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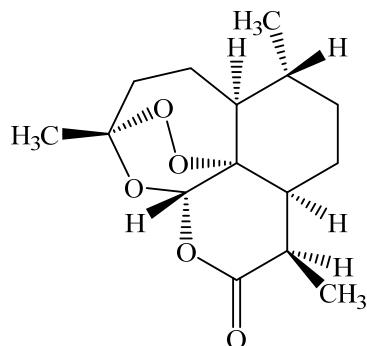
**SPREADING OF
ARTEMISIA ANNUA L. AND ITS CONTENT OF ARTEMISININ**

Abstract. This article presents the results of study resources and the assessment of the raw material base of the industrial source of the antimalarial drug “Artemisinin” derived from *Artemisia annua L* in the Almaty region of the Republic of Kazakhstan. Phytocenotic characteristic of the communities was carried out, the operational stock and the possible volume of annual raw materials harvesting of *Artemisia annua L* were determined.

The content of pharmacologically active sesquiterpene lactone artemisinin in the raw material of *Artemisia annua L* from different coenopopulations was discussed. It also provides recommendations for the exploitation of the natural reserves of *Artemisia annua L*.

Key words: *Artemisia annua L.*, coenopopulation, operating stock, raw materials, CO₂-extraction, sesquiterpene lactone, artemisinin.

Introduction. *Artemisia annua L.* (one-year warmwood) is the main source of raw materials for the production of the antimalarial drug artemisinin, developed on the basis of the sesquiterpene lactone with the same name (**1**) [1].



Artemisinin (**1**)

The World Health Organization (WHO) estimates that amount of artemisinin to provide at least 120 million treatments is 96,000 kg. At the same time, around 500 million cases of malaria are reported every year around the world [2].

The main suppliers of artemisinin in the world are Chinese and Indian companies, such as: Maysar Herbals (Haryana, India), KPC Pharmaceuticals (Kungming, China), Xi'an Lyphar Biotech Co., Ltd. (Xian, China), Guangzhou Quanao Chemical Co., Ltd. (Guangzhou, China), Nanjing Zelang Medical Technology Co., Ltd. (Nanking, China), Kerui nanhai (Chongqing, China) [3]. Despite the ongoing cultivation of *Artemisia annua L.* [4] and obtaining one-year species with artemisinin content up to 2% with regard to air-dry raw materials [5], as well as developing a semi-synthetic method for producing

artemisinin, pharmaceutical companies are still dependent on harvesting plant materials in nature due to the high cost of these methods [6].

In the Republic of Kazakhstan, the habitat of *Artemisia annua L* passes through a narrow strip of South and South-East Kazakhstan: the Zaisan depression, the valleys of the Chu, Sarysu, Syrdarya rivers, the Prikaratau piedmont plain, the foothills of the Dzungarian, Ili and Kyrgyz Alatau [7, 8]. Reconnaissance surveys of one-year-old wormwood thickets were conducted earlier [9] in the Almaty region of the Republic of Kazakhstan.

The purpose of the research is to identify and evaluate the raw material base of *Artemisia annua L* in the Almaty region for the preservation and balanced use of renewable plant materials, as well as to determine the content of sesquiterpene lactone artemisinin in coenopopulation different of different places of growth.

Materials and methods. Object of study - natural populations of *Artemisia annua L.* in the Almaty region of the Republic of Kazakhstan (figure 2).

Artemisia annua L. is a widespread cosmopolitan plant, which grows on sandy places, gardens and in settlements. The stalks of the *Artemisia annua L* reach a height of 30-100 cm, erect. Stem leaves are short; lower leaves sessile twice pinnate; periston-cut ovoid segments. The leaves are alternate, broadly ovate shape, 2.5–10 cm long and 2.5–4 cm wide, with 2-3 segments on each side. Petioles of lateral segments widely bordered. End of nibs quickly acuminate. The flowers are yellow. Baskets on drooping thin legs 1-3 mm long, hemispherical, form a common loose, wide densely leafy paniculate inflorescence; naked wrapper, linear filmy leaves. Edge flowers in a thread-like basket; median - glass and tubular. The fruit is an oblong flat achene without a crest, it blooms in July - August. The fruits ripen in August - September. The longevity of populations of *Artemisia annua L* ranges from 1 to 7-9 years, after which the species is displaced [10].



Figure 2 – *Artemisia annua L.* on territory of Almaty region

Traditional methods of geobotanical [11-13] and resource research were used during carrying out the work [14].

Supercritical CO₂-extraction was carried out at the USFE-5/2 installation (production GORO-engineering, Russia). Carbon dioxide food GOST 8050-64 was used As the extractant.

Extraction of raw material (weight in all experiments was 100 g) was carried out at a pressure of 25 MPa, extraction time 3 hours and a temperature of 60 °C.

The quantitative content of artemisinin in the extracts was determined by HPLC on an Agilent 1260 chromatograph (USA) compared to an external standard, under the following conditions: Zorbax SB-C18 sorbent column, 4.6 x 150 mm, particle size 5 μm , acetonitrile-water mobile phase 60: 40, detection at 235 nm, the column temperature is room temperature, the speed of the mobile phase is 0.5 ml/min.

Results and discussion. In August 2018 we made route-reconnaissance survey and found coenopopulations in the village Uzynagash Zhambyl district, near the village of Shilikbay Zhambyl district, near the village of Sayan Karasai, near the village of Kainar Karasai, village Kaskelen Karasai in the neighborhood Akshi village of Enbekshikazakh district, in the vicinity of the village of Avat, Enbekshikazakh district, village of Turgen of the Enbekshikazakh district and in the vicinity of the village of Enbek Alm Atinsky region of Enbekshikazakh district with a total length of 300 km. As a result of the expeditionary work in the surveyed area, 10 fishing arrays of *Artemisia annua* L were identified (table 1).

Table 1 – Productivity and raw material reserves of the aboveground mass of the generative organs *Artemisia annua* L. in Almaty region

Location of coenopopulation	Square	Plant density, pcs/m ²	Model plant weight, g	Productivity (air dry weight)		Operating stock of air-dry raw materials, t	Volume of possible annual procurement of air-dry raw materials, t
				g/m ²	c/hectare		
2 km from vill. Uzynagash Zhambyl district	2	1,2±0,03	14,75±0,1	17,7±0,5	1,7±0,05	0,34	0,14
Vicinity round village Shilikbai of Zhambyl district	1,5	3,4±0,05	36,9±2,3	125,5±4,6	12,5±0,4	1,88	0,75
Vicinity round village Shilikbai of Zhambyl district	1,7	3,2±0,04	35,7±2,2	114,2±3,7	11,4±0,3	1,94	0,78
Vicinity round village Sayan Karasay district	2,2	2,7±0,03	36,8±4,1	99,4±3,2	9,9±0,3	2,18	0,87
Vicinity round village Kainar Karasay district	0,9	2,2±0,02	24,8±3,2	54,5±3,1	5,4±0,3	0,49	0,19
Village Kaskelen Karasay district	1,2	2,7±0,04	34,5±2,6	93,1±3,3	9,3±0,3	1,12	0,45
Village Akshi of Enbekshikazakh district	1	1,1±0,02	14,1±0,2	15,5±1,3	1,5±0,1	0,15	0,006
Village Avat, Enbekshikazakh district	1,1	2,1±0,01	25,5±0,9	53,5±2,9	5,3±0,2	0,58	0,23
Village Turgen Enbekshikazakhskogo area	1,3	2,9±0,03	30,9±2,1	89,6±3,2	8,9±0,3	1,16	0,46
Vicinity round village Enbek Enbekshikazah area	2,5	2,3±0,03	24,2±1,4	55,6±3,3	5,5±0,3	1,38	0,55
Total	15,4					11,22	4,48

Coenopopulation 1 (C3 1) is located 2 km from the village. Uzynagach, Zhambyl district, 43°22'16,74 " N, 76°98'52,79 " E, forming a weedy community along the edge of the field. Co-dominants: *Cichorium intybus* L., *Agropyron cristatum* (L.) Beauv. First tier 85-130 cm - *Artemisia annua* L., *Cannabis ruderalis* Janisch., *Urtica dioica* L.; second 40-80 cm - *Cichorium intybus* L., *Acroptilon repens* (L.) DC., *Lactuca tatarica* (L.) C.A.Mey.; third 25-40 cm - *Agropyron cristatum* (L.) Beauv., *Achillea nobilis* L.; fourth - *Trifolium pratense* L., *Plantago lanceolata* L., *Malva pusilla* Sm. The height of *Artemisia annua* L ranges from 90 to 135 cm, the number of commodity individuals is 1.2 ± 0.03 pcs/m². The area of thickets is 2 hectares, the operating stock is estimated at 3.4 centners, of which the volume of possible blanks is 1.4 centners.

Coenopopulation 2 (CP 2) 43°13'44,16" N, 76°22'14,46" E. Thicket number 2 is found near by village Shilikbay Zhambyl district. The thicket was located on the edge of an abandoned field, with a total area of 1.5 hectares. Co-dominants: *Lactuca tatarica* (L.) C.A.Mey., *Achillea nobilis* L., *Cirsium arvense* (L.) Scop. Ярусность не выражена. Types of tiers are not pronounced. The average height of specimens of the *Artemisia annua* L was 111.7 ± 2.4 cm, diameter 51.1 ± 1.2 cm. The number of commercial

specimens per 1 m² was 3.4 ± 0.05 pieces. The operational stock of raw materials on an area of 1.5 hectares of CP amounted to 18.8 centners, of which the harvesting can be carried out at a level of 7.5 centners.

Coenopopulation 3 (CP 3) 43°14'48,17" N, 76°24'16,53" E. is located 400 meters from the thickets number 2 and covers an area of 1.7 hectares. The vegetation is growing on a hill side. In this community, 2 tiers are expressed: the first tier (110cm): dominants - *Artemisia annua* L. second tire (till 55 cm) - *Bromopsis inermis* (Leyss.) Holub., *Lactuca tatarica* (L.) C.A.Mey., *Tripleurospermum inodorum* (L.) Sch. Bip. The number of commodity plants per 1 m² was 3.2 ± 0.04 pieces, with an average height of plants of wormwood 106.9 ± 3.1 cm and a diameter of 39.5 ± 0.7 cm. c, the amount of possible harvest - 7.8 c.

Coenopopulation 4 (CP 4) 43°17'54,50" N, 76°44'90,21" E. was discovered near the Sayan village of the Karasai district and covers an area of 2.2 hectares, located on abandoned fields. Co-dominants: *Glycyrrhiza uralensis* Fisch., *Arctium tomentosum* Mill.. Marked 2 tiers: upper tier 80 cm and higher - *Artemisia annua* L.; *Arctium tomentosum* Mill.; bottom 30-50 cm - *Glycyrrhiza uralensis* Fisch., *Polygonum aviculare* L. The yield was 9.9 centners per hectare. The operational stock of air-dry raw materials is 21.8 centners, the volume of possible harvest is estimated at 8.7 centners.

Coenopopulation 5 (CP 5) 43°16'19,64" N, 76°44'53,00" E. grows in the vicinity of the village Kainar of the Karasai district and covers an area of 0.9 hectares, located on abandoned fields. Edifier in the community *Artemisia annua* L., Co-dominants: *Cannabis ruderalis* Janisch., *Onopordum acanthium* L. in high tier (80-130 cm) and *Xanthium strumarium* L., *Achillea millefolium* L. in bottom tier (20-35 cm). The average plant height was 125.4 ± 3.1 cm, diameter - 21.6 ± 0.8 cm. The operational stock was 4.9 centner of air-dry raw material, of which up to 1.9 centner can be harvested.

Coenopopulation 6 (CP 6) 43°21'62,71" N, 76°69'23,08" E. was found 2 km from the village. Kaskelen of Karasay district towards Almaty with a total area of 1.2 hectares. The number of commodity plants is 2.7 ± 0.04 pcs/m². The yield was 93.1 g/m², the operational stock of raw materials was estimated at 11.2 centners, the amount of possible raw material harvesting was 4.5 centners.

Coenopopulation 7 (CP 7) 43°50'92,64" N, 77°65'50,18" E. grows in the vicinity of the village. Akshi of Enbekshikazakh district, and covers an area of 1 hecatr. The height of *Artemisia annua* L in the CP was 140.4 ± 4.0 cm, diameter 12.6 ± 0.3 cm. Co-dominants - *Artemisia vulgaris* L., *Cirsium vulgare* (Savi) Ten. The presence of tiers is not marked. the operational stock of raw materials was estimated at 1.5 centners, the amount of possible raw material harvesting was 0.6 centners.

Coenopopulation 8 (CP 8) 43°40'48,1"N, 77°29'76,03" E. is located in the vicinity of the village of Avat of the Enbekshikazakh district on the edge of a corn field on an area of 1.1 hectares, the operational reserve is estimated at 5.8 centners, of which 2.3 centners can be harvested annually. Co-dominants *Xanthium strumarium* L., *Cannabis ruderalis* L. The average height of wormwood in the CP is 130.0 ± 3.1 cm, diameter - 34.2 ± 0.9 cm.

Coenopopulation 9 (CP 9) is located in the vicinity of the village of Turgen, Enbekshikazakh district 43°41'11,62"N, 77°61'14,19" E. on an abandoned field of 1.3 hectares, the operational reserve is estimated at 11.6 centners, of which 4.6 cent can be harvested annually. Edifier *Artemisia annua* L., Co-dominants *Xanthium strumarium* L., *Polygonum aviculare* L. The average height of wormwood in the CP is 129.3 ± 3.6 cm, diameter - 35.8 ± 0.7 cm.

Coenopopulation 10 (CP 10) was marked near the village of Enbek Enbekshikazakh district 43°40'48,1"N, 77°29'76,03" E. 43°47'46,69" N, 77°45'27,44" E. in an abandoned field, the total area was 2.5 hectares, the operational reserve is estimated at 13.8 centners, of which 5.5 cent units can be harvested annually. Edifier *Artemisia annua* L., Co-dominants - *Cichorium intybus* L., *Polygonum aviculare* L. The average height of thicket in the CP is 117.4 ± 2.9 cm, diameter - 25.6 ± 0.8 cm.

Thus, according to the results of resource studies on the territory of the Almaty region, 10 *Artemisia annua* L. coenopopulations were found, the total operating stock was 112.2 centners, of which the annual harvesting volume was 44.8 centners (figure 3).

The most promising for the procurement of raw materials is the CP 4 with an operating stock of 21.8 c, but, due to the increased economic activity, we can see a noticeable decrease in the raw material stocks of *Artemisia annua* L. in the Almaty region of the Republic of Kazakhstan. In the event of an increase in the demand for raw materials of *Artemisia annua* L., cultivation is recommended under conditions acceptable to growing, since one-year wormwood successfully goes through all phases of growth and gives a high yield of above-ground mass in Kazakhstan [15].

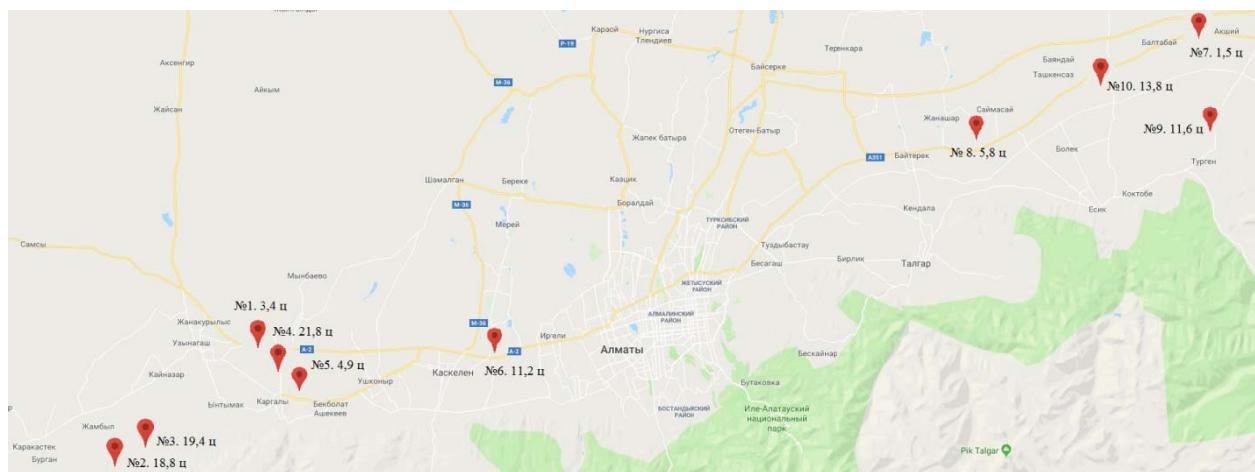


Figure 3 – Map of *Artemisia annua* L raw materials on the territory of Almaty region



– Marker designation of sites for the collection of raw materials.

To determine promising coenopopulations in terms of the content of the target substance artemisinin (1), we conducted studies to determine the optimal modes of supercritical fluid extraction of *Artemisia annua* L. collected in 10 coenopopulations to obtain an extract with quantitative content of artemisinin (1).

The results of supercritical fluid extraction and quantification of artemisinin are shown in table 2, which shows the average values for 3 experiments.

Table 2 – Comparison of the output of CO₂-extract of wormwood annual and the volume of the possible production of artemesinin (1) from the place of collection of raw materials

Place of collection	Output of extract, %	Content of Artesemisinin, % (g)	Volume of artemesinin production, kg/year
neighborhood of the village Avat	7,2	5,48 (0,40)	0,92
neighborhood of the village Turgen	6,5	9,44 (0,62)	2,85
neighborhood of the village Enbek	7,6	9,48 (0,72)	3,96
neighborhood of the village Sayan	5,2	13,30 (0,70)	6,09
neighborhood of the village Kainar	4,33	17,47 (0,76)	1,44
neighborhood of the village Kaskelen	7,5	17,92 (1,34)	6,03
neighborhood of the village Akshi	6,4	8,10 (0,52)	0,99
neighborhood of the village Uzunagash	10,0	0,80 (0,08)	0,11
neighborhood of the village Shilikbay	5,0	3,03 (0,15)	1,13
neighborhood of the village Shilikbay (2 km away from first thicket)	6,5	1,36 (0,10)	0,78

As can be seen from table 2, the content of artemisinin (1) varies from 0.1% (neighborhood of Shilikbay) to 1.34% (neighborhood of Kaskelen), depending on the place of collection, therefore promising in terms of industrial production of artemisinin (1) are coenopopulations of *Artemisia annua* L. in the vicinity of Kaskelen village with artemisinin content up to 1.34%, Sayan village - up to 0.7% and Enbek village - up to 0.72%, which is 6.03 kg per target substance (1), 6.09, kg and 3.96 kg respectively.

Conclusion. On the territory of the Almaty region in the Republic of Kazakhstan promising for industrial workpieces in terms of the amount of raw material harvested are thickets of *Artemisia annua* L. in the vicinity of the Shilikbai and Sayan settlements (coenopopulations 2, 3, 4). In general, the total operational stock of air-dry raw wormwood in the surveyed cenopopulations was 11.22 tons, with a possible annual harvest of 4.48 tons, while the average content of artemisinin in air-dry raw materials is 0.26%, which is on the target substance is 8.0 kg of artemisinin. The total area for collecting air-dry raw

wormwood was 15.4 hectares. At the same time, according to the content of artemisinin in raw materials, the most promising are coenopopulations in the vicinity of the villages of Kaskelen, Sayan, Enbek (1.34%, 0.7% and 0.72%, respectively) with the possibility of total annual procurement of air-dry raw wormwood in an annual amount of 1,87 tons, while the average content of artemisinin in air-dry raw materials is 0.92%, which in terms of the target substance is an average of 16.0 kg of artemisinin. That is why, *Artemisia annua L.* with rational use of the identified thickets in the Almaty region is provided with a raw material base and its operational reserves in the vicinity of the villages of Kaskelen, Sayan and Enbek are the basis of the industrial production of the antimalarial drug «Artemizinin».

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БІРЖЫЛДЫҚ ЖУСАННЫҢ (ARTEMISIA ANNUA L.) ТАРАЛУЫ ЖӘНЕ ОНЫҢ ҚҰРАМЫНДАҒЫ АРТЕМИЗИНИН

Аннотация. Мақалада безекке қарсы «Артемизинин» препаратының өнеркәсіптік көзі болып табылатын біржылдық жусанның (*Artemisia annua L.*) Қазақстан Республикасы Алматы облысындағы шикізат қорына баға беріліп, оның ресурстарын зерттеу нәтижелері келтірілген. Біржылдық жусан өсімдік бірлестіктеріне фитоценоздық сипаттама берілді, оны пайдалану коры және шикізатын жыл сайын дайындаудың ықтимал көлемі белгіленді.

Әртүрлі ценопопуляциялардағы біржылдық жусан шикізатының құрамындағы фармакологиялық белсенді артемизинин сесквитерпенді лактонының мөлшері талқыланады. Біржылдық жусанның табиғи корларын пайдалану бойынша ұсынымдар берілді.

Түйін сөздер: *Artemisia annua L.*, ценопопуляция, пайдалану қоры, шикізат, CO₂-экстракциялау, сесквитерпенді лактон, артемизинин.

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Аннотация. В статье приводятся результаты изучения ресурсов и оценка сырьевой базы промышленного источника антималярийного препарата «Артемизинин» полыни однолетней (*Artemisia annua L.*) в Алматинской области Республики Казахстан. Проведена фитоценотическая характеристика сообществ, определен эксплуатационный запас и возможный объем ежегодных заготовок сырья полыни однолетней.

Обсуждается содержание фармакологически активного сесквитерпенового лактона артемизинина в сырье полыни однолетней из разных ценопопуляций. Приведены рекомендации по эксплуатации природных запасов полыни однолетней.

Ключевые слова: *Artemisia annua L.*, ценопопуляция, эксплуатационный запас, сырье, CO₂-экстракция, сесквитерпеновый лактон, артемизинин.

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