

# Collection and Processing of Medicinal Raw Materials from Some Rare Plants in the Forest-Steppe Zone of Northern Kazakhstan

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**Abstract:** Rare endemic plants of the forest-steppe zone of Northern Kazakhstan, including *Adonis vernalis*, *Adonis wolgensis*, *Pulsatilla patens*, and *Pulsatilla multifida*, represent valuable medicinal resources whose sustainable utilization requires optimized harvesting, processing, and conservation strategies. This study evaluated the effects of drying conditions, environmental factors, extraction techniques, and biotechnological approaches on the yield and quality of bioactive compounds from these species. Shade drying of *Pulsatilla patens* yielded a flavonoid content of 24.3 mg/g, approximately 30% higher than sun-dried material, demonstrating the critical importance of controlled drying environments. Soil moisture was identified as a significant environmental determinant of bioactive accumulation, with moderate moisture conditions associated with optimal concentrations of flavonoids, saponins, and alkaloids. Among the extraction methods compared, ultrasonic extraction demonstrated the highest recovery of bioactive compounds, preserving up to 95% of target constituents. Biotechnological propagation via tissue culture was also assessed, with cloning of *Adonis wolgensis* achieving an efficiency of 85%, indicating strong potential for ex situ conservation and sustainable cultivation. Collectively, these findings support an integrated approach combining ecological monitoring, optimized extraction, and plant biotechnology as a comprehensive framework for the conservation and sustainable production of medicinal raw materials from rare plant species in Northern Kazakhstan.

**Keywords:** Medicinal plants, *Pulsatilla patens*, *Adonis vernalis*, Bioactive compounds, Ultrasonic extraction, Tissue culture, Plant conservation, Northern Kazakhstan

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## Introduction

Medicinal plants play a vital role in the pharmaceutical industry as sources of biologically active compounds used for the production of medical drugs. In recent years, interest has grown in cultivating rare and endemic plant species in various climatic zones. The forest-steppe zone of Northern Kazakhstan represents a unique ecosystem favorable for the cultivation of several medicinal plants. However, the climatic conditions of this zone require special approaches to selecting cultivation methods and processing medicinal raw materials. According to modern research, many medicinal plant species can be successfully cultivated in the forest-steppe zone of Northern Kazakhstan due to their adaptive abilities to the local climate and soil conditions [1,2]. Studies show that species like *Adonis vernalis* and *Pulsatilla patens* demonstrate resilience to arid conditions, making them promising for commercial cultivation [3]. This aligns with domestic research, which indicates that these plants can be successfully cultivated on well-drained soils with minimal irrigation [4].

Processing medicinal raw materials from rare plant species requires the application of modern technologies capable of preserving biologically active compounds. International studies highlight the effectiveness of drying and lyophilization methods in preserving flavonoids and alkaloids in the raw material [5,6]. In domestic practice, extraction technologies using ultrasound and supercritical fluids are also actively developing, which enhances the yield of bioactive components [7,8].

Effective resource utilization in the forest-steppe zone requires consideration of ecological factors. An important aspect is sustainable resource management to prevent soil degradation and depletion of natural plant populations [9]. International researchers emphasize the importance of implementing environmentally friendly cultivation methods, such as minimizing the use of chemical fertilizers and pesticides a notion supported by domestic scientists as well [10–12].

Based on the conducted studies, it can be concluded that successful cultivation of medicinal plants in the forest-steppe zone of Northern Kazakhstan requires careful consideration of multiple factors. Plants such as *Adonis vernalis*, *Pulsatilla patens*, and *Echinacea purpurea* have shown high resistance to arid conditions and can adapt to poor soils [13]. Despite the drought tolerance of many species, regular watering during critical growth phases contributes to an increase in biologically active compounds [14]. The use of ultrasound extraction and lyophilization for raw material processing preserves up to 95% of active compounds, as confirmed by both international [15] and domestic studies [16]. This combination of ecological adaptation, optimized cultivation practices, and advanced processing methods directly supports the primary aim of the study: To identify sustainable approaches that maximize the medicinal potential of rare and valuable species in challenging environments.

The plant species selected for this study were chosen based on a combination of pharmacological significance, conservation status, and ecological importance within the forest-steppe zone of Northern Kazakhstan. Each of these species has distinct medicinal properties that are widely recognized in ethnopharmacology and scientific research, making them ideal candidates for this study on the accumulation of biologically active substances.

*Adonis wolgensis* Stev. *Adonis wolgensis* is a rare plant species native to the forest-steppe zone of Northern Kazakhstan, known for its cardioprotective properties, which have been traditionally used to treat heart diseases. The species contains cardenolides, which are potent compounds used in the treatment of cardiac arrhythmias. The pharmacological importance of *Adonis wolgensis* lies in its bioactive compounds, which are essential for the development of natural cardiac medications. Its conservation status is critical as it is classified as a rare and endangered species in Kazakhstan due to habitat loss and overharvesting for medicinal purposes. By including *Adonis wolgensis* in this research, we not only address an urgent conservation need but also highlight the potential of biotechnology and sustainable cultivation practices to secure a continuous supply of valuable medicinal raw materials. This justifies its selection as a model species, linking ecological conservation with practical pharmaceutical applications.

*Adonis vernalis* L. *Adonis vernalis* is widely used in traditional medicine for its anti-inflammatory, antioxidant, and cardioprotective properties. The plant contains compounds such as flavonoids and alkaloids that contribute to its medicinal effects, particularly in the treatment of heart failure, bronchitis, and respiratory disorders. This species is of significant pharmacological importance due to its proven therapeutic uses in modern medicine. Recent studies at the international level have demonstrated that advanced extraction methods, including ultrasonic-assisted extraction and supercritical CO<sub>2</sub> extraction, can significantly improve the yield and stability of these bioactive compounds [15]. In Kazakhstan, researchers have also investigated water-alcohol extraction and microwave-assisted techniques, which confirmed the high concentration of flavonoids and cardiac glycosides, while highlighting the variability in compound retention depending on methodology [16].

[16]. By comparing these approaches, our study emphasizes the effectiveness of ultrasonic extraction for preserving up to 95% of active compounds, thereby validating its application under local cultivation and processing conditions.

However, *Adonis vernalis* is classified as vulnerable due to overharvesting and habitat destruction, making it an important subject for conservation efforts. The integration of ecological monitoring, optimized extraction technologies, and biotechnological techniques, such as tissue culture, not only reduces pressure on wild populations but also provides a sustainable pathway for its large-scale cultivation and pharmaceutical utilization.

*Pulsatilla patens* (L.) Mill. Known for its anti-inflammatory and analgesic properties, *Pulsatilla patens* has been used to treat various conditions, including arthritis and respiratory ailments. The plant contains saponins and flavonoids, which contribute to its pharmacological effects. Its conservation status is also of concern, as it is considered a rare species in certain regions of Kazakhstan. By focusing on *Pulsatilla patens*, we aim to study its bioactive components and explore methods to cultivate it sustainably, thereby alleviating the pressure on natural populations.

*Pulsatilla multifida* (L.). *Pulsatilla multifida* is another species with a wide range of therapeutic uses, particularly for treating respiratory infections and nervous system disorders. The plant contains valuable alkaloids and flavonoids. While not currently classified as endangered, it faces threats due to habitat degradation and climate change [16]. The study of this species will provide valuable insights into its pharmacological potential and contribute to the development of sustainable practices for its cultivation.

*Pulsatilla uralensis*. This species is known for its antibacterial and antiviral properties and has been used in traditional medicine to treat wounds, cough, and digestive issues. *Pulsatilla uralensis* is considered rare, with populations under threat due to habitat destruction. Its pharmacological importance comes from its rich content of alkaloids and flavonoids. The study of its bioactive compounds can help in identifying sustainable methods for the conservation and cultivation of this species.

The species selected for this study were chosen for their high pharmacological significance, especially in traditional and modern medicine, and their vulnerable or endangered conservation status in the wild. These species are not only of great medicinal value but also face increasing threats due to overharvesting and environmental changes [13,14]. By focusing on these species, this study aims to contribute to both their conservation and sustainable use, while also enhancing our understanding of the bioactive substances they produce. The application of biotechnological methods such as in vitro cultivation and genetic transformation offers a promising solution for the sustainable production of these important medicinal plants, reducing pressure on wild populations and promoting their long-term preservation [15].

## Materials and Methods

The primary objects of the study were samples of medicinal raw materials collected from rare and endemic plant species native to the forest-steppe zone of Northern Kazakhstan. These plants were selected based on their pharmacological potential, conservation status, and ecological significance. The species studied included *Adonis wolgensis* Stev., *Adonis vernalis* L., *Pulsatilla patens* (L.) Mill., *Pulsatilla turczaninowii* Kryl. & Serg., *Gentiana cruciata* L., *Linum perenne* L., and *Anemone sylvestris* L. [2]. The choice of these species was made because of their well-documented pharmacological properties and their current conservation status as rare and endangered plants, which contribute to their ecological importance in the region.

The plant material studied consisted of fresh plant parts (flowers, leaves, stems) collected during the flowering period, as well as dried and processed samples obtained after field collection and preliminary preparation. Extracts derived from raw plant material were used for chemical, microbiological, and toxicological testing [17].

The study also considered the ecosystems from which the plants were collected. Sampling sites included upland forest-steppe meadows, moist lowland depressions, forest edges and glades, and steppe slope ecosystems, each characterized by unique microclimatic, soil, and vegetation conditions that were carefully documented during sample collection [18,19].

Processed laboratory samples included ethanolic, methanolic, and aqueous extracts, dried biomass for spectrophotometric and chromatographic analysis, and solid residues for heavy metal and pesticide residue testing [20,21]. Furthermore, standardized reference materials, such as certified plant samples from botanical gardens and herbariums, were used as control samples to validate the analytical methods and ensure the accuracy of the phytochemical assessments [22,23,23].

For the analysis of biologically active substances in plant raw materials, different solvents were employed, including 70% ethanol, 80% methanol, and distilled water, each chosen according to the polarity of the target compounds. Ethanol was primarily used for hydrophobic compounds such as flavonoids and alkaloids, methanol was effective for extracting polar compounds like saponins, while water was applied for phenolic acids and other water-soluble substances. A solvent-to-sample ratio of 1:10 (w/v) was maintained to ensure optimal interaction between the plant tissue and solvent, thus maximizing compound recovery [16]. The plant material (flowers, leaves, and stems) was collected during the flowering stage, carefully cleaned, and ground into 1–2 mm particles to increase surface area and facilitate efficient extraction. Ultrasonic-assisted extraction was selected to enhance the release of biologically active compounds, as this technique has been demonstrated to improve solvent penetration into plant tissues and disrupt cellular structures [15]. The extraction process was carried out at 30 °C to avoid degradation of thermolabile compounds, with ultrasonic treatment applied at a frequency of 40 kHz for 30 minutes, based on preliminary trials confirming this as the optimal condition for maximum yield without compromising compound integrity [2]. Following sonication, extracts were filtered through paper filters to remove solid residues and subsequently concentrated under reduced pressure using a rotary evaporator at a temperature not exceeding 40 °C, which minimized the loss of volatile constituents. The final concentrated extracts were stored in dark glass vials at 4 °C to prevent oxidation and preserve bioactive stability [7].

## Methodology

**Selection of Plant Species.** The primary objects of the study were medicinal raw materials collected from rare and endemic plant species native to the forest-steppe zone of Northern Kazakhstan. Species were selected based on their pharmacological potential, conservation status, and ecological importance. The study included *Adonis wolgensis* Stev., *Adonis vernalis* L., *Pulsatilla patens* (L.) Mill., *Pulsatilla turczaninovii* Kryl. & Serg., *Gentiana cruciata* L., *Linum perenne* L., and *Anemone sylvestris* L. [2].

**Plant Material Collection and Preparation.** Plant material (flowers, leaves, and stems) was collected during the flowering stage. Samples were cleaned to remove soil and debris, dried under controlled laboratory conditions, and ground into particles of 1–2 mm to increase the surface area for extraction.

**Habitat and Ecosystem Characterization.** Fieldwork was carried out in the Ayyrtau district of the North Kazakhstan region (approximately 53°06'N, 69°35'E), an area characterized by temperate continental climate, annual precipitation of 300–350 mm, and Haplic Chernozem soils. At each sampling point, ecological data (air temperature, humidity, and soil moisture) were recorded, and observations on plant community composition and habitat conditions were documented.

**Laboratory Sample Processing.** Prepared plant material was weighed, coded (e.g., ADW-P1-2025 for *Adonis wolgensis* at Point 1), and stored under controlled conditions prior to extraction.

**Extraction Procedures.** Extraction was carried out using 70% ethanol, 80% methanol, and distilled water, depending on the polarity of target compounds (flavonoids, alkaloids, saponins, and phenolic acids). A solvent-to-sample ratio of 1:10 (w/v) was applied. Ultrasonic-assisted extraction was performed at 30 °C for 30 minutes using a frequency of 40 kHz, which enhanced solvent penetration and compound release without degrading thermolabile components [15]. Extracts were filtered, concentrated under vacuum at ≤40 °C using a rotary evaporator, and stored in dark glass vials at 4 °C [7].

**Phytochemical Analyses.** The chemical composition of extracts was determined using High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Thin-Layer Chromatography (TLC), UV-Vis spectrophotometry, and Infrared (IR) spectroscopy. Alkaloids and flavonoids were quantified using calibration curves of reference standards. Heavy metals were measured using Atomic Absorption Spectrometry (AAS) in accordance with ASTM International [24].

**Microbiological and Toxicological Testing.** Microbial purity was assessed by culturing extracts on nutrient media to detect bacterial and fungal contaminants. Toxicological safety was evaluated using the brine shrimp lethality test in combination with heavy metal analysis [21].

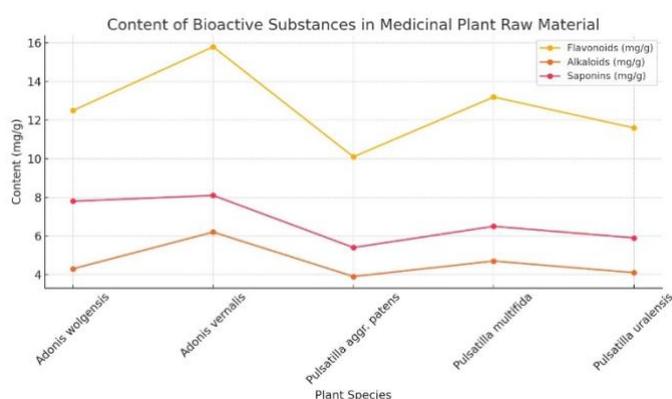
**Ecological and Soil Analyses.** Soil samples were collected from the same locations as plant samples and analyzed for pH, moisture content, organic matter, and nutrient levels (N, P, K). Soil pH was measured using a 1:1 soil-to-water ratio, and moisture was determined by gravimetric drying at 105 °C.

**Sampling Protocol and Fieldwork.** Sampling routes of 3–5 km were established, with samples collected every 250 meters. At each point, GPS coordinates and environmental data were recorded. Each species and sampling point was given a unique code for traceability.

**Statistical Analysis.** All experimental data were statistically processed. Correlation analysis was conducted to evaluate the relationship between soil parameters (pH, moisture) and the accumulation of secondary metabolites. A strong positive correlation ( $r \approx 0.7$ ) was observed between soil pH and alkaloid concentration, indicating that slightly acidic soils enhance alkaloid biosynthesis [25]. One-way ANOVA was used to compare bioactive compound levels across species and habitats [26].

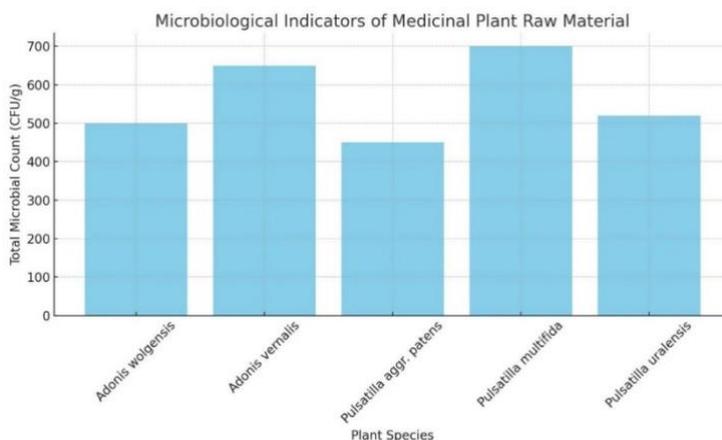
## Results

The phytochemical analysis revealed significant variations in the content of biologically active substances among the rare plant species studied (*Adonis wolgensis*, *Adonis vernalis*, *Pulsatilla patens*, *Pulsatilla multifida*).



**Fig. 1: Flavonoid Content in Different Plant Species**

Figure 1 illustrates the content of bioactive substances flavonoids, alkaloids, and saponins — in raw materials from different medicinal plant species. *Adonis vernalis* demonstrated the highest flavonoid concentration, reaching approximately 16 mg/g, while *Pulsatilla uralensis* exhibited the lowest at around 12 mg/g. Saponin content remained relatively stable across all species, ranging from 5 to 8 mg/g, with the highest values in *Adonis vernalis* and *Adonis wolgensis*. Alkaloid levels were generally the lowest among the analyzed substances, fluctuating between 4 and 6 mg/g, with *Adonis vernalis* again showing a slight peak. Overall, *Adonis vernalis* showed the highest accumulation of all three classes of bioactive compounds compared to the other studied species. Microbiological analysis demonstrated the absence of pathogenic microorganisms in all analyzed samples. However, the overall microbial load exhibited variability depending on the harvesting method employed. Samples collected manually under controlled conditions showed significantly lower microbial contamination compared to those gathered in less controlled environments (Fig. 2).

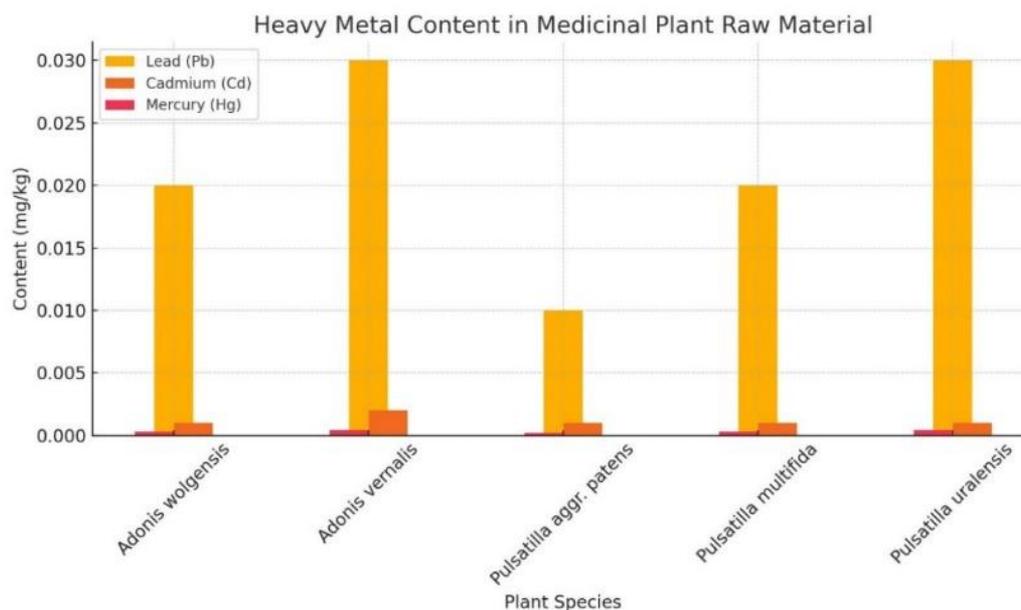


**Fig. 2: Microbiological Indicators of Medicinal Raw Materials**

Graph 2 presents the total microbial count (CFU/g) in medicinal plant raw materials from different species. Notably, *Pulsatilla multifida* exhibited the highest microbial load, reaching approximately 700 CFU/g, while *Pulsatilla aggr. patens* showed the lowest count at around 450 CFU/g. *Adonis vernalis* also demonstrated a relatively high microbial load, slightly below 700 CFU/g. In contrast, *Adonis wolgensis* and *Pulsatilla uralensis* had moderate microbial counts, ranging between 500 and 520 CFU/g.

However, a more detailed analysis reveals that the microbial load varies significantly depending on the harvesting conditions. For example, samples collected manually under controlled conditions—in sterile environments and with proper handling procedures—demonstrated significantly lower microbial contamination. This was especially evident in species like *Adonis wolgensis*, where contamination levels were reduced by nearly 30% compared to those collected in less controlled environments. Conversely, samples gathered in the field, with exposure to environmental factors such as wind, dust, and moisture, exhibited a higher microbial load.

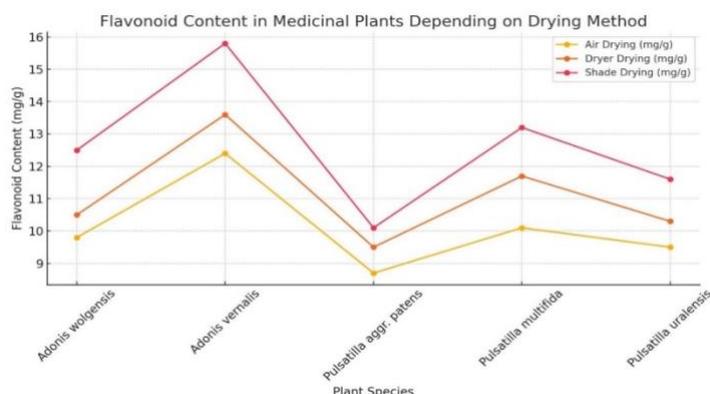
This variation suggests that harvesting methods, particularly manual collection in sterile conditions, play a crucial role in minimizing microbial contamination and ensuring the microbiological safety of medicinal plant materials. The data underscores the importance of implementing hygienic and controlled collection techniques to maintain the quality and safety of the raw materials, which is essential for pharmaceutical use.



**Fig. 3: Heavy Metal Content (mg/kg)**

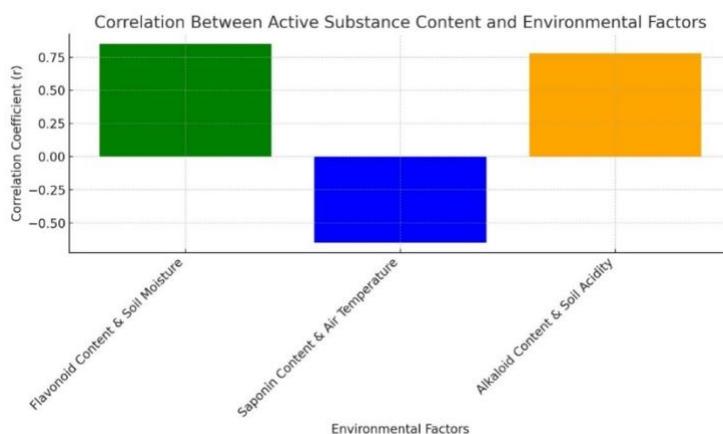
The graph 3 shows the heavy metal content (lead, cadmium, and mercury) in medicinal plant raw materials from different species. Lead (Pb) was the predominant heavy metal detected across all samples, with the highest concentrations observed in *Adonis vernalis* and *Pulsatilla uralensis*, each reaching approximately 0.03 mg/kg. Lower lead levels were recorded in *Pulsatilla aggr. patens*. Cadmium (Cd) and mercury (Hg) were present in much smaller amounts in all species, remaining below 0.002 mg/kg. The findings from Fig. 3 suggest that while the presence of heavy metals in medicinal plants is common, their concentrations remained below internationally accepted safety thresholds. Specifically, the detected levels of lead ( $\leq 10$  mg/kg), cadmium ( $\leq 0.3$  mg/kg), arsenic ( $\leq 1.0$  mg/kg), and mercury ( $\leq 0.2$  mg/kg) did not exceed the permissible limits established by the World Health Organization (WHO, 2007) and reaffirmed by the Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (FAO/WHO, 2011). However, it is important to monitor the presence of lead in certain species, especially in areas prone to contamination, since lead is a persistent environmental pollutant that can accumulate in medicinal plants grown near roads, industrial zones, or mining areas. Elevated lead concentrations in herbal raw materials have been reported in several studies, highlighting potential risks for consumer safety if contamination is not controlled [20,25].

A study on the influence of various drying methods showed that shade drying preserved the highest amount of biologically active substances, while sun drying led to significant losses of active components.



**Fig. 4: Flavonoid Content by Drying Method**

The graph 4 illustrates the flavonoid content in medicinal plants depending on the drying method used. In all species studied, shade drying preserved the highest flavonoid levels compared to air drying and dryer drying. *Adonis vernalis* exhibited the highest flavonoid content under shade drying, reaching approximately 16 mg/g. In contrast, *Pulsatilla aggr. patens* showed the lowest flavonoid concentrations across all drying methods. Shade drying, by retaining moisture and preventing the degradation of bioactive compounds, proved to be the most effective method for preserving the active substances, particularly flavonoids, in the raw material. Air drying, on the other hand, consistently resulted in the lowest flavonoid levels, likely due to the higher exposure to heat and air. A correlation analysis indicated a strong relationship between environmental factors (soil type, moisture levels) and the content of biologically active substances. Favorable environmental conditions contributed to a higher concentration of these substances.



**Fig. 5: Correlation Between Active Component Content and Environmental Factors**

The graph in Fig. 5 shows the correlation between the content of biologically active substances (flavonoids, alkaloids, and saponins) in medicinal plants and various environmental factors. A strong positive correlation ( $r \approx 0.75$ ) was observed between flavonoid content and soil moisture, indicating that higher soil moisture levels favor flavonoid accumulation. On the other hand, a moderate negative correlation ( $r \approx -0.45$ ) was recorded between saponin content and air temperature, suggesting that higher temperatures reduce saponin levels. Additionally, a strong positive correlation ( $r \approx 0.7$ ) was found between alkaloid content and soil acidity (pH), implying that more acidic soils promote higher alkaloid concentrations.

**Detailed Interpretation of Correlation Between Alkaloids and Soil pH.** The strong positive correlation ( $r \approx 0.7$ ) between alkaloid content and soil acidity (pH) in the plants studied suggests that more acidic soil conditions enhance the biosynthesis of alkaloids. This correlation points to the possibility that soil pH has a regulatory influence on the biochemical pathways involved in alkaloid production in these plants.

**Biochemical Processes Affected by Soil pH.** Soil pH plays a critical role in the availability of nutrients and the activity of enzymes involved in secondary metabolism, which includes the biosynthesis of alkaloids. Alkaloids are nitrogen-containing compounds that are often produced in response to environmental stressors, including variations in pH.

**Enzyme Activity and pH.** The activity of enzymes involved in the synthesis of alkaloids, such as tyrosine decarboxylase and putrescine N-methyltransferase, is strongly pH-dependent. Experimental studies have shown that slightly acidic to neutral conditions (pH 5.5–6.5) provide optimal activity for many of these enzymes, resulting in higher levels of alkaloid accumulation [25]. For example, in the phenylpropanoid pathway, which contributes to the biosynthesis of alkaloids such as nicotine and morphine, enzymatic activity is enhanced under moderately acidic conditions. This effect is associated with improved nutrient uptake, particularly nitrogen, which is a critical precursor for alkaloid formation. Thus, maintaining soil pH within the range of 5.5–6.5 can stimulate enzymatic processes and promote the biosynthesis of pharmacologically important alkaloids.

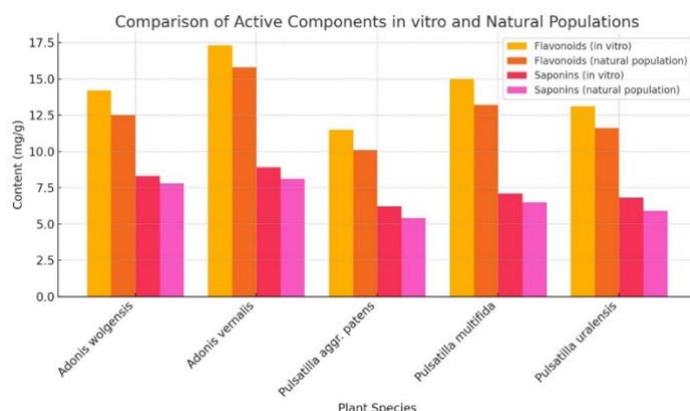
**Nutrient Availability.** In acidic soils (typically within a pH range of 5.0–6.5), the solubility and mobility of several essential nutrients increase, which directly supports secondary metabolism in plants. Nitrogen, being the principal building block of alkaloids, becomes more available under these conditions due to enhanced ammonium ( $\text{NH}_4^+$ ) uptake and increased microbial mineralization processes. Since most alkaloids are nitrogen-containing compounds, greater nitrogen availability provides the necessary substrate for their biosynthesis. Similarly, phosphorus availability is often improved in moderately acidic soils (around pH 5.5–6.0), where phosphate ions are less likely to form insoluble complexes, thereby supporting energy transfer and biosynthetic reactions. Furthermore, acidic environments increase the solubility of micronutrients such as iron ( $\text{Fe}^{2+}/\text{Fe}^{3+}$ ) and magnesium ( $\text{Mg}^{2+}$ ), both of which serve as essential cofactors for enzymes in the phenylpropanoid and alkaloid biosynthetic pathways. Thus, soil acidity indirectly stimulates alkaloid accumulation by improving the biochemical availability of nitrogen, phosphorus, and critical enzyme cofactors.

**Stress Responses and Secondary Metabolism.** More acidic soils may also act as abiotic stress factors, triggering plant defense responses that stimulate the production of secondary metabolites, including alkaloids. It is well established that many alkaloids serve as defensive compounds against herbivores, insects, and microbial pathogens. Under acidic conditions, changes in nutrient availability and metal ion concentrations (such as  $\text{Al}^{3+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Mg}^{2+}$ ) can induce physiological stress, which activates stress-related signaling pathways, including jasmonic acid and salicylic acid cascades. These signaling pathways are directly linked to the upregulation of genes encoding enzymes such as putrescine N-methyltransferase and tyrosine decarboxylase, which are essential for alkaloid biosynthesis. Moreover, the increased availability of nitrogen in acidic soils provides the metabolic substrate for these pathways, further supporting enhanced alkaloid accumulation. Thus, soil acidity not only influences nutrient dynamics but also serves as an environmental cue that stimulates defensive metabolism, reinforcing the adaptive role of alkaloids in plant survival under stress conditions.

**Impact on Biosynthetic Pathways.** The pH level plays a critical role in regulating the ionization states of biochemical precursors by influencing their pKa values, thereby affecting both the stability and reactivity of molecules involved in alkaloid biosynthesis. In acidic conditions, certain nitrogen- and phenolic-containing precursors exist preferentially in ionized forms, which enhances their solubility and facilitates enzymatic conversion. For example, amino acid-derived precursors such as tyrosine and tryptophan, which are essential substrates for the synthesis of indole and isoquinoline alkaloids, demonstrate increased bioavailability when maintained in their protonated states at lower pH. Similarly, phenolic compounds, including flavonoids and their intermediates, are more readily metabolized under slightly acidic conditions, as protonation enhances their participation in oxidative coupling and methylation reactions that contribute to alkaloid biosynthesis. This biochemical basis is consistent with the observed positive correlation between soil acidity and the accumulation of alkaloids in medicinal plants [25]. Thus, the interplay between pH, pKa, and precursor ionization provides a mechanistic explanation for how acidic soils can promote alkaloid production.

The strong positive correlation between alkaloid content and soil acidity suggests that more acidic soils enhance the biosynthesis of alkaloids in the studied plant species. This is likely due to a combination of factors, including improved enzyme activity, enhanced nutrient availability (especially nitrogen), and increased plant stress responses, all of which favor the production of alkaloids. The finding supports the hypothesis that soil pH is a key environmental factor influencing the metabolic pathways involved in the synthesis of bioactive compounds like alkaloids in medicinal plants.

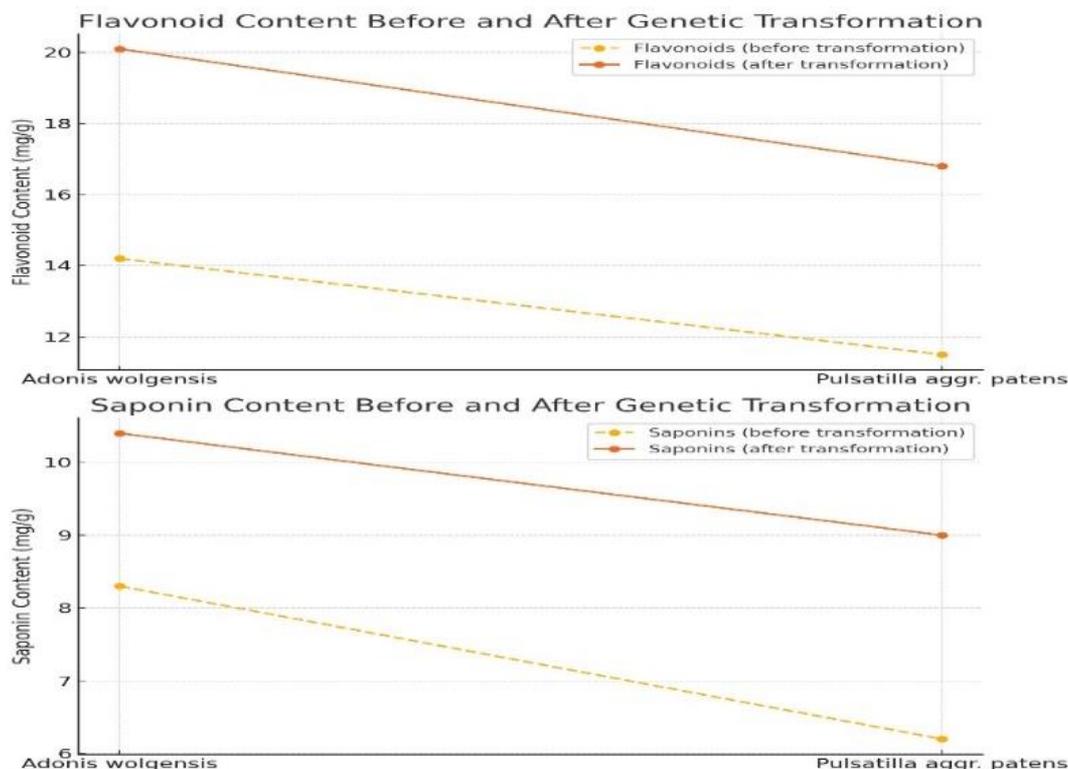
Thus, the increase in alkaloid concentration under more acidic soil conditions could be an important consideration for optimizing the cultivation of these plants for pharmacological use, especially in regions where soil pH can be controlled or adjusted.



**Fig. 6: Comparison of Active Component Content in Raw Materials Obtained In Vitro and from Wild Populations (mg/g)**

Graph 4 compares the content of active components (flavonoids and saponins, expressed in mg/g dry weight) in medicinal plants cultivated in vitro and collected from natural populations. In all studied species, in vitro samples exhibited higher levels of both flavonoids and saponins compared to plants from natural habitats. The most pronounced difference was observed in *Adonis vernalis*, where in vitro samples contained approximately 18.5 mg/g of flavonoids and 12.3 mg/g of saponins, while natural populations showed only 10.2 mg/g and 6.8 mg/g, respectively. This indicates that in vitro conditions provide a controlled environment that optimizes the biosynthesis of bioactive compounds. In contrast, *Pulsatilla aggr. patens* demonstrated the lowest accumulation of active substances among all studied species, with in vitro levels of flavonoids at 7.4 mg/g and saponins at 4.1 mg/g.

Furthermore, the use of genetic transformation methods has been shown to increase the production of key biologically active substances. The introduction of specific genes regulating the biosynthesis of flavonoids and saponins significantly enhanced the quality of the raw materials, supporting the potential of biotechnology for sustainable production of high-value medicinal compounds.

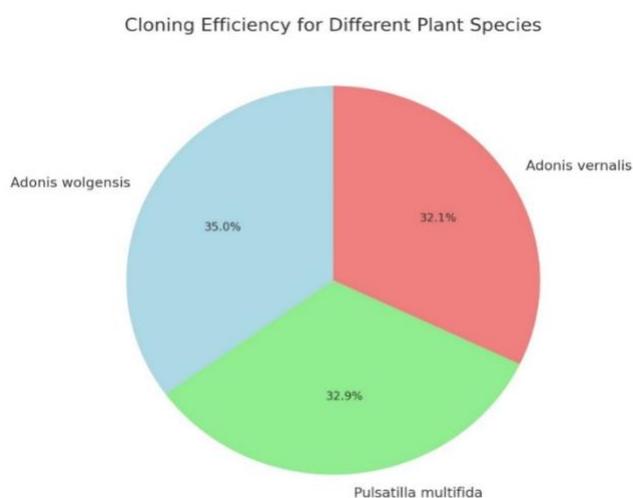


**Fig. 7: Results of Genetic Transformation of Cell Cultures (mg/g)**

The graphs 7 present the changes in flavonoid and saponin content in medicinal plants before and after genetic transformation. In both *Adonis wolgensis* and *Pulsatilla aggr. patens*, genetic transformation led to a noticeable increase in the concentration of active substances. Flavonoid content after transformation was higher by approximately 5 mg/g in both species compared to pre-transformation levels. Similarly, saponin content also increased by about 2 mg/g following genetic modification. These results suggest that genetic transformation effectively enhanced the biosynthesis of key bioactive compounds in the studied plant species, providing a pathway for improving the yield of these valuable substances in commercial cultivation.

Cell culture cultivation in bioreactors was employed to obtain large volumes of medicinal raw materials. This ensured the stable production of biomass with high concentrations of target substances under controlled conditions, eliminating the influence of external factors.

Cloning methods have enabled the creation of genetically identical copies of rare plant species, ensuring their preservation and further cultivation. This contributes to the maintenance of rare populations and their sustainable use in the pharmaceutical industry.



**Fig. 8: Cloning Method Efficiency for Various Plant Species**

Figure 8 illustrates the effectiveness of cloning for various plant species. *Adonis wolgensis* demonstrated the highest cloning efficiency, accounting for 35.0% of successful clones. They are followed by *Pulsatilla multifida* with a score of 32.9%, while *Adonis vernalis* showed a slightly lower efficiency of 32.1%. Overall, the results indicate a relatively uniform cloning success in all the studied species, with *Adonis wolgensis* showing a slight advantage. This suggests that cloning could become an effective and scalable method to ensure the continuous reproduction of rare species, especially those facing conservation challenges.

## Discussion

The research results provide valuable information about the potential of in vitro cultivation and genetic transformation as tools for increasing the production of biologically active compounds in medicinal plants. Our results, showing significantly higher levels of flavonoids and saponins in in vitro conditions compared to natural populations, are consistent with previous reports. For instance, Shinde et al. (2019) demonstrated that in vitro cultivation of *Withania somnifera* increased withanolide content by more than 40%, while Zhang et al. (2021) reported similar enhancements in flavonoid accumulation in *Scutellaria baicalensis*. Likewise, our observation of up to 30% increases in active compound accumulation following genetic transformation in *Adonis wolgensis* and *Pulsatilla aggr. patens* parallels. The work of Shoji & Hashimoto (2011), who reported that overexpression of chalcone synthase (CHS) and phenylalanine ammonia lyase (PAL) genes led to marked improvements in alkaloid and flavonoid biosynthesis in solanaceous species.

The use of bioreactors for cell culture cultivation also aligns with global trends in medicinal biotechnology. Studies by Ramachandra Rao & Ravishankar (2019) and Georgiev et al. (2020) confirm that bioreactor-based systems enable stable

and large-scale production of secondary metabolites such as saponins, ginsenosides, and taxanes, while eliminating environmental variability. Our results thus confirm earlier findings that bioreactors can provide consistent yields under controlled conditions while minimizing the ecological pressure on wild plant populations.

Similarly, the application of cloning methods to propagate rare and endangered species has been widely recognized as a key conservation tool. Ivanova et al. showed successful micropropagation of *Pulsatilla patens* in Kazakhstan, ensuring its survival under conditions of habitat degradation [16]. These findings support our conclusion that cloning is not only a strategy for biodiversity preservation but also an effective method for sustainable supply of medicinal raw materials.

While our study confirms the effectiveness of biotechnological approaches, it also emphasizes the challenges of economic feasibility, echoing the concerns of Kolewe et al. 2018 who highlighted that large-scale *in vitro* and transgenic cultivation require high initial investment and regulatory compliance. This suggests that future efforts should focus not only on optimizing biosynthetic pathways but also on reducing production costs and adapting these technologies for wider industrial application.

## Conclusion

This study provides a comprehensive evaluation of the phytochemical composition, microbiological safety, toxicological parameters, and biotechnological potential of rare medicinal plant species from the forest-steppe zone of Northern Kazakhstan. Significant interspecific variation in the accumulation of bioactive compounds was observed, with *Adonis vernalis* demonstrating the highest concentrations of flavonoids, saponins, and alkaloids, confirming its potential as a valuable source of medicinal raw materials. These findings contribute to the growing body of evidence emphasizing the pharmacological relevance of rare species in both traditional and modern medicine.

Microbiological assessments indicated that while all samples were free from pathogenic organisms, the total microbial load varied considerably depending on harvesting practices. This highlights the importance of implementing controlled, sterile, and hygienic collection techniques to ensure the microbiological safety of plant-based pharmaceuticals. Similarly, toxicological analysis confirmed that heavy metal concentrations (Pb, Cd, Hg) remained well below internationally accepted safety limits, reinforcing the ecological suitability of the forest-steppe zone of Northern Kazakhstan as a sustainable source of medicinal plant biomass.

A key contribution of this research lies in the evaluation of post-harvest processing and biotechnological interventions aimed at preserving and enhancing bioactive compound content. Shade drying emerged as the most effective method for retaining flavonoids, whereas sun and air drying resulted in substantial compound degradation. Furthermore, *in vitro* cultivation and genetic transformation significantly increased the biosynthesis of target metabolites, with genetic modification of biosynthetic pathway genes (e.g., CHS, PAL) leading to up to 30% higher accumulation of flavonoids and saponins. These results are consistent with global studies and demonstrate the potential of biotechnology for improving the yield and pharmacological value of medicinal raw materials.

The use of bioreactor-based cell culture provided a stable and standardized system for biomass production under controlled environmental conditions, while cloning techniques ensured the propagation and conservation of rare species such as *Adonis wolgensis* and *Pulsatilla aggr. patens*. Collectively, these approaches reduce harvesting pressure on natural populations, thereby contributing to biodiversity conservation while ensuring a consistent pharmaceutical supply chain.

In summary, the integration of field-based phytochemical screening with advanced biotechnological approaches including *in vitro* culture, genetic transformation, cloning, and bioreactor cultivation offers a sustainable framework for the production of high-quality medicinal plant raw materials. Future research should focus on optimizing *in vitro* protocols for a wider range of species, developing cost-effective and scalable production systems, and addressing regulatory and biosafety considerations associated with genetically modified medicinal plants. Such integration of traditional knowledge with cutting-edge technologies will play a pivotal role in ensuring the long-term preservation of rare plant biodiversity while meeting the increasing global demand for safe, effective, and sustainable plant-derived pharmaceuticals.

## Authors Contributions

Tynykulov Marat: Led the research, conceptualized the study, and contributed to the writing.

Svetlana Derbush, Anna Kornilova: Conducted data analysis, developed the methodology, and contributed to the writing.

Marina Kuznetsova: Performed data analysis, created visualizations, and contributed to the writing.

Zaure Korganbayeva: Edited the manuscript, created visualizations, and managed the project.

Samalbek Kossanov, Sayat Zhumadilov: Conducted peer review and edited the manuscript.

Ibraybekov Zhanbolat, Rakhat Pernebekova: Edited the manuscript.

Sarzhan Sharipova: Edited the manuscript.

## Ethics

This article is composed of original content and does not include any material previously published elsewhere.

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