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Life Characteristic of Cistanche Salsa (C.A. Mey.) G. Beck – Perspective Medicinal Plant of the South Peri–Balkhash Lake Saxaul Forests

N. G. Gemejiyeva, Zh. Zh. Karzhaubekova

Institute of Botany & Phytointroduction Ministry of Education and Science, Almaty, Kazakhstan

Email address:
ngemed58@mail.ru (N.G. Gemejiyeva), zhanna1322@mail.ru (Zh. Zh. Karzhaubekova)

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Abstract: The present study was carried out within the framework of grant financing of scientific surveys per subject: Restoration potential of producing vegetation of solonchak cistanche (Cistanche salsa (C. A. Mey.) G. Beck) in the South Peri–Balkhash Lake area (2012–2014). The survey object is perspective medicinal plant encountered within the South Peri–Balkhash Lake area solonchak cistanche of the Orobanchaceae Vent. family, which in Kazakhstan is produced only at the territories of Almaty and Zhambyl regions and then it is exported to Korea and China as raw stock for production of a number of pharmacologically active compounds having wide spectrum of effect such as hypertension, sexual vigor, antioxidant activity, where at present time, the producing areas and reserves in these countries have diminished. The life characteristic of this species was studied for the purpose of balanced use and preservation of natural populations of cistanche as perspective source for production of plant-based preparations of tonic and antioxidant effect. As a result of surveys, the peculiarities of distribution and floristic composition of vegetation communities including solonchak cistanche located within the South Peri–Balkhash Lake area totaling at least 48 species of plants of 16 families were identified. As per results of field surveys, the inventory and monitoring of producing areas of cistanche were carried out before and after raw stock production. The reserves of raw stock materials were calculated and schematic maps on distribution of identified producing areas of cistanche within the surveyed area were compiled. The total hydroalcoholic extracts were received and preliminary phytochemical screening of cistanche vegetable stock collected in 2013–2014 in the area was provided. The stolons of studied plant were found to contain main groups of biologically active compounds such as flavonoids, coumarines, carbohydrates, amino acids, organic acids, alkaloids, triterpenoids, saponins. The structures of two substances were extracted and identified.

Keywords: Cistanche Salsa, Medicinal Plant, Kazakhstan

1. Introduction

Kazakhstan flora has great potential as a source of raw materials for production of contemporary and efficient phytopreparations. Among 6000 species of Kazakhstan flora nearly 1500 species are characterized by medicinal properties of which only 230 species are used in official medicine [1]. Among such poorly studied and unused in Kazakhstan official medicine species is wild-growing medicinal plant Cistanche salsa (C.A. Mey.) G. Beck of Orobanchaceae Vent. family, highly popular at foreign producers of medicinal products as a feedstock for production of a number of pharmacologically active compounds of wide range of effect such as tonic, sexual vigor and antioxidant [2].

In Kazakhstan this plant is harvested only at the territories of Almaty and Zhambyl regions and then it is exported to China, where to date the production areas and reserves have reduced [3]. Therefore, without scientific approach and balanced use of natural thickets, reserves of cistanche will be depleted in quick time.

The resource characteristic of this species was for the first time studied for the purpose of balanced use and preservation of natural populations of cistanche as perspective source for production of plant-based preparations of tonic and antioxidant effect being the foundation for development of scientifically-based algorithm of rational use of raw plant. The resource characteristic of this species includes description of ecologically-cenotic and biological features of

species, dynamics of yielding capacity by years, data on raw stock in different phytocenosis, qualitative composition of raw materials, methods and duration of population restoration and methods of their protection [4].

2. Study Area & Methods

The goal of the study is analysis of life characteristics of perspective medicinal plant of Cistanche salsa (C. A. Mey.) G. Beck (fam. Orobanchaceae Vent.) which grows in saxaul forests the South Peri–Balkhash Lake area.

Cistanche order Cistanche Hoffmgg. et Link of the family Orobanchaceae Vent. includes nearly 20 species distributed in the Mediterranean, West and Central Asia countries. In «Kazakhstan Flora» [5], Cistanche Hoffmgg. is represented by three species: C. ambigua (Bunge) G. Beck, C. flava (C. A. Mey.) Korsh., C. salsa (C. A. Mey.) G. Beck), by last data [6, 7] there are five species among those which have already been mentioned above and C. mongolica G. Beck, and C. fissa (C. A. Mey.) G. Beck.

The most widely-spread is Cistanche salsa – perennial parasite plant with the height 10–40 cm, thick tufted stem (Fig. 1). The flower head has short cylindrical form of the height 5–25 cm and 5–8 cm wide and lush. The flowers are in spicate raceme, attached. The corolla is obtusely campanulate. Blossoms and fruits in April-May. The fruits represent egg-shaped capsules opening with two shells [5]. This species grows on solonetz and solonchaks, in desert steppes of almost all desert, small hummocky and piedmont Kazakhstan areas.

Cistanche practically has neither root system nor stems. The stolon (stem) is covered with chaffs. The plant vegetates on soil surface approximately two weeks. For this period the ripen seeds quickly break up and the plant dries out.

(a) (b) (c)

a – saxaul forest, b – Cistanche salsa in bloom stage, c – stolon

Figure 1. *Cistanche salsa (C. A. Mey.) G. Beck (family Orobanchaceae Vent.) in the South Peri–Balkhash area.*

Cistanche mainly parasites on the roots of the host plants Haloxylon Bunge order of Chenopodiaceae Vent. family, order Calligonum L. of Polygonaceae Juss. family, order Tamarix L. of Tamaricaceae Link family, thus attaching to the host roots and sucking out its nutritional substances. The relationship system formed between the parasitic plant and host plant is complicated which includes a number of adaptations from the side of the first plant and defense reactions from the second one.

It is very important for a parasite to ensure the contact with host plant beginning from seed germination. The seeds of many parasitic species do not germinate until they are found to be near the roots of host plant which exudes into the soil substances stimulating germination of seeds of a parasite and determining direction of its haustorium growth [8].

Survey methods include general resource, geobotanical and phytochemical investigations [9–12]. Resource survey was carried out by route–recognostic method [13] by using cartographic materials. The surveys were conducted in the south-east of Kazakhstan, in South Peri-Balkhash Lake area (Fig. 2) which located as consistent with botanic–geographic zoning within East–North Turan subprovince of North–Turan province of Iran–Turan subregion of Sakhara–Gobi desert area [14].

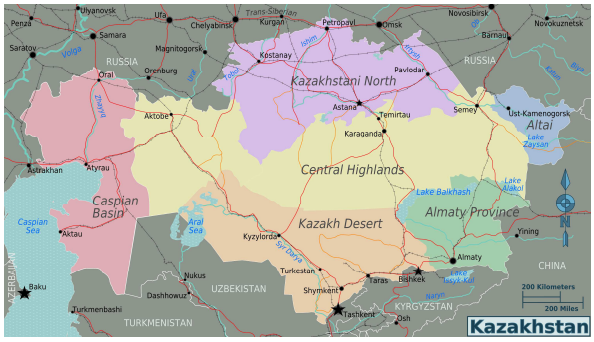

Figure 2. *Republic of Kazakhstan.*

The survey area characterized by continental climate, plain and hummocky-ridged relief. Soil cover formed by hydrogenic soils, solonchaks, takyrs, takyr–like and desert sandy soils. The vegetation cover characterized by wide development of black saxaul (Haloxylon aphyllum) and saltwort (Salsola orientalis) communities. Small sand areas occupied by saxaul–teresken–wormwood (Ass. Artemisia terrae-albae – Krascheninnikovia ceratoides – Haloxylon aphyllum) communities [15].

3. Results & Discussion

In autumn, 2012, route–recognostic survey was carried out for observation of natural plant population areas of cistanche from v. Bakanas to v. Kokzhide including outskirts of v. Kokzhide, Akkol, Akzhar, Ushzharma, Bereke and Bakanas. There were identified location areas of cistanche and pilot index plots for recording of cistanche intended for further monitoring of wild–growing populations in 2013–2014. Field surveys aimed at inventory of producing areas and monitoring of raw material base of cistanche were conducted before (April) and after (end of May–June) harvesting within Balkhash district of Almaty region.

As a result of surveys, the resource characteristic of natural populations was for the first time ever provided for Cistanche

salsa in the South Peri–Balkhash Lake area within Balkhash district of Almaty region. It was established that the populations of cistanche growing in rhubarb–wormwood–saxaul (Bereke area), zhuzgun–saxaul (Ushzharma area) and wormwood–saxaul (Akzhar area) communities has high yielding capacity which are confined to takyr–like–solonetz and solonchak grey soils, and at some areas – to clayey, sandy soils.

Floristic composition of vegetation communities including C.salsa consists of at least 48 species of plants of 16 families having the biggest number of representatives of families: Chenopodiaceae (14), Asteraceae (6), Tamaricaceae (4), Poaceae (3), Limoniaceae (3) etc. Among associated cistanche species, 15 species were recorded: Allium schubertii, Arnebia decumbens, Cardaria repens, Erodium oxyrhynchum, Microcephala subglobosa, Nepeta micrantha, Nonea caspica, Scorzonera sericeo-lanata, Scorzonera purpurea, Strigosella africana, Hyoscyamus pusillus, Hypecoum parviflorum, Lepidium perfoliatum, Trigonella arcuata, Ziziphora tenuior. The vegetation communities including solonchak cistanche growing at pilot index plots of Akzhar, Ushzharma and Bereke were rich in floristic composition.

During field survey of natural populations of cistanche, the blooming specimens suitable for harvesting at the areas of 100 x 100 m^2 were identified and recorded prior to completion of large-scale harvesting of raw materials at the end of April 2013–2014, in addition, the density of raw stock, phytomass and size of model specimens of cistanche, yielding capacity of raw stock were identified at each index plot (Table 1).

Table 1. *Dynamics of yielding capacity of Cistanche salsa at key plots before harvesting (April, 2013–2014)*

Location of index plot and date of observation	Quantity of blooming cistanche specimens, units per 100x100 m^2	Density of raw stock reserve, spec./m^2	Yielding capacity of raw stock, kg/ha	
			fresh	dry
Bereke index plot (1). At 1.5 km westwards of turn to v. Bereke among saxaul forests. 30.04.2013	35	0.004	11.06±1.44	-
Bereke index plot (1). 29.04.2014	43	0.004	14.55±1.89	2.47±0.32
Kokzhide index plot (2). At 1 km from the turn of Bakanas-Kokzhide motor road to v. Kokzhide, by both sides of the road among saxaul forests. 1.05.2013	18	0.002	5.53±0.66	-
Kokzhide index plot (2). At 3 km from the turn of Bakanas-Kokzhide motor road to v. Kokzhide towards v. Akkol (9.4 km to Akkol) among saxaul forests not far from motor road. 30.04.2014	35	0.004	11.34±1.58	2.03±0.26
Akkol index plot (3). At 1 km from the turn of Kokzhide–Bakanas motor road to v. Akkol, by both sides of the road among saxaul forests. 1.05.2013	7	0.0007	1.94±0.25	-
Akkol index plot (3). At 1 km from gates to v. Akkol 30.04.2014	10 (left) 7 (right)	0.001 0.0007	2.83±0.37 1.98±0.27	0.40±0.06 0.28±0.04
Akzhar index plot (4). Turn to v. Akzhar, at 300 m to guidance sign to v. Akzhar 1.05.2013	35	0.0035	14.72±1.77	-
At 7–8 km southwards of v. Akzhar (4 a) (Tekturmys) on sand drifts among saxaul forests 1.05.2013	2	3	4	5
Akzhar index plot (4). At 800 m from the turn to v. Akzhar 30.04.2014	70	0.007	25.76±3.35	-
Ushzharma index plot (5). At 1.6 km from the turn to v. Ushzharma, by both sides of the road. 1.05.2013	44 (91) (left) 21 (right)	0.0044 0.0021	14.37±2.01 6.86±0.96	2.49±0.32 1.19±0.17
Ushzharma index plot (5). 0.04.2014	28 42	0.0028 0.0042	11.04±1.21 14.72±1.91	-

According to our observations, in 2013, the number of specimens at pilot index plot of the size 100 x 100 m^2 ranged from seven to 70 thus averaging 49 specimens per hectare. However, the weight of raw stock of one specimen has

significantly reduced ranging from 106.0 to 872.0 (at an average 276.6±30.42) g.

In 2014, the number of specimens at pilot index plots of the size 100 x 100 m^2 varied from seven to 73 thus averaging 47 specimens per hectare.

The weight of one raw plant specimen varied from 116.0 to 692.0 g (at an average 393.4±55.08), i.e. during 2013–2014, no significant changes per weight of raw plant specimens and density of reserves were detected.

As a result of field surveys, the inventory of producing areas of cistanche was done, the reserves of raw stock were calculated and location maps of cistanche producing areas recorded during 2013-2014 within the investigated territory of Balkhash district of Almaty region were compiled. The most productive areas were recorded in the outskirts of Akzhar village (within the territory of Kurtinskiy SI) and v. Ushzharma, Bereke, Kokzhide (within the territory of Bakanas SI).

The overall available inventory of raw stock of cistanche within the territory of Bakanas forestry during 2013–2014 amounted to 280–295 t at the area of 34835.0 ha respectively. Considering biological peculiarities of solonchak cistanche, the volume of possible annual production will not exceed 56-59 t of fresh raw stock (Table 2).

Table 2. *Information on results of resource survey of Cistanche salsa at the territory of Balkhash district of Almaty region*

Location of producing areas at the territory of Balkhash district forestries	Area occupied by Cistanche salsa, ha	Overall available inventory of raw stock, t	Volume of possible annual production of raw stock, t
2005 (as per results of works under economic agreement)			
Bakanas State Forestry Institution Bakanas Forestry (plots unknown)	41.500	111.2	22.2
Akkol Forestry (plots unknown)	7.300	38.1	7.6
Total	48.800	149.3	29.8
2013			
Bakanas State Forestry Institution Bakanas Forestry (plots 203, 204, 205, 213, 214, 215)	34.835	280.4	56.1
Akkol Forestry (plots 298, 299)	4.558	6.6	1.3
Kurtinskiy State Forestry Institution Akzhar Forestry (plots 1–35, 83)	6.333	99.9	20.0
Total	45.726	386.9	77.4
2014			
Bakanas State Forestry Institution Bakanas Forestry (plots 203, 204, 205, 213, 214, 215)	34.835	295.03	59.0
Akkol Forestry (plots 298, 299)	4.558	9.02	1.8
Kurtinskiy State Forestry Institution Akzhar Forestry (plots 1–35, 83)	6.333	91.01	18.2
Total	45.726	395.1	79.0

At the territory of Kurtinskiy SI of Akzhar forestry, the available inventory of raw stock of cistanche amounted to 91–99.9 t at the area of 6.333 ha respectively. Considering biological peculiarities of cistanche, the volume of possible annual production will not exceed 18–20 t of fresh raw stock. The archive data obtained in 2005 during implementation of works under economic agreement were used to compare them with the newly obtained data. Thus, in 2005, the weight of raw stock of one cistanche specimen reached up to 3 kg, while the average number of solonchak cistanche specimens per one hectare varied from seven to 23.

By the example of Akkol forestry, it may be noted that the area occupied by cistanche as well as available inventory and volume of raw stock harvesting have reduced from 2005 to 2013, although for 2005, the numbers and areas of plots at which cistanche reserves were recorded were unavailable. Unfortunately, it is impossible to compare by other data due to absence of specific and equal data.

The monitoring of producing areas of cistanche before and after harvesting of raw stock within the surveyed territory of Balkhash district of Almaty region showed that at index plots after harvesting of raw stock there were left "traces" in the form of deep hollows – pits, which need to be backfilled in order to prevent the roots of host plants such as Haloxylon Bunge, Calligonum L.,Tamarix L. from drying–out. Likewise, the remains of cut crowns of cistanche blossom clusters with immature seeds were recorded. As it turned out, local producers leave these cut blossom clusters purposely near the hollows in order that seeds would subsequently get into the soil once they are ripen and then attach to host plant thus indirectly contributing to preservation of seed regeneration.

Almost all detected prior to harvesting specimens were dug out, only a few specimens of cistanche were left intact at index plots due to later aftergrowth, i.e after large-scale collection of stolons.

At Ushzharma index plot, the aftergrowth of fresh cistanche specimens was recorded near the remaining dried-out last year specimens.

Continuation of monitoring surveys of the state of cistanche populations in 2014 allowed to establish that no

significant changes were recorded per weight of raw stock and density of cistanche reserves during two vegetation seasons. The difference between obtained indices ranges within the error. However, this year, the factual harvesting areas were noticed to be shifted from the territory of selected index plots towards remote saxaul forests. In this connection, the local increase of yielding capacity of raw stock was recorded at certain index plots such as Bereke and Kokzhide.

The results of two-year observations (2013–2014) of the state of cistanche populations at index plots before and after harvesting of raw stock showed that density of raw stock at all index plots (Bereke, Kokzhide, Akzhar, Ushzharma) did not exceed 0.004 spec./m^2, except Akkol area where this index was the lowest ranging from 0.0007 to 0.001 spec./m^2.

The raw weight of specimens varied from 122.0 to 692.0 g (average 393.4±55.08) at Bereke index plot and from 178.0 to 550.0 g (average 326.6±45.71) at Akzhar index plot. The length of stolon without blossom cluster ranged from 13.0 to 45.0 cm, diameter of stolon varied from 2.5 to 6.5 cm. The length of blossom cluster reached up to 11–25 cm.

During two-year monitoring, the lowest yielding capacity of cistanche populations totaling 1.98±0.27 kg/ha was recorded in Akkol forestry. The highest yielding capacity of cistanche populations (14.55±1.89 kg/ha and 14.37±2.01) was recorded at Bereke and Akzhar index plots. The yielding capacity of cistanche populations at Ushzharma index plot was less totaling (8.52±1.10 and 9.25±1.20 kg/ha), as compared to 2013 it reduced at an average to 23-30% although the density of raw stock was the same as before. Evidently, this is due to the fact that vegetation is harvested without "resting" at one and the same areas.

The comparison of morphometric indices of model specimens of cistanche showed that populations of cistanche at Akkol plot had the least size and phytomass (224.3±29.16g). The biggest phytomass had specimens collected at Bereke plot (393.4±55.08 g), Akzhar (326.6±45.71 g) and Kokzhide (283.4±39.68 g). The average yielding capacity of cistanche at Bereke index plot in 2014 has increased for 30% thus totaling 14.55±1.89 kg/ha under the same density 0.004 spec./m^2.

At Kokzhide index plot, the productive populations of cistanche shifted at the distance of 3 km from last year harvesting areas as a result of which density of reserves and yielding capacity of raw stock (11.34±1.58 kg/ha) has increased almost twice as compared to 2013 data.

By yielding capacity, the most stable were populations of cistanche at Akzhar forestry whose maximal yielding capacity during 2013–2014 did not change much thus constituting 14.37±2.01 (14.72±1.77) kg/ha, although producing areas shifted inward saxaul forests for 800–1000 m. The difference between obtained data ranges within the error.

Overall, available local 2005 data per Bakanas and Akkol forestries obtained during works implemented under economic agreement are indicative of the fact that by now the areas occupied by cistanche at the territories of above–indicated forestries have reduced in a great measure and twice at certain areas. In order to determine the factual restoration potential of studied species, it is necessary to conduct multiyear monitoring of the state of natural populations of cistanche as well as dynamics of their reserves density and yielding capacity and morphometric indices.

For the purpose of preservation of natural populations of Cistanche salsa, it is recommended that next harvesting of raw stock at used producing areas should only be done after the land is allowed to rest for 4-5 years as new underground sprouts of cistanche are formed from the so-called root tubers –tuber–like formations at the place of contact with host plant during at least 3–4 years.

The herbal plant material was collected in natural populations for further production of extract from Cistanche salsa raw stock. The collected Cistanche salsa plant material at the territory of Bakhash district of Almaty region was dried–out, ground, weighed down and determined for good quality of studied plant raw material. The solvents for extraction of raw material were selected. Air–dry ground mass of plant material was treated with hydroalcoholic solvent three times. The extraction lasted three days under room temperature. The obtained total hydroalcoholic extract was filtered and then reduced to minimal volume and prepared for initial phytochemical screening.

Phytochemical survey of parasitic plants which are adapted to "host plants" is of big interest as many of these plants are used by local community for treatment of certain diseases but they are not included in the arsenal of scientific medicine due to their low chemical and biological study.

The first steps towards studying parasitic plants came out in the proceedings in [16, 17]. Reference [18] was described the early studies of the researchers of plant resources laboratory which shows the results of qualitative reactions for coumarines and saponins at parasitic plants exept Cistanche flava produced in the South Peri-Balkhash Lake area at the territory of Almaty region.

The preliminary phytochemical screening of Cistanche salsa samples collected during 2013–2014 in Almaty region was carried out. The fractional extraction of total water-alcohol extract was done by using ethyl acetate and n-butanol. The compounds β-sitosterol-β-D-glicoside was isolated from butanol extract. The β-sitosterol ($C_{29}H_{50}O$) was isolated from ethyl acetate extract [19]. These compounds were earlier extracted by [20] from Cistanche salsa. In addition, free monosaccharides, xylose, glucose and amino acids were found in the extracts, the study of amino-acid composition was started. The main groups of biologically active substances were established in stolons of studied objects such as flavonoids, coumarins, carbohydrates, amino acids, organic substances, alkaloids, triterpenoids, saponins which are consistent with reference materials [21].

4. Conclusion

Thus, the resource characteristic was provided for the first time ever for perspective medicinal plant Cistanche salsa grown in the South Peri–Balkhash Lake area, in addition, biological, phytocenotic as well as phytochemical peculiarities of this species and the most productive

communities including Cistanche salsa were identified. The reserves and volume of possible harvesting of raw stock were calculated, distribution maps of detected producing areas of Cistanche salsa within the surveyed territory were compiled.

The recommendations were developed on optimal regime of harvesting and rational use of Cistanche salsa raw stock in the South Peri–Balkhash Lake area having all backgrounds for creation of domestic production of up-to-date dietary supplements, herbal preparations and efficient plant medicinal products based on renewable feedstock whose main exporter is Kazakhstan.

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