed similar trends (Figure 5).

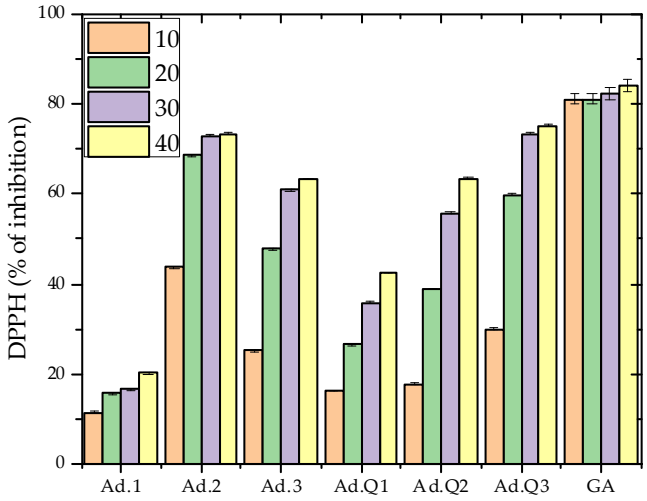


Figure 5. Anti-radical scavenging activity in *Adonis volgensis* extracts at four concentrations (10–40 μg/mL). Values are expressed as mean ± SD. Significant differences were determined by one-way ANOVA with the Fisher (F) test, and results are presented as *p*-values. Aerial samples from the Akmola region: Ad.1—$CHCl_3$ extract; Ad.2—ethyl acetate extract; Ad.3—EtOH extract. Aerial samples from the Kostanay region: Ad.Q1—$CHCl_3$ extract; Ad.Q2—ethyl acetate extract; Ad.Q3—EtOH extract. The following are the abbreviations of the examined parameters: DPPH—2,2-diphenyl-1-picrylhydrazyl; GA—gallic acid.

Ethyl acetate extracts from both regions (Ad.2 and Ad.Q2) exhibited strong anti-radical activity at higher concentrations, with Ad.2 reaching 73.32% and Ad.Q2 reaching 63.37% at 40 μg/mL. Among the Kostanay region extracts, the EtOH extract (Ad.Q3) showed the highest anti-radical activity, achieving 75.15% at 40 μg/mL (Figure 5).

However, the DPPH radical scavenging ability observed in Figure 5 is lower than that of nine methanolic (MeOH) extracts prepared from *Adonis* species, including *A. volgensis* (IC_{50} = 3.8 ± 0.6 μg/mL) [7]. Conversely, it is higher compared to Turkish MeOH extracts of *A. paryardica* from different plant parts (IC_{50} ranging from 0.144 to 0.312 mg/mL) [68]. Similarly, a study by Mohadjerani et al. [20] reported an IC_{50} value of 27.45 μg/mL for a hydro-MeOH extract, which is comparable to or, in some cases, lower than the results presented in Figure 5 of our study.

These findings indicate that the antioxidant potential of *A. volgensis* extracts varies significantly depending on the solvent and the sampled region, as confirmed by studies on *Adonis* and related plant species [7,68–70]. Ethyl acetate and EtOH extracts generally exhibited higher antioxidant activity compared to $CHCl_3$ extracts, aligning with our previous findings [71,72]. This suggests that polar solvents are more effective at extracting antioxidant compounds from *A. volgensis*, consistent with earlier reports [73,74]. This aligns with previous studies on related species, where the aerial parts typically contain higher concentrations of bioactive compounds, such as flavonoids and phenolic acids, known for their antioxidant properties. Furthermore, both plant anatomy and extraction techniques significantly influence the efficiency of antioxidant compound recovery [69,70].

3.4.2. *Artemia salina* Toxicity Assay

Based on the results presented in Table 3, the *Artemia salina* toxicity of the various extracts of *A. volgensis* was evaluated at working concentrations of 0.10, 0.05, and 0.01 mg/mL, with Actinomycin D used as the reference compound. Actinomycin D exhibited the highest toxicity, causing 96% mortality at all tested concentrations (0.10, 0.05, and 0.01 mg/mL).

Table 3. Results of the *Artemia salina* toxicity test for the tested plant extracts.

Test Substances	Concentration, mg/mL	No. of Larvae in Control		No. of Larvae in the Test Sample			% of Surviving Larvae in Control	% of Surviving Larvae in the Sample	Mortality, A, %	Presence of Neurotoxicity, %
		Survived	Dead	Survived	Dead	Par.				
Actinomycin D	0.10	24	1	0	22	0	96	0	96	0
	0.05	24	1	1	25	0	96	4	92	0
	0.01	24	1	9	18	0	96	33	63	0
Extracts of *A. volgensis* plants from the Akmola region										
$CHCl_3$ extract (Ad.1)	0.10	24	1	23	3	0	96	88	8	0
	0.05	24	1	30	1	0	96	96	0	0
	0.01	24	1	28	0	0	96	96	0	0
Ethyl acetate extract (Ad.2)	0.10	24	1	22	1	0	96	96	0	0
	0.05	24	1	26	0	0	96	96	0	0
	0.01	24	1	26	0	0	96	96	0	0
EtOH extract (Ad.3)	0.10	24	1	24	0	0	96	96	0	0
	0.05	24	1	22	0	0	96	96	0	0
	0.01	24	1	24	0	0	96	96	0	0
Extracts of *A. volgensis* plants from the Kostanay region										
$CHCl_3$ extract (Ad.Q1)	0.10	24	1	25	0	0	96	96	0	0
	0.05	24	1	21	0	0	96	96	0	0
	0.01	24	1	24	0	0	96	96	0	0
Ethyl acetate extract (Ad.Q2)	0.10	24	1	18	5	0	96	78	18	0
	0.05	24	1	20	3	0	96	87	9	0
	0.01	24	1	26	0	0	96	96	0	0
EtOH extract (Ad.Q3)	0.10	24	1	26	1	0	96	96	0	0
	0.05	24	1	24	0	0	96	96	0	0
	0.01	24	1	26	1	0	96	96	0	0

Among the plant extracts, the $CHCl_3$ extract from the Akmola region (Ad.1) demonstrated significant toxicity at 0.10 mg/mL, with 88% mortality, while lower concentrations showed no toxic effects. Furthermore, the ethyl acetate extract from the Kostanay region (Ad.Q2) exhibited moderate toxicity, with 18% mortality at 0.10 mg/mL and 9% at 0.05 mg/mL. In contrast, tested EtOH extracts (Ad.3 and Ad.Q3), as well as most $CHCl_3$ and ethyl acetate extracts at lower concentrations, exhibited no toxicity, with 100% survival of larvae. Notably, at 0.01 mg/mL, no toxicity was observed, indicating a concentration-dependent toxic effect. This trend aligns with the dose–response patterns observed in other studies on plant extracts, where toxicity increases proportionally with concentration [41,75].

The remaining *A. volgensis* extracts, specifically ethyl acetate and EtOH, exhibited no significant toxicity across all tested concentrations. Extracts from both the Akmola and Kostanay regions showed similar inactivity in terms of mortality rates. This suggests that $CHCl_3$ may extract specific bioactive compounds responsible for the observed toxicity, while other solvents are less effective in isolating these compounds. The $CHCl_3$ extract showed notable toxicity at higher concentrations, with mortality rates increasing as the concentration rose. The extract from the Kostanay region exhibited stronger toxic and neurotoxic effects than that from the Akmola region, particularly at higher concentrations (Table 3). This comparison highlights the potential for regional variation in the bioactivity of *A. volgensis* extracts.

The substantial toxicity of the $CHCl_3$ extract may be attributed to its ability to isolate non-polar compounds, such as flavonoids or terpenes, which have demonstrated toxicity in previous studies [76–79]. Supporting this, the presence of neurotoxic coumarins like umbelliferone and scopoletin could further explain these findings. Both compounds,

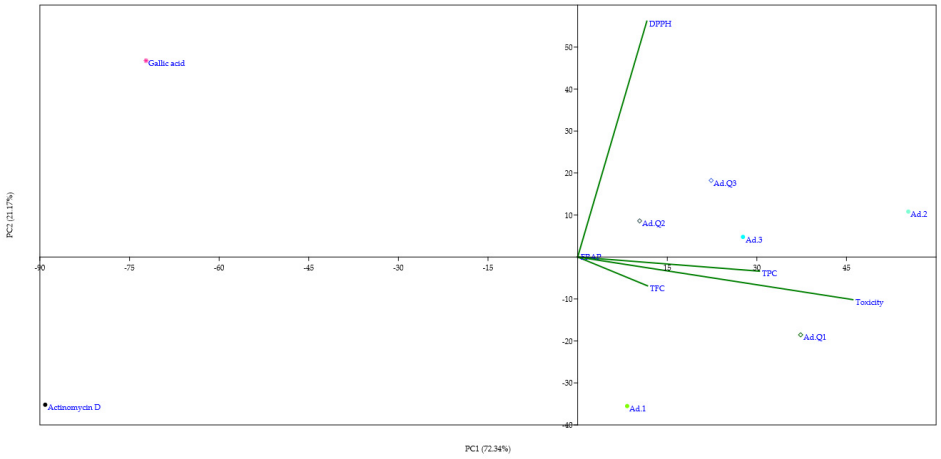
isolated from other *Adonis* species, are recognized for their bioactivity, including toxic effects, and likely contribute to the high efficacy of the $CHCl_3$ extract.

Similar to our previous findings [42], where non-polar compounds exhibited toxicity, we suspect that non-polar compounds in the $CHCl_3$ extract of *A. volgensis* play a significant role in its observed toxicity.

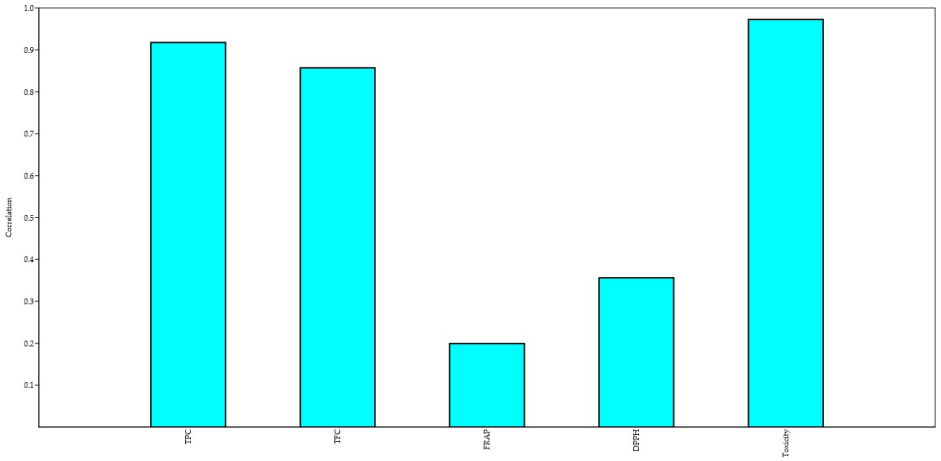
In conclusion, the results indicate that the toxicity observed in *A. volgensis* is primarily attributed to specific non-polar compounds present in the $CHCl_3$ extract. Additionally, no neurotoxic effects were observed in any of the tested samples. These findings suggest that the toxicity of *A. volgensis* extracts varies depending on the solvent used for extraction and the geographical origin of the plant material. To fully understand their potential, further studies are needed to isolate and identify these compounds, which could reveal valuable applications in pharmacology and highlight the broader significance of the tested extracts of *A. volgensis*.

3.4.3. Principal Component Analysis (PCA)

The Principal Component Analysis (PCA) was conducted to evaluate the relationship between six tested extracts (Ad.1 to Ad.Q3), two standard compounds (Gallic acid, GA, and Actinomycin D), and five observed parameters: TPC, TFC, two antioxidant assays (FRAP and DPPH), and *Artemia salina* toxicity assay (Figure 6).



(**a**)



(**b**)

Figure 6. Principal Component Analysis of six analyzed *Adonis volgensis* extracts, quantified compounds including TPC and TFC and antioxidant and toxicity analysis. Distribution of variables on (**a**) score plot

and (**b**) loading plot of the first two principal components. Aerial samples from the Akmola region: Ad.1—$CHCl_3$ extract; Ad.2—ethyl acetate extract; Ad.3—EtOH extract. Aerial samples from the Kostanay region: Ad.Q1—$CHCl_3$ extract; Ad.Q2—ethyl acetate extract; Ad.Q3—EtOH extract. The following are the abbreviations of the examined parameters: TPC—total phenolic content; TFC—total flavonoid content; FRAP—Ferric Reducing Antioxidant Power; DPPH—2,2-diphenyl-1-picrylhydrazyl; GA—gallic acid.

The first two principal components (PCs) explained 93.51% of the total variance, with PC1 accounting for 72.34% and PC2 for 21.17%, indicating that most of the dataset variability is well represented within these two dimensions.

PC1 primarily reflects the influence of phenolic and flavonoid content on antioxidant activity, as TPC, TFC, FRAP, and DPPH are strongly positively correlated along this axis. Extracts from Akmola region (Ad.1, Ad.2, and Ad.3) exhibit a strong positive correlation with TPC, TFC, and the antioxidant assays, suggesting that these samples contain higher levels of phenolics and flavonoids, contributing to their potent reducing and free radical scavenging capacities. GA, a well-known antioxidant, aligns closely with these parameters, reinforcing this association.

PC2 separates toxicity from antioxidant properties, with the *Artemia salina* toxicity assay positioned in opposition to the antioxidant-related variables. Actinomycin D, a known cytotoxic compound, is distinctly associated with the toxicity assay, confirming its strong bioactivity in this regard. EtOH extract Ad.Q3 (Kostanay region) clusters closer to Actinomycin D, suggesting that it exhibits relatively higher toxicity compared to the other extracts. In contrast, other two extracts prepared from samples from Kostanay region (Ad.Q1 and Ad.Q2) display intermediate positioning, indicating a balance between moderate antioxidant potential and toxicity.

4. Conclusions and Future Perspectives

The four cenopopulations of *Adonis volgensis* examined in this study appear to be in satisfactory condition, demonstrating both vegetative and seed reproduction capabilities. These populations, which are moderately developed and not located in protected areas, show adaptive responses to varying environmental conditions. This highlights the importance of further investigation into the genetic and ecological factors that could inform targeted conservation strategies. Grazing was observed to negatively affect plant viability and seed production, underscoring the need for long-term monitoring and more detailed recording of individual plants to strengthen conservation efforts. Our findings emphasize the significant role that local environmental conditions play in shaping the phytocenotic structure and phenotypic traits of *A. volgensis*, insights which are crucial for developing effective conservation approaches.

The study also identifies notable regional and solvent-based variations in the phytochemical composition, antioxidant potential, and toxicity, measured as lethality, of *A. volgensis* extracts.

FTIR analysis underscores the impact of solvent choice on extraction outcomes: EtOH extracts polar antioxidant compounds, ethyl acetate captures esterified and aromatic compounds, and $CHCl_3$ isolates non-polar compounds such as lipids and terpenoids. The relatively high TPC and TFC in the $CHCl_3$ and ethyl acetate extracts suggests that these solvents may be optimal for obtaining antioxidant-rich extracts from this plant.

Toxic effects were most pronounced in the $CHCl_3$ extracts of tested aerial parts, displaying a dose-dependent lethality. These results indicate that *A. volgensis* is a source of bioactive compounds with potential antioxidant and toxic properties, particularly flavonoids and coumarins.

Future research should focus on isolating and characterizing these compounds to explore their potential applications in developing pharmacologically relevant antioxidant and cytotoxic agents. Our study provides valuable regional insights into the phytochemical profile and ecological resilience of *A. volgensis*, while also acknowledging the need for further research to fully understand its broader pharmacological potential.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17050352/s1, Figure S1: GC–MS chromatogram of the EtOH extract (Ad.Q3) of *A. volgensis* aerial parts of the plant from the Kostanay region.

Author Contributions: Conceptualization, M.Z. and Z.I.; methodology, M.Z. and Z.I.; software, Z.I.; validation, A.M. and M.Z.; formal analysis, M.Z., G.T. and M.R.; investigation, M.Z., A.M., S.M., G.T., J.M., M.R. and Y.G.; resources, M.Z. and S.K.; data curation, M.Z., G.T. and M.R.; writing—original draft preparation, M.Z., S.K., J.M., M.R. and Y.G.; writing—review and editing, Z.I., S.K., A.M., G.T., S.M., J.M., M.R. and Y.G.; visualization, G.T., J.M., M.R. and Y.G.; supervision, M.Z. and S.K.; project administration, M.Z.; funding acquisition, M.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Project No. AP23488845, funded by the Committee of the Ministry of Education and Science of the Republic of Kazakhstan.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflict of interest.

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