

## ABSTRACT

thesis for the degree of Doctor of Philosophy (PhD) in the specialty  
“8D05108 – Geobotany”

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**"Study of botanical and molecular-genetic features of *Hedysarum theinum* Krasnob. in the conditions of East Kazakhstan"**

**Relevance of the dissertation.** Biodiversity conservation plays an important role in identifying priority areas for environmental protection. Efficient use and conservation of natural resources require the development and implementation of strategies aimed at assessing the state of plant communities. The aim of the present research project was to study morphological, anatomical, phytochemical, and molecular genetic features of *Hedysarum theinum* Krasnob., a rare plant species with medicinal properties. The material for the study was collected in the natural populations of East Kazakhstan. In 2020-2022, the populations of *Hedysarum theinum* Krasnob. found on the Ivanovo Ridge of the Kazakhstan Altai at an altitude of 1470-1938 meters above sea level and in the GPS coordinate interval of 50°N and 83°E were studied by the method of the route reconnaissance survey. *Hedysarum theinum* is a perennial plant of the legume family (Fabaceae). It is endemic to Altai, the mountains of Central Asia and Mongolia. Valuable medicinal and endemic plants of East Kazakhstan have not been sufficiently studied, and their ranges are constantly shrinking. In this regard, there is a need for an in-depth study of *Hedysarum theinum*. To increase the effectiveness of research work and enable a comprehensive study of *H. theinum*, a similar species, *Hedysarum neglectum* Ledeb, was selected for comparison.

Conservation of biological diversity requires a deep understanding of all its facets at the local and regional scales. Biodiversity provides valuable biological resources that support human life on earth. The use of plants with valuable medicinal properties, such as *H. theinum*, is of particular interest to medicine. Plants of the genus *Hedysarum* are widely used in folk medicine and are practically not used in official medicine due to poorly understood phytochemical composition.

However, modern medicine recognizes that *H. theinum* has medicinal properties and can be used to treat many diseases, since it contains combined biologically active compounds. All over the world, products of plant origin based on biologically active compounds are widely used for therapeutic and prophylactic purposes. Valuable drugs made from plants are known to be more beneficial to human health than chemically synthesized drugs. Medicines derived from plant raw materials do not have side effects, so the population prefers to use products derived from medicinal plants with known medicinal properties. In this regard, the study of such plants is of particular importance.

Altai is one of the richest floristic regions of Kazakhstan. More than 2,500 species of higher plants grow here, which is 50% of the total number of species of the flora of the Republic of Kazakhstan. The floristic diversity is due to the specifics of the climate in the region, which contributed to the formation of various ecotopes

with meadows, swamps, deserts, and semi-deserts. We can say that Kazakhstan's Altai is a storehouse of medicinal plants. However, today, despite the increased interest in medicinal raw materials, there is no modern assessment of the species diversity of the regional medicinal flora and its resource potential.

*H. theinum* is used in folk medicine as an analgesic, anti-inflammatory agent for kidney inflammation, and acute and chronic nephrological diseases. It also plays a very important role in energy replenishment and contains anti-tumor components.

In the wild, the similarity between *H. theinum* and *H. neglectum* often causes confusion. To solve the problem, it is necessary to conduct comprehensive morphological, anatomical, and genetic studies, considering the habitat conditions. Plants of the genus *Hedysarum* need in-depth study of their phytochemical composition, from chemical and environmental points of view.

The territory of Kazakhstan has a huge stock of medicinal plants widely used in traditional folk medicine, but not recognized by official medicine. *H. theinum* and *H. neglectum* from the family Fabaceae belong to this group of plants. These plants have been used for centuries by the inhabitants of Altai in folk medicine to treat a wide range of human diseases. They are widely used as a food additive created on the basis of biologically active compounds obtained from medicinal plants. Mountain Altai is an inexhaustible source of plant raw materials with a large number of plants rich in biologically active compounds. Many medicinal plants are unique in that they are practically not found in other regions of Kazakhstan. Since they grow in extreme conditions of high-mountain climate, they are characterized by a large content of highly effective biological compounds. There is a growing interest in studying the ecological state of the highlands. Such studies occupy an important place in the study of vegetation cover.

The poor knowledge of the ecological and morphological features of *Hedysarum theinum* is a prerequisite for scientific research, therefore a comprehensive study of the species is relevant.

**Study object:** *Hedysarum theinum* Krasnob. and *Hedysarum neglectum* Ledeb. of the family Fabaceae, found in the Ivanovo Ridge.

**Objective:** To study the morphological, anatomical, phytochemical, and molecular genetic features of *Hedysarum theinum* Krasnob. growing in East Kazakhstan, in order to preserve the biodiversity of this region.

**The objectives are as follows:**

To achieve this goal, the following tasks were implemented:

1. Floristic composition of the Ivanovo Ridge plant communities with *Hedysarum theinum* Krasnob. was determined.
2. Analysis of herbarium collections of *H. theinum* and *H. neglectum* was carried out.
3. The germination rate of *H. theinum* seeds was determined, and the basic age spectrum of the species was established.
4. Morphological and anatomical structure of *H. theinum* and *H. neglectum* found in East Kazakhstan was studied.
5. The phytochemical composition of *H. theinum* and *H. neglectum* was studied to determine biologically active components.

6. A phylogenetic tree with two species of the genus *Hedysarum* was built based on the ITS nuclear marker.

**Research methods:**

Geobotanical, morpho-anatomical, mass spectrophotometric, molecular genetic, and gas chromatography methods were used in the work.

**Scientific novelty of the dissertation.** The results of the study reflect a new understanding of the morpho-anatomical, phytochemical, and molecular genetic features of the species and will serve as the basis for the conservation and inclusion of the species in the Red Book of Kazakhstan.

1. For the first time, the habitat of *H. theinum* on the Ivanovo Ridge was described, a schematic map was created, and the floristic composition of plant communities was determined. As a result of the study, the herbarium of the Institute of Botany and Phytointroduction was supplemented with the collections of *H. theinum* and *H. neglectum*.

2. The seed germination rate and the species basic age spectrum were determined, and the foundations for preservation of the plant in the wild have been laid. The scarification method showed good results in determining the laboratory germination rate of seeds.

3. Statistical processing of morphometric and anatomical parameters of the species was carried out. During the anatomical study, the presence of idioblasts in the structure of the studied plant species was established.

4. As a result of phytochemical studies, biologically active substances have been identified in the aboveground and underground organs of *H. theinum*. For the first time, squalene was found in the plant, which has antitumor and antioxidant properties.

5. For the first time, *H. theinum* was studied by the molecular genetic method. A study based on the ITS DNA marker was carried out, and the genetic relationship of the species with other species was revealed.

**Scientific and practical significance of the research work.**

The obtained scientific results allow us to assess the current state of the endangered plant *H. theinum* found on the Ivanovo Ridge. Herbarium specimens of *H. theinum* and *H. neglectum* obtained during the dissertation work were transferred to the herbarium of the RSE on the REM "Institute of Botany and Phytointroduction" of the Forestry and Wildlife Committee of the Ministry of Ecology, Geology and Natural Resources of the Republic of Kazakhstan and to the herbarium of the Faculty of Biology and Biotechnology, Al-Farabi KazNU. *H. theinum* seeds were transferred to the seed bank of the Institute of Botany and Phytointroduction.

The results obtained during the dissertation work are a prerequisite for the proposal to protect the species and list it in the Red Book of Kazakhstan.

**Key provisions of the thesis put forward for consideration are the following:**

1. In the floristic composition of the Ivanovo Ridge plant communities with *Hedysarum theinum*, 176 species from 41 genera have been identified.

2. The results of a comprehensive study of the morphological and anatomical structure supplement the available information on the morphological and anatomical characteristics of *H. theinum* and *H. neglectum*.

3. The results of a phytochemical analysis of the studied species, qualitative and quantitative parameters of biologically active substances contained in the aboveground and underground organs. The plant's Latin name is *H. theinum*, but pharmaceutical companies that use it in dietary supplements call it *H. neglectum*, leading to widespread misconception.

4. The location of *H. theinum* and *H. neglectum* species in the phylogenetic genealogy based on the nucleotide sequence of the ITS DNA marker indicates the polyphyletic origin of the species.

5. As a result of research, it was revealed that the most numerous were groups of middle-aged generative individuals of *H. theinum*. A base age spectrum with a maximum on middle-aged generative individuals has been identified. A small number of juveniles is the result of anthropogenic factors, so the species *H. theinum* is endangered, and therefore it is recommended to be listed in the Red Book of Kazakhstan.

**Relationship with the plan of the main scientific works.** The dissertation work was carried out within the framework of the grant project AP09561631 "Research of botanical and molecular genetic features of the species *Hedysarum theinum* Krasnob. (Fabaceae Lindl.) for biodiversity conservation", 2021-2023 (State registration No. 0121RK00516).

**Personal contribution of the author to the work.**

The author of the dissertation fully contributed to the choice of the form and concept of research, the definition of the purpose of the work, the formulation of the research problem, the planning and execution of experiments, and the generalization and processing of the data obtained.

**Approbation of the work.**

The results and main provisions of the thesis were presented and discussed at international scientific conferences:

- "International Scientific Conference of Students and Young Scientists Farabi Alemi" (Almaty, Kazakhstan, 2021);

- "International Scientific Conference of Students and Young Scientists Farabi Alemi" (Almaty, Kazakhstan, 2022);

- "International Scientific Conference of Students and Young Scientists Farabi Alemi" (Almaty, Kazakhstan, 2023).

**Publications**

Based on the results of the study, the author published 7 scientific papers; including 1 articles in journals indexed in the Web of Science and Scopus databases, 3 articles in the journal recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, and 3 publications in collections of materials of international scientific conferences.

**Structure of the thesis.** The dissertation consists of 110 pages, introduction, literature review, materials and methods, results and discussion, conclusion, list of 187 references, 4 appendices, 14 tables, and 35 figures.