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Quantitative Determination of Metabolites of Turkestan Soaproot (*Allochrusa gypsophiloides* (Regel) Schischk.) Grown in Kazakhstan

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Abstract

Allochrusa gypsophiloides (Regel) Schischk. (Caryophyllaceae Juss.), Turkestan soaproot (TSR) is a super producer of triterpene saponins with a wide spectrum of pharmacological activity and high surface-active properties. The realization of his full commercial potential requires biochemical studies with a focus on the metabolites dynamics at various phases of plant development and in connection with the storage duration of raw plant material. Spectrophotometric determination of saponins in terms of oleanolic acid was carried out in roots with different morphometric parameters and the aerial part from wild plants collected in the south of Kazakhstan. The content of saponins, carbohydrates, phenols, and flavonoids as well as the foaming properties of ethanol extracts obtained from air-dried TSR roots before and after their long-term storage were evaluated. The maximum quantity of saponins was revealed in average roots from middle-aged and mature generative plants in fruiting. A high level of saponins was detected in the aerial part of plants during the flowering, which decreased twice at the fruiting stage. After long-term storage of the roots, an increase in the initial content of saponins and flavonoids combined with a decrease in carbohydrates was noted. Saponins accumulation was confirmed by a 12-fold increase in the foaming index in the aqueous extract from the deposited roots relative to its initial value in the extract from the original roots. The data obtained indicate the possibility of alternative use of the aerial part of the original plants of *A. gypsophiloides* at the flowering stage to obtain triterpene saponins.

Key words: *Allochrusa gypsophiloides*; Turkestan soaproot; triterpene saponins

1. Introduction

Allochrusa gypsophiloides (Regel) Schischk., Turkestan soaproot (TSR), an endemic Central Asian species from the family Caryophyllaceae Juss. is an herbaceous perennial that grows up to 90 cm high with a relatively limited range of growth in foothill desert loess steppes in the Western Tien Shan at 400-1300 m above sea level (Eisenman et al., 2012). Since 1981, TSR as a rare species is listed in the Red Data Book of the Kazakh SSR (1981). At the same time, botanical studies have shown that in places that have not been subjected to anthropogenic pressure for a long time, natural populations of soap roots are restored and selective harvesting can be practiced (Kuzmin et al., 2001). To restore natural populations, it is necessary to resume the introduction of TSR, which has been tested in Kazakhstan and Turkmenistan (Bespaev, 1966; Gladyshev and Mishchenko, 1990).

TSR surpasses similar plants-producers (*Saponaria officinalis* L., *Gypsophila paniculate* L., etc.) by the content of the oleanolic type triterpene saponins, of which acanthophyllazides B, C, and D were identified (Kondratenko et al. 1981; Böttger and Melzig, 2011; Khatuntseva et al., 2012). A literature review showed a variety of pharmacological and technical applications of TSR: as expectorants, diuretics, laxatives (Grudzinskaya and Gemejieva, 2014) a detergent in the household, in the manufacture of shampoos, and other uses, such as a foaming agent in obtaining fire mixtures, in the manufacture of halva, fizzy drinks, in the manufacture of foam concrete, in the mining industry for flotation and effective separation of minerals from waste the rock, etc. (Balandin, 1996).

Saponins, chemically are high molecular weight steroid or triterpene glycosides consisting of hydrophilic carbohydrate part and hydrophobic aglycone or sapogenin (El Aziz et al., 2019). From the biological point of view, saponins are considered secondary metabolites which act as phytoprotectors against a wide range of pathogens, from eating by insects and herbivores (Guglu-Ustundag, et al., 2007; Faisal and Geelen, 2013). Plant saponins have high foaming, and emulsifying properties and exhibit different pharmacological effects: antibacterial, virucidal, anti-inflammatory, anti-leishmaniosis, anti-cancer, etc. (Desai et al., 2009). The wide range of biological activity of saponins is based on their specific chemical properties: high affinity to phospholipids of cell membranes, the ability to form insoluble complexes with sterols and proteins, lytic activity, etc. (Singh and Kaur, 2018).

The secondary metabolism of plants is known to be characterized by taxonomic specificity. Steroid saponins were mainly found in representatives of monocotyledonous families: Agavaceae, Dioscoreaceae, and Liliaceae. Triterpene saponins were found mainly in dicotyledons of the Leguminosae, Araliaceae, and Caryophyllaceae families (Vincken et al., 2007). According to the literature sources, the level of saponins varies significantly depending on the plant species (El Aziz et al., 2019).

The ability to synthesize secondary metabolites is a genetically determined feature of differentiated tissues and is characteristic of specialized organs and at certain phenological phases of plants (Vincken et al., 2007). The level of accumulation of secondary metabolites changes depending on the plant age, environmental and agronomic factors of growth, storage conditions of raw materials, etc. (Paseshnichenko, 2001).

Phytochemical studies on TSR are extremely scarce and were mostly conducted in the 60s-80s of the last century (Bespaev, 1966; Putieva et al., 1979; Kondratenko et al., 1981). To realize the full scientific and commercial potential of TSR, further study of physiological and biochemical features of TSR secondary metabolism is required to determine the optimal growing conditions of the species, effective terms of harvesting plant raw materials, and storage conditions with the maximum yield of the biology active metabolites.

The purpose of the research was a phytochemical study of *A. gypsophiloides* from natural populations in southern Kazakhstan territory during vegetation, after harvesting, and after long storage.

2. Material and Methods

2.1. Plant material and physicochemical evaluations

The source material *Allochrusa gypsophiloides* (Regel) Schischk. was collected from natural populations in the desert zone in southern Kazakhstan (Gemejiyeva et al., 2016). Experimental plants were collected on an elevated undulating plain between 375 and 670 m above sea level (latitude: 42˚02'52.0" N. longitude: 069˚28'40.9" E). The growing conditions of plants in this zone are characterized by a significantly dry climate with a high heat supply and the prevalence of light sierozem (Rachkovskaya et al., 2003). The plant material of *A. gypsophiloides* was identified in the Institute of Botany and Phytointroduction and a herbarium specimen of *A. gypsophiloides* number - 2499 was given (Almaty, Kazakhstan). Roots were excavated and the aerial part was collected from young, middle-aged, and mature generative plants during the stages of active vegetation (May-June), flowering (July), and fruiting (August). Each analyzed group included plant material of roots or aerial parts from three plants, being on the same phase of vegetation and having similar morphometric parameters. The collected TSR samples were pre-treated and dried at room temperature to the requirements of State Standard (SS) 24027. Roots were further stored in parchment bags at room temperature and 40% humidity. Macroscopic analysis of TSR raw material was carried out according to SS 344878. Physicochemical parameters like a loss on drying and yield of extractive substance were estimated according to SS 24027. 2-80.

2.2. Preparation of plant extracts and estimation of total saponins content

To obtain extracts, 2 g of dry crushed raw material was pre-treated twice with chloroform to remove fatty substances from the plant material. After evaporation of chloroform, the remaining raw material was extracted with 90% ethanol in a water bath with a reflux condenser for 2 h. Ethanol extractions were filtered and total solution A was obtained, from which an aliquot was taken for saponins hydrolysis, and the remaining volume was further evaporated under vacuum. The aliquot (10 ml) taken from solution A was dried and the dry residue was hydrolyzed in a 10 ml mixture: glacial acetic acid - hydrochloric acid - purified water (3.5: 1: 5.5). After that, the hydrolysis mixture was diluted with twice as much water and filtered. The precipitate on the filter was washed with water and dissolved in 25 ml of hot 90% ethanol and collected in a 25 ml volumetric flask (solution B) for quantitative analysis of saponins.

The total content of triterpene saponins was determined spectrophotometrically after the reaction with concentrated sulfuric acid as a result of which the triterpenoids are protonated at the double bond to form a

carbocation, and the subsequent lactonization occurs in the presence of a carboxyl group at C-28. In this case, the characteristic absorption maximum at 310 nm is observed (Oganesyan, 1980; Lim et al., 2020). Spectrophotometric determination of triterpene glycosides in the reaction with sulfuric acid is used for *Aralia mandshurica* Rupt.et Maxim. (Pisarev et al., 2009; Kurkin et al., 2017), *Anthyllis vulneraria* L. (Shadrin et al., 2011), *Solidago caucasica* Kem.-Nath. (Fedotova et al., 2014), *Ferula hermonis* Boiss. (Fedoseeva and Bashar, 2016), etc. (El Mabrouki et al., 2014). This method makes it possible to quantify the total sum of triterpene glycosides, and oleanolic acid derivatives, regardless of the number and structure of carbohydrate residues in the composition of their molecules (Mironenko et al., 2011; Zhang and Qu, 2013).

One ml of solution B was taken in a test tube, and 4 ml of concentrated sulfuric acid was added and incubated for 10 min. The absorbance of the solution was determined on a spectrophotometer (Jenway, England) at λ = 310 nm against the blank, concentrated sulfuric acid. In parallel, the absorbance of the oleanolic acid (OA) standard (Sigma. USA) was determined under similar experimental conditions. Calculation of the content of the saponins in percentage (X) in terms of OA on absolutely dry raw material was carried out according to the formula: $X = (A_x \times m_o \times 250 \times 25 \times 100 \times 100) / (A_o \times m_x \times 25 \times (100-W)$, Ax – absorbance of the plant sample; m_o – the weight of standard OA; m_x – sample weight; A_o – absorbance of standard OA; W- loss in mass of the raw material during drying (%).

2.3. Determination of total carbohydrate content

Total carbohydrates were estimated by the phenol sulfuric acid method (Nielsen, 2010). The exact weight of the sample was mixed with hot water (1:100) and incubated in an 80 °C water bath for one hour. Proteins were precipitated by 10% lead acetate solution, followed by filtration. From the filtrate, 10 ml was selected for the precipitation of carbohydrates with ethanol. The precipitate was separated by centrifugation and then with small water portions was transferred to a 25 ml flask. The resulting solution was brought to the desired volume and filtered. A 0.5 ml aliquot was taken from the resulting filtrate, 0.5 ml of 90 % ethanol solution of phenol (freshly distilled), and 2.5 ml of concentrated sulfuric acid were added, stirred, and left for half an hour for color development and the absorbance of the solutions was measured at λ = 490 nm. The same procedure was repeated for the dilution series of glucose (0.02, 0.04, 0.08, 0.1, 0.12, 0.16 and 0.2 mg/ml) for calibration line construction. The equivalent concentrations of samples were determined from a linear equation of a standard curve of glucose ($y=0.0081x + 0.0758$, $R^2 = 0.989$).

The carbohydrates content in percentage (X) was calculated according to the formula: $X = (C \times 25 \times 100 \times 100 \times 100) / m \times V \times 10 \times (100-W)$, C - concentration according to the graduation curve of glucose (g ml^{-1}); m – sample weight (g); V – the volume of the aliquot (ml); W - loss in weight when drying the raw material (%).

2.4. Determination of total flavonoid content

The total content of flavonoids in the ethanol extract was determined according to the reaction of complexation with aluminum chloride (Lomboeva et al., 2008; Chirikova et al., 2010; Kurkina et al., 2022). An analytical crushed sample (1 g) was placed in a 250 ml flask, poured with 100 ml of 70% ethanol, and extracted

three times in a water bath under a reflux condenser for an hour. After cooling, the extracts were combined and filtered and the total volume was brought to 200 mL with ethanol (solution A). An aliquot (2 ml) of solution A was transferred into a 25 ml flask, 2 ml of 2 % aluminum chloride solution dissolved in 95% ethanol, and 1 drop of 5% acetic acid was added. The volume of solution was brought to the mark with 95 % ethanol (solution B) and was incubated for 40 minutes at room temperature. The absorbance of solution B was determined by a spectrophotometer at λ = 414 nm, using rutin (Sigma, USA) as a standard sample. The exact weight of rutin (0.05) g was dissolved in 95% ethanol in a volumetric flask of 100 ml. From the rutin solution obtained, 2 mL was taken and the components of the reaction mixture were added as described above. The flavonoids content in terms of rutin standard and absolutely dry raw materials in percentage (X) was calculated by the formula: $X = 100 \times (D \times K_s \times m_s \times 100) / m \times D_s \times K_s \times (100 - W)$, D – absorbance of the test solution; D_s – absorbance of the rutin standard; m – the sample weight; m_s – rutin weight; K - dilution factor of the test solution (2500); K_s - dilution factor of the rutin solution (1250); W - loss in weight during drying of raw material (%).

2.5. Determination of total phenols content

The content of phenolic compounds was determined by spectrophotometric method with Folin Denis reagent using gallic acid as a standard (Mechnikova et al., 2007). Extraction with 70 % ethanol was performed twice (raw material: extractant, 1: 100) in a water bath with a reflux condenser. After each extraction, the contents of the flask were cooled to room temperature and filtered. The resulting filtrates were pooled and, if necessary, the total volume was adjusted to 200 ml with 70% ethanol (solution A). An aliquot (0.5 ml) was taken from solution A, 20 ml of Folin-Denis reagent (Sigma-Aldrich, USA), and 10 ml of 20 % sodium carbonate were added and shaken until no more bubbles were formed. The total solution volume was brought to 50 ml with water (solution B). The flasks with the reaction mixture were closed and incubated in an 80° C water bath for 30 minutes. The result of the reaction was evaluated by the change in the absorbance of solution B at λ = 760 nm. The phenols content (X) in % was estimated as mg equivalent of gallic acid per g of absolutely dry raw material according to the formula: $X = (D \times m_s \times 200 \times 50 \times 0.5 \times 100 \times 100) / (D_s \times m \times 0.5 \times 250 \times 50 \times (100 - W)$, D – absorbance of the test solution; D_s – absorbance of the gallic acid standard; m - sample weight; m_s - standard weight; W - loss in mass during drying of raw material, %.

2.6. Foaming properties

The initial aqueous extractions from the roots were prepared in the ratio of 1 g:100 ml and 1g:1000 ml, and a series of dilutions (from 1:2000 to 1:80000) was performed to carry out the foaming reaction. The foam number was determined as the lowest dilution of extract that forms a foam that does not disappear within 15 minutes (El Aziz et al., 2019).

2.7. Statistical analysis

All experiments were performed in three biological and three technical replicates. The processing of data and graphing were performed using Microsoft Excel (Microsoft Corp., Redmond, Washington, DC, USA). Atypical

values were excluded from the data based on t-tests, and the standard error of the average sample was calculated using the parametric Student's test according to State Pharmacopoeia (2010). Differences were considered significant at $p < 0.05$.

3. Results and Discussion

The macroscopic analysis showed that the collected raw material of TSR roots fully complied with the standard requirements: in appearance, the collected raw material represented heavy, hard, cylindrical-shaped pieces of excavated roots, mostly spirally twisted with an irregular wrinkled surface, covered with a network of numerous small transverse depressions (in the form of thin circular lines), deep longitudinal grooves and cracks, with traces of rounded scars left after removal of lateral roots (Figure 1A).

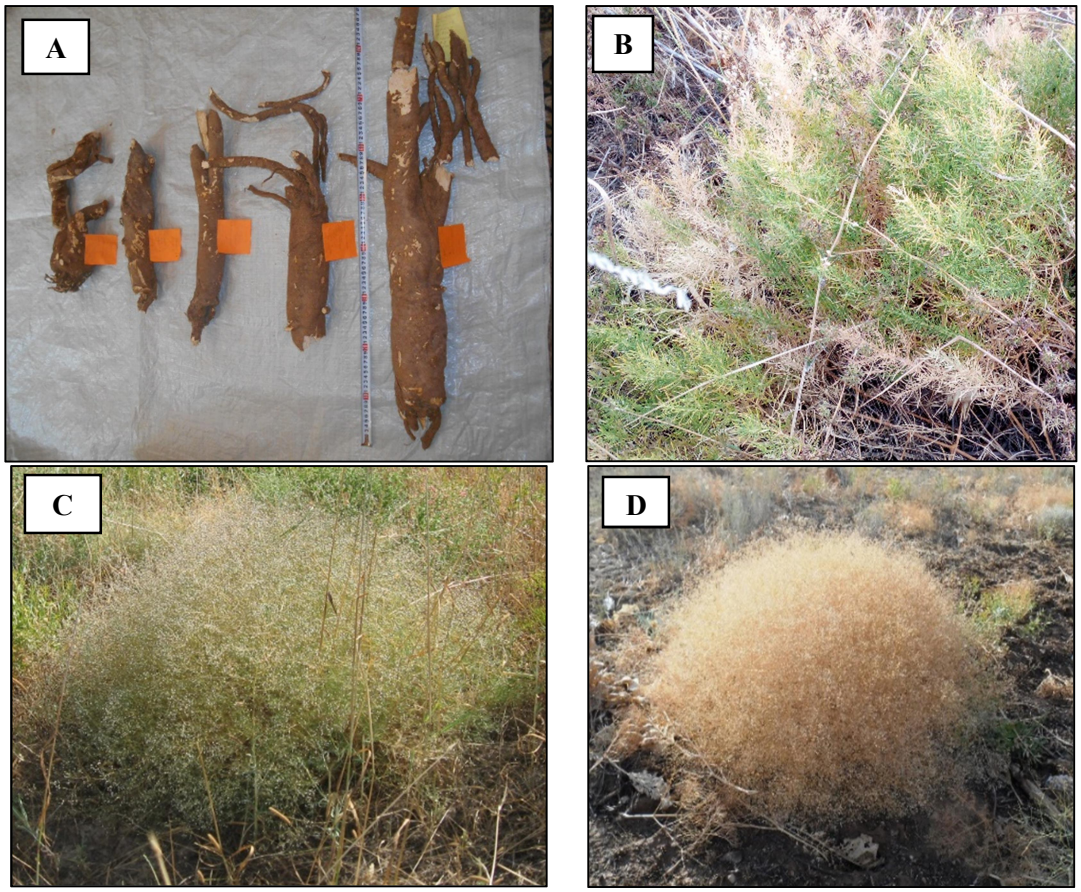

Figure 1. Turkestan soaproot *Allochrusa gypsophiloides*: (A) different size roots; (B) at the beginning of vegetation; (C) flowering; (D) fruiting.

The aerial part changed during the plant's growth and development. At the beginning of vegetation, these were strongly deflected branches 10-15 cm in height with opposite-leaved linear green leaves (Figure 1B). At the flowering stage, the plant looked like a spherical green bush 50-70 cm high, and 60-100 cm in diameter, with leaves and numerous paniculate inflorescences of whitish-pink small flowers (Figure 1C). The fruiting plant was a characteristic "tumbleweed" bush with dried shoots with numerous seed bolls in the upper part (Figure 1D).

The moisture content of the aerial part of the plant and roots did not differ significantly and averaged 9.4

%. The average yield of extractive substances when using 90% ethanol was 13.7% in the roots and in the aerial part 7.9% (Table 1).

Table 1. Moisture Content and Extraction Yield of the Different Parts of *Allochrusa gypsophiloides.*

Plant part	Moisture (%)	Extraction yield (%)
Root	9.00 ± 2.40	13.7 ± 1.38
Aerial	9.84 ± 0.14	7.9 ± 1.07

In earlier studies, it was found that the morphometric indicators of the aerial part cannot always accurately determine the age state of the plant. With very close growth parameters of bushes (height from 55 to 64 cm, diameter from 105 to 165 cm), morphometric parameters of their roots varied significantly, particularly root diameter from the minimum average value of 2.4 cm to the maximum of 9.2 cm (Gemejiyeva et al., 2016). In this regard, determining the age state of the TSR plants by morphometric parameters of the root seemed to be more accurate.

All excavated root samples were divided according to their biometric parameters into three groups: small ones from young generative plants, medium ones specific to mature medium generative individuals, and large ones from older generative plants. It was found that visually allocated groups of roots significantly varied among themselves in terms of diameter. Small roots differed in length and air-dry weight only from large roots, which did not differ from medium-sized roots in these parameters (Table 2).

Table 2. Saponin Content in *Allochrusa gypsophiloides* Roots with Different Morphometric Parameters**.**

Root size and parameters	Length (cm)	Diameter (cm)	Fresh weight (kg)	Dry weight (kg)	Saponins content (%)
Small	35.0 ± 2.8a	2.07 ± 0.60a	0.28 ± 0.05a	0.21 ± 0.09a	3.67 ± 0.52a
Medium	44.2 ± 8.8ab	4.55 ± 1.07b	1.56 ± 0.7a	0.80 ± 0.32ab	8.33 ± 0.86b
Large	52.3 ± 2.84b	7.83 ± 1.04c	2.63 ± 1.4a	1.87 ± 0.75b	6.17 ± 0.75b

Note: different letters represent significant differences in column at $p < 0.05$, n= 5 roots for each parameter

The lack of reliability on other parameters is probably because absolute data on length and weight do not always correspond to their actual values, since they depend on the depth of root digging. The peculiarity of TSR plants is that the tap root system of generative individuals penetrates to a considerable depth, up to six meters, which makes it difficult to extract them completely from the soil. When harvesting the raw material, the root is exposed to the point of branching at a depth of 60-80 cm and cut off (Kuzmin et al. 2001).

Analysis of the data in Table 2 shows that higher saponin levels were found in middle- and large-size roots with an average root diameter in a range of 4.55 - 7.83 cm. The large-size samples of roots from mature generative plants were not significantly different from middle-aged samples (t 1.89). In the roots of young plants

two or three times smaller in diameter, the average level of saponins was significantly lower and did not exceed 3.67%.

The dependence of saponins level on the root diameter indicator, which is determined by the age of the donor plant, was revealed. The maximum amount of saponins was found in the roots of middle-aged generative plants at the phase of flowering-fruiting. It was shown that the root diameter with an average value 4-8 cm, which is characterized by a high content of saponins, is an important parameter of the selection for the initial raw material of the TSR.

To study the effect of the plant vegetation phase on the saponin content, mature roots of a medium diameter and the aerial part of medium-aged generative individuals collected at the beginning of the vegetation, flowering, and fruiting stages were taken.

It was found that the dynamics of saponin levels in roots and aerial parts of plants differed during the growing season. The saponin content in roots was found to increase from 6.0% to 9.6% during the transition of plants from the beginning of vegetation to fruiting. The maximum level of saponins was observed in the roots at the end of the growing season, which significantly exceeded the initial level (t 2.72 at n=8, p 0.05), but without significant differences with the stage of flowering. The average amount of saponins in the roots during the growing period of TSR was 7.5% (Figure 2).

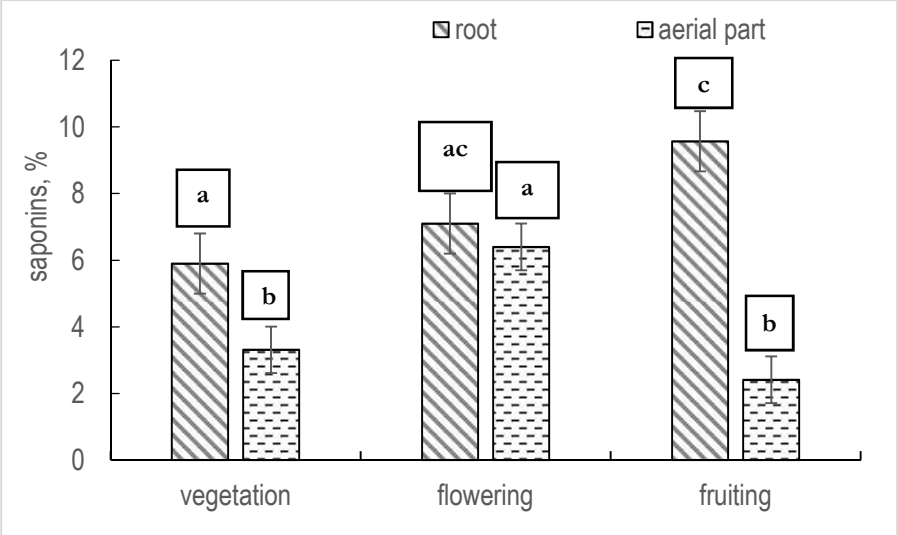

Figure 2. Saponin content in roots and aerial parts of TSR at the different vegetation phases. Different letters represent significant differences between all the data at $p < 0.05$ (by Student's test).

In the aerial part during the regrowth of green shoots, the saponins content was about 3%, while in the flowering bush the level of saponins with high confidence (t 6.2 at n=6) rose to a maximum value of 6.4%. During further development, at the stage of fruiting, the saponins level decreased to 2.4 % (t 8.11, p 0.01). At the same time, saponin content at the beginning of vegetation of plants represented by green shoots and at the end of vegetation, mostly dry shoots with seeds, was minimal and did not differ significantly from each other.

It was found that at the beginning and the end of the vegetation the saponin level prevails in the roots, but at the flowering stage, the saponin content in the aerial part is almost equal (6.4% and 7.1%, respectively).

In studies previously conducted with *A. gypsophiloides* growing in Uzbekistan, the content of saponins by the gravimetric method in the aerial part was 0.79 %, in roots from 14.19 to 22.16% depending on their age (Bespaev, 1966; Putieva et al., 1979). Experimental data on the content of saponins in TSR depending on the age and vegetation phase of plants are very scarce. Thus, it is stated that the amount of saponins in the roots increases with plant age, from 9.5% in annuals to 23% in five-year-old plants (Kuzmin et al., 2001). The results of the introduction of *A. gypsophiloides* in Turkmenistan showed that the maximum accumulation of saponins in the plant roots (up to 20%) was observed only by the 5th year of cultivation (Gladyshev and Mishchenko, 1990).

Literature data on the content of saponins depending on the phase of plant vegetation for other plants - producers of triterpene saponins are rather contradictory. It was revealed that the maximum amount of triterpene saponins in *A. vulneraria* is contained in the root at the flowering stage (Shadrin et al., 2011). It was shown for *Saponaria officinalis* that the vegetation phase had no significant effect on the saponin content in the roots. It was revealed that with the age of the plant, the decrease in its saponins content was accompanied by an increase in polysaccharides (Chrevach et al., 2009).

The obtained data on the TSR analysis growing in Kazakhstan confirm the value of this crop as a commercial source of triterpene saponins, the content of which is higher than that of other plant producers. Thus, the maximum amount of saponins of oleanolic acid derivatives in the root *A. vulneraria* did not exceed 3.46% in the flowering phase (Shadrin et al., 2011). In the underground organs of *F. hermonis,* the content of saponins estimated by the spectrophotometric method was 7.27% (Fedoseeva and Bashar, 2016). High content of 9.14 % was detected in the rhizomes of *A. mandshurica* grows mainly in the Far East (Pisarev et al., 2009). The results of our research indicate that the aerial part of the flowering plant can also serve as a high-grade source of saponins, which greatly facilitates the harvesting of the plant material without digging and allows the saving of the original specimen due to the annual renewal of growth from the root buds.

Table 3 presents data on the content of the main metabolites in TSR roots and the foaming activity of their aqueous extracts after collection and after their long-term storage for 3 years.

Table 3. Metabolites Contents in Native TSR from Natural Populations in the Collection Year and after Long Storage.

Roots	Carbohydrates (%)	Phenols (%)	Flavonoids (%)	Saponins (%)	Foaming (dilution)
Before storage	7.41±1.37a	0.047±0.007a	0.04±0.003a	7.63±0.31a	1: 6,660
After storage	3.63± 0.43b	0.03±0.009a	0.12±0.03b	14.46±3.08b	1:80,000

Note – different letters in column represent significant differences at $p < 0.05$, n= 5 roots for each parameters

The obtained results indicate that after long-term storage, a significant increase in the content of saponins and flavonoids was observed in the TSR roots, while the initial high level of sugars decreased by half,

the minor amount of phenols practically did not change during storage. The results of the analysis indicate a possible redistribution of metabolites during the long-term storage of roots. In particular, the content of carbohydrates decreased as a result of their conversion to triterpene glycosides, and the associated increase in the level of saponins. The increased level of saponins in the roots after long-term storage was confirmed by the foaming reaction results. After storage, the foaming number of the aqueous extract from the roots increased 12-for relatively to the foaming rate of the original roots (1: 6660). Long-term stable foaming was noted at the final dilution of the initial extract (1:80 000), indicating an increase in the saponins content in the original raw material.

4. Conclusion

The phytochemical analysis of the wild-growing raw material *A. gypsophiloides* revealed a significant content of triterpene saponins in the roots during the flowering-fruiting stage and in the aerial part of plants during the flowering phase. Roots, 4-8 cm in diameter, from middle-aged and mature generative plants should be selected for the raw material preparation. As an alternative source of saponins the aboveground part of the plant can be used, which is annually recovered from the preserved root. Long-term storage of the roots leads to increases in the saponin content and enhances their surfactant properties.

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Conflicts of Interests

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

Statement contribution of the authors

This study's experimentation, analysis and writing, etc. all steps were made by the authors.

References

Balandin, M. F. (1996). Commercial Utilization of Plant-Derived Saponins: An Overview of Medicinal, Pharmaceutical, and Industrial Applications. In: Waller, K., Yamasaki, K. (Eds). Saponins Used in Traditional and Modern Medicine. New York: Plenum Press, pp.1-14. https://doi.org/10.1007/978-1-4899-1367-8

Bespaev, S. B. (1966). *Acanthophyllum gypsophiloides* in Kazakhstan: Morphology, Taxonomy, Phytosociology, Testing in Culture. Abstract Thesis on The Scientific Degree of Candidate of Biological Sciences. Alma-Ata, Kazakhstan.

Böttger, S., & Melzig, M. (2011). Triterpenoid saponins of the Caryophyllaceae and Illecebraceae family. *Phytochemistry Letters, 4*, 59-68. https://doi.org/10.1016/j.phytol.2010.08.003

Chirikova, N. K., Olennikov, D. N., & Tankhaeva, L. M. (2010). Quantitative determination of flavonoid content in the aerial part of Baical scullcap (*Scutellaria baicalensis* Georgi). *Russian Journal of Bioorganic Chemistry, 36,* 915–922 https://doi.org/10.1134/S1068162010070204

Chrevach, E., Yudina, T., Frolova, G., Zhivchikova, R. (2009). Dynamics and biological activity saponins in extracts from roots *Saponaria officinalis* L. *Izvestiya vuzov. Food Technology, 4,* 25-28.

Desai, S., Desai, D., & Kaur, H. (2009). Saponins and their biological activities. *Pharma Times, 41*(3),13-16.

Eisenman, S., Strume, D., Zaurov D (2012). Medical Plants of Central Asia: Uzbekistan and Kyrgyzstan. Springer Science & Business Media, USA.

El Aziz, M. M. A., Ashour, A. S., & Melad, A. S. G. (2019). A review on saponins from medicinal plants: chemistry, isolation, and determination. *Journal Nanomedicine Research, 7*(4), 282-288. https://doi.org/10.15406/jnmr.2019.08.00199

El Mabrouki H., Kaukhova, L., Sorokin, V., & Minina, S. (2014). The development technique of quantitative definition saponins in the grass *Herniaria glabra* L. B*elgorod State University Scientific Bulletin Medicine Pharmacy, 24*(195), 235-238.

Faisal, A., & Geelen, D. (2013). Saponins and their role in biological processes in plants. *Phytochemical Review, 12,* 877-893. https://doi.org/10.1007/s11101-013-9322-4

Fedotova, V.V., Oganesyan, E. T., & Chelombitko, V. A. (2014). Triterpene glycosides of the herb of *Solidago caucasica* Kem. Nath. *Pharmacy & Pharmacology, 4*(5), 52-56. https://doi.org/10.19163/2307-9266-2014-2-4(5)-52-56

Fedoseeva, L. M., & Bashar, D. B. (2016). Study of saponins in roots of *Ferula hormones*. *Khimiya Rastitel'nogo Syr'ya, 1,* 181-184. https://doi.org/10.14258/jcprm.201501122

Gemejiyeva, N., Mursaliyeva, V., & Mukhanov, T. (2016). Assessment of the current state of *Allochrusa gypsophiloides* (Regel) Schischk natural populations in the South-Kazakhstan region. News of National Academy of Science of the Republic of Kazakhstan. *Series of Biological and Medical, 1*(313), 22-29.

Gladyshev, A., & Mishchenko, A. (1990). *Acanthophyllums* of Turkmenistan, Their Biology and Prospects for Economic Use. Science, Ashgabat. ISBN 5-8338-0165-6.

Grudzinskaya, L. M., & Gemejieva, N. G. (2014). List of Medicinal Plants in Kazakhstan. Almaty, Kazakhstan ISBN: 978-601-80248-6-3.

Guglu-Ustundag, O., Mazza, G., & Balsevich, J. (2007). Saponins: properties, applications, and processing. *Critical Reviews in Food Science and Nutrition, 47,* 231-258. https://doi.org/10.1080/10408390600698197

Khatuntseva, E., Men'shov, V., Shashkov, A., Tsvetkov, Y., Stepanenko, R., Vlasenko, R., … & Nifantiev, N. (2012). Triterpenoid saponins from the roots of *Acanthophyllum gypsophiloides* Regel. *Beilstein Journal of Organic Chemistry, 8,* 763-775. https://doi.org/10.3762/bjoc.8.87

Kondratenko, E. S., Putieva, Z. M., & Abubakirov, N. K. (1981). Triterpene glycosides of plants of the family *Caryophyllaceae*. *Chemistry of Natural Compounds, 17,* 303–317. https://doi.org/10.1007/BF01185255

Kuzmin, E. V., Tugelibaev, G. T., & Sitpaeva, G. T. (2001). Plant Population Reconstruction of *Allochrusa gypsophiloides* Rgl. in Southern Kazakhstan. (Abiev S, Fursova, N, Veselova, P, Sitpaeva, G, Aidarbayeva, D.) (Eds.). A Study of Kazakhstan Flora and its Protection. Collection of Scientific Articles. Aidana LLP, Almaty, Kazakhstan, pp. 91-194.

Kurkin, V. A., Ryazanova, T. K., & Zulkarnyaeva, L. V. (2017) Quantitative determination of saponins in the drugs of *Aralia elata*. *Khimiya Rastitel'nogo Syr'ya,* 3, 163-168. https://doi.org/10.14258/jcprm.2017031309

Kurkina, A. V., Savel'eva, A. E., & Kurkin, V.A. (2022). Development of quantitative determination methods for total flavonoids in *Tagetes patula* L. herbs. *Pharmaceutical Chemistry Journal, 56,* 657-660 https://doi.org/10.1007/s11094-022-02691-2

Lomboeva, S. S., Tankhaeva. L. M., & Olennikov, D. N. (2008). Method for the quantitative determination of the total content of flavonoids in the aerial part of *Orthilia secunda* (L. House). *Khimiya Rastitel'nogo Syr'ya,* 2, 65-68.

Lim, J. G., Park, H. M., & Yoon, K. S. (2020). Analysis of saponin composition and comparison of the antioxidant activity of various parts of the quinoa plant (*Chenopodium quinoa* Willd.). *Food Science & Nutrition, 8*, 694-702.

Mechnikova, G. Ya., Stepanova, T. A., & Zaguzova, E. V. (2007). Quantitative determination of total phenols in strawberry leaves. *Pharmaceutical Chemistry Journal, 41*(2), 38-41. https://doi.org/10.1007/s11094-007-0021-6

Mironenko, I. V., Brezhneva, T. A., & Selemenev, V. F. (2011). UV-spectrophotometric determination of triterpene saponins - derivatives of oleanolic acid. *Khimiya Rastitel'nogo Syr'ya,* 3, 153-157.

Nielsen, S. S. (2010). Phenol-Sulfuric Acid Method for Total Carbohydrates. (Nielsen S.S.) (Eds). Food Analysis Laboratory Manual. Food Science Texts Series. Springer, Boston, MA., pp. 47-53 https://doi.org/10.1007/978-1-4419-1463-7_6

Oganesyan, E. T. (1980). Mechanism of the reaction of triterpenoids with sulphuric acid. *Chemistry of Natural Compounds, 16*, 464-468. https://doi.org/10.1007/BF00571039

Paseshnichenko, V. A. (2001). Plants as producers of bioactive compounds. *Soros Educational Journal 7*(8), 13-19.

Pisarev, D. I., Martynova, N. A., Netrebko, N. N., Novikov, O. O., & Sorokopudov, V. N. (2009). Saponins and their definition in the rhizomes *Aralia Manchurian* from the Belgorod region. *Himija Rastitel'nogo Syr'ja, 4,* 197-198.

Putieva, Zh. M., Gorovichu, T. T., Kondratenko, E. S., & Abubakirov, N. K. (1979). Triterpene glycosides of *Acanthophllum gypsophiloides* VI. Structure of acanthophylloside D. *Chemistry of Natural Compounds*, *15,*148–151. https://doi.org/10.1007/BF00570786

Rachkovskaya, E., Volkov, E., & Khramtsov, V. (2003). Botanical Geography of Kazakhstan and Middle Asia (Within The Desert Zone). St. Petersburg, pp. 157-162.

Red Data Book of the Kazakh SSR (1981). Science, Alma-Ata, Kazakhstan, pp. 67-68.

Singh, B., & Kaur, A. (2018). Control of insect pests in crop plants and stored food grains using plant saponins: a review. *Food Science and Technology, 87,* 93-101. https://doi.org/10.1016/j.lwt.2017.08.077

Shadrin, D. M., Pylina Yu. I., Volodina, S. O., & Volodin, V. V. (2011). Chemical analysis of plants *Anthyllis vulneraria* L. growing in the European Northeast of Russia. *Izvestiya of the Samara Scientific Center of the RAS,* 13, 1(4), 945-947.

State Pharmacopoeia of the RF (2010). XII. Vol 2. Moscow, Russia.

Vincken, J. P., Heng, L., Groot, A de, & Gruppen, H. (2007). Saponins, classification, and occurrence in the plant kingdom. *Phytochemistry,* 68(3), 275-297. https://doi.org/10.1016/j.phytochem.2006.10.008

Zhang, J., & Qu, F. (2013). Methods for Analysis of Triterpenoid Saponins. In: Ramawat, K. G., Merillon, J. M. (Eds) Natural products. Springer-Verlag Berlin Heidelberg, pp. 3311-3323.