

Study of the Elemental Composition and Water-Soluble Vitamins of Cistanche Salsa of Karakalpakstan

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Abstract The content of water-soluble vitamins and the elemental composition of the stolon of Cistanche salsa (Cistanche salsa) growing in Karakalpakstan were studied. Using high-performance liquid chromatography, the presence of 7 water-soluble vitamins (C, PP, B1, B2, B6, B9 and B12) and inductively coupled plasma mass spectrometry - 30 macro- and microelements were established. Among the vitamins, vitamin C is dominant, with vitamin B12 having the lowest content. The quantitative content of the sum of vitamins is 6.23 mg/μl, of which vitamin C accounts for 2.6 mg/μl (41.73%). For saline cistanche, the predominance of macroelements K, Ca is characteristic. Of the microelements, iron and aluminum predominate; an abnormally high content of silver has been established. The concentrations of toxic elements do not exceed the permissible limits for a living organism.

Keywords Medicinal plant, Cistanche salina, Elemental composition, Microelements, Macroelements, Water-soluble vitamins, Inductively coupled plasma mass spectrometric method, High performance liquid chromatography

1. Introduction

One of the promising plants for the world pharmaceutical market are plants of the genus Cistanche Hoffm. et Link, which belongs to the Orobanchaceae family and, according to [1], includes 27 holoparasitic species. In Uzbekistan, it is represented by 9 species, of which 4 species are found in Karakalpakstan [2,3].

This plant has been known since ancient times as being widely used in traditional Chinese medicine. It is registered in the Chinese and Japanese Pharmacopoeias as Cistanches Herba (Rou Cong Rong), an excellent tonic drug obtained from the dried succulent stems of Cistanche deserticola YC Ma or Cistanche tubulosa (Schenk) Wight [4-6]. Its traditional functions include the treatment of kidney failure, impotence, female infertility and senile constipation, and in recent years, the treatment of neurodegenerative diseases [4].

Chemical analysis of Cistanches Herba showed that the main constituents are phenylethanoid glycosides, iridoids, lignans, oligosaccharides, and polysaccharides [7-9].

Pharmacological studies have confirmed that cistanche has lipid-lowering, estrogenic, neuroprotective, immunomodulatory, antitumor, anti-inflammatory, hepatoprotective, antioxidant, antibacterial, antiviral, and tonic properties [10-13].

To date, more than 100 compounds have been isolated from this genus and more than 100 Chinese patent medicines are registered in the Chinese Pharmacopoeia Commission (2015) and other official standards [13].

Along with pharmacopoeial species from China, other Cistanche species that grow outside of China, in particular in Kazakhstan and Uzbekistan, have recently attracted the attention of local scientists as a promising raw material resource for the creation of new pharmacological agents and analogues of Cistanches Herba [14-22].

It is natural to assume that the therapeutic effect of this medicinal plant may depend not only on the biologically active substances contained in it, but also on the concentration of amino acids, vitamins, macro- and microelements, information on the quantitative content of which in plants of the genus Cistanche is rare or absent [21-23].

The possibility of using cistanche growing on the territory of Karakalpakstan as a source of raw materials for the isolation of biologically active substances and the production of herbal preparations dictates the need to study its main and secondary metabolites, macro- and micronutrients.

Previously, we reported on the study of macro and microelements (underground and aboveground parts, and seeds) and the amino acid composition of the stolon of Cistanche salina from Karakalpakstan [19,20].

In this paper, we present the results of studies of the elemental composition and water-soluble vitamins of stolons of Cistanche salsa, growing in the southern region of Karakalpakstan.

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Earlier, we briefly noted the relevance of research and the role of macro- and microelements in the biochemical processes of the human body [19]. Equally important are vitamins, which are low molecular weight organic compounds of various chemical nature and structure, synthesized mainly by plants and partially by microorganisms [24].

The main role of vitamins is to ensure the normal course of biochemical and physiological processes by participating in the regulation of metabolism. Vitamins are not included in the structure of human and animal tissues and are not used as an energy source.

In the human body, some vitamins are not synthesized completely, others are synthesized by the intestinal microflora and tissues in insufficient quantities, and therefore the body must constantly receive vitamins from food or directly, or their closest biochemical precursors - provitamins, which in human and animal organisms are easily converted into vitamins [24].

It should be noted that no food product contains all or even most of the vitamins, each group of products serves as a source of individual vitamins. The lack of vitamins, or their lack in the body, leads to the development of various diseases - hypo- or avitaminosis, and in the case of microelements, microelementoses. Considering that plants are the main source of vitamins, research on the vitamin composition of medicinal plants, along with other biologically active substances, is relevant [25,26].

The qualitative and quantitative composition of vitamins, as well as macro- and microelements of most medicinal plants of Karakalpakstan, including cistanche species, are not studied.

Therefore, we considered it expedient to supplement our research on the saline cistanche, growing in stressful, environmentally unfavorable conditions of Karakalpakstan, with the determination of its vitamin and elemental composition.

2. Materials and Methods of Research

The objects of our study were the stolons of *Cistanche salsa* collected in April 2022. in the flowering phase in the Kipchak territory of the state forestry "Nazarkhan" of the Republic of Karakalpakstan. When harvesting stolons, the plant was dug up, cleaned with a brush from the ground, the inflorescences were separated from the stems, which were washed lightly from the remnants of dirt in running cold

water. The dried plant stolons were cut transversely into thin slices. Drying was carried out by the air-shadow method, spreading the cut raw materials in one layer on racks of nylon nets, periodically turning.

The analysis of water-soluble vitamins of stolons of *Cistanche salina* was carried out by high performance liquid chromatography (HPLC), on an Agilent Technologies 1200 chromatograph on an Exlipse XDB C18 column (reversed phase), 3.5 μm , 4.6x150 mm. Diode array detector (DAD), 254, 290 nm. Solution A: 0.5% acetic acid, pH 1.7: B: CH₃CN (acetonitrile). Flow rate 1 ml/min. Gradient %B/min: 0-5min/96:4%, 6-8min/90:30%, 9-15min/80:20%, 15-17min/96:4%. Thermostat 250C.

The elemental composition was analyzed using inductively coupled plasma mass spectroscopy (ICP-MS) on a Perkin Elmer ISP-MS instrument (Nexion 2000) using a multi-element standard for 29 elements and a Hg standard. Preliminary sample preparation was carried out using a Berghoff microwave decomposition system with MWS-3+ software (Germany).

3. The Discussion of the Results

The content of 7 water-soluble vitamins was determined in the studied sample of the stolon *Cistanche salsa* (Cistanche salsa), growing in Karakalpakstan, using high-performance liquid chromatography:

The results of the study on the study of the qualitative and quantitative content of vitamins by HPLC are presented in Fig. 1 and in Table 1.

Table 1. Water Soluble Vitamins *Cistanche salsa*

vitamins	Concentration	
	mg/ μg	%
B1 (thiamine)	1,02	16,37
B2 (riboflavin)	0,56	8,99
B6 (pyridoxine)	1,15	18,46
B9 (Bc) (folic acid)	0,51	8,18
B12 (cyanocobalamin)	0,39	6,26
B3 (PP) (nicotinamide)	0	0
C (vitamin C)	2,6	41,73
Sum	6,23	

As can be seen from Table 1, the stolons of *Cystanthe salina* contain vitamins of group B, which have the properties of coenzymes, and vitamin C, which has antioxidant activity.

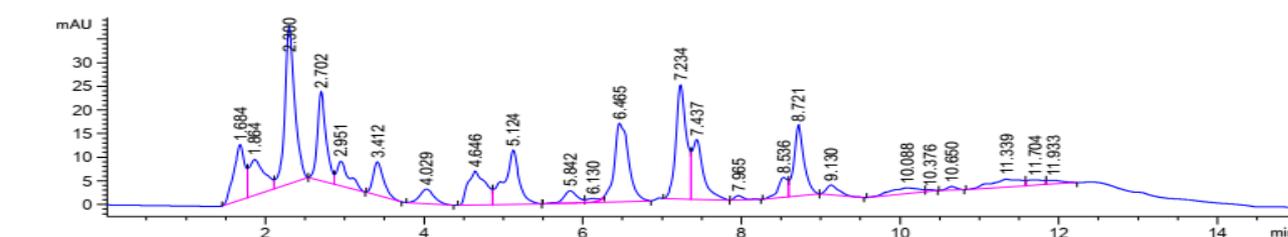


Figure 1. Хроматограмма витаминов столонов *Cistanche salsa*

The quantitative content of the sum of vitamins is 6.23 mg / μg ., of which vitamin C accounts for 2.6 mg / μg (41.73%), the rest are vitamins of group B. Among them, vitamin C is dominant, the lowest content falls on vitamin B12, and vitamin PP was not detected.

An overestimated content of ascorbic acid was also found for underground stems of *Cistanche mongolica* from the Ferghana region of Uzbekistan, which amounted to 57% of the total mass of water-soluble vitamins, which is approximately 15% higher than its content in saline *cistanche* [21].

In addition, it is worth noting that a number of B vitamins, namely B1, B6, B12, are higher in the *cistanche* solonchak plant than in the Mongolian *cistanche*.

The B vitamins are important cofactors in many metabolic reactions. The specific function of B vitamins in the body is that they form coenzymes and prosthetic groups of enzymes that carry out the most important metabolic processes.

Among the vitamins of group B, the maximum content falls on vitamin B6 - Pyridoxine, (18.46%) in relation to the total amount of vitamins. Such a high content of Vitamin B6 allows the use of *cistanche* in violations of the normal activity of the nervous system, hematopoietic organs, and the liver.

Vitamin B1 or thiamine makes up 16.37% of the total vitamin content, and the content of vitamins B2 (8.99%) and vitamin B9 (8.18%) are close to each other.

Vitamin B12 has the lowest content among vitamins (6.26%). Usually, cyanocobalamin is not found in plant foods or is found in very small amounts. And in this case, its presence in *C. salina* has a certain value as a plant resource of this vitamin.

Thus, the study of water-soluble vitamins of the stolon of *Cistanche salina*, growing in stressful, environmentally unfavorable conditions of Karakalpakstan, showed that it can serve as a raw material source of vitamin C and B vitamins.

Along with vitamins, minerals are essential nutrients and must also be ingested through food and water in certain amounts.

As a result of studying the elemental composition of the *Cistanche salsa* stolon, the composition of 30 macro- and microelements was determined by inductively coupled plasma mass spectrometry on an ICP-MS instrument (Nexion 2000). Table 2 presents the results of the analysis.

Potassium predominates in a number of macronutrients, calcium is almost two times less, and sodium has the lowest concentration. We observed a similar low accumulation of sodium earlier in the seeds and aboveground stems of *cistanche*, while in the underground parts sodium usually predominated [19]. In addition, a comparison of the quantitative indicators of macroelements of the studied stolons of *cistanche* with the indicators of *cistanche* of other regions of Karakalpakstan shows a strong discrepancy, which once again confirms the opinion of many researchers that the main trends in the formation of the elemental composition of plants are associated with the habitat [27].

Of the essential trace elements, iron predominates, the content of zinc and copper are close to each other, and the lowest content is selenium.

We observed a characteristic overestimated content of iron earlier [19], and noted that they can serve as a raw material source for replenishing iron deficiency in the body.

In the case of conditionally essential elements, only Ni, V, Li As were determined, among which the nickel concentration prevails (0.1157 mg/l).

Among the potentially toxic and toxic elements, an anomalously high content of silver (1035.06 mg/l) and the predominance of aluminum should be noted. We observed a similar high aluminum content in earlier studies [19]. The concentrations of other potentially toxic and toxic elements for the majority are about hundredths or thousandths, or have trace amounts, and do not exceed the permissible limits for a living organism.

Thus, the content of elements of the stolons of *Cistanche salina*, growing in the southern region of Karakalpakstan, differ significantly in quantitative composition, being much inferior to the samples of *Cistanche* from the northern region and the elemental composition of seeds.

Table 2. Macro- and microelements in *Cistanche salsa*, mg/l

№	Bioelements-macroelements		№	Conditionally essential		№	Toxic trace elements	
1	Na	14,8336	1	Ni	0,1157	1	Al	5,5983
2	K	158,3469	2	V	0,0496	2	Pb	0,0920
3	Ca	86,5329	3	Li	0,0291	3	Ba	0,4231
4	Mg	52,7207	4	As	0,0045	4	Bi	0,0459
	Essential trace elements			Potentially toxic trace elements		5	Cd	0,0097
1	Fe	4,3301	1	Rb	0,1795	6	Hg	0,0083
2	Zn	2,3705	2	Ag	1035,06	7	Be	0,0009
3	Cu	2,4858	3	Ga	0,0017	8	Tl	0,0028
4	Mn	0,8202	4	Sr	1,5652	9	U	0,0049
5	Co	0,5608				10	In	0,0009
6	Cr	0,1219				11	Cs	0,0037
7	Se	0,0415						

4. Conclusions

Thus, the study of water-soluble vitamins of the stolon of *Cistanche salina*, growing in stressful, environmentally unfavorable conditions of Karakalpakstan, showed that medicinal raw materials can serve as a raw material source of vitamins C and B.

As a result of the analysis of the macro- and microelement composition of the stolon of the saline *cistanche* growing in the southern region of the Republic of Karakalpakstan, it can be stated that its quantitative composition is significantly inferior to the content of the elements of the saline *cistanche* from the northern regions of the republic.

The accumulation of toxic trace elements has been found to meet regulatory requirements or are contained in safe trace amounts.

The obtained results indicate the need to continue research on the chemical composition of Karakalpakstan species of the genus *Sistanche* Hoffing. et Link depending on the place and growing conditions to assess the prospects for their practical use as herbal remedies.

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