OnLine Journal of Biological Sciences

Original Research Paper

# Comparison of Anatomical Characteristics and Phytochemical Components Between Two Species of *Hedysarum* (*Fabaceae*)

**[1]Adil Kusmangazinov, [1]Meruyert Sakenovna Kurmanbayeva, [1]Irina Zharkova, [1]Dina Karabalayeva, [1]Raushan Kaparbay and [2]Tursynbek Kaiyrbekov**

[1]*Department of Biodiversity and Bioresources, Al-Farabi Kazakh National University, Almaty, Kazakhstan*
[2]*Department of Botany, Ondokuz Mayis University, Samsun, Turkey*

*Article history*
Received: 09-03-2023
Revised: 19-05-2023
Accepted: 23-05-2023

Corresponding Author:
Meruyert Sakenovna Kurmanbayeva
Department of Biodiversity and Bioresources, Al-Farabi Kazakh National University, Almaty, Kazakhstan
Email: kurmanbayevakz@gmail.com

**Abstract:** *Hedysarum* L. is one of the large genus of the *Fabaceae* Lindl. family, consisting of 200 species. Species in the genus *Hedysarum* are valuable forage and medicinal resources. In this study, we studied *Hedysarum theinum* Krasnob and *Hedysarum neglectum* Ledeb growing in the western Altai of Eastern Kazakhstan. *H. theinum* is a perennial, herbaceous, valuable medicinal plant, a rare species, an Altai-Dzungarian endemic, and a globally endangered species. *H. neglectum* is a perennial plant of high medicinal and fodder value, growing mainly in the sub-alpine zone. The purpose of the work is to identify the anatomical features and determine the phytochemical composition of the components of two species of *Hedysarum*. Route-reconnoitering methods were used in a field search of two species of *Hedysarum* for comparative study in 2020-2021. The extracts were analyzed by gas chromatography with mass spectrometric detection. The location of biologically active substances in the anatomical structure of the stem has been established. Biologically active substances are concentrated around the conductive bundles of the leaf and stem. The comparative anatomical structure of the stem in *H. theinum* revealed a thick ring of sclerenchyma under the cambium, which is absent in *H. neglectum*. In determining the phytochemical compounds of the aboveground organs of two *Hedysarum* species, the total number of components was 74, of which 55 compounds were found in *H. theinum* and 57 in *H. neglectum*. In both species, 37 components were detected, in *H. neglectum* 11 components had significant content, in *H. theinum* 9 components contained a significant percentage of compounds. Analysis of the root extract showed that *H. theinum* contains 27 and *H. neglectum* contains 29 compounds. Of these, 15 compounds are characteristic of both species. The phytochemical data obtained confirm the anti-inflammatory, antibacterial, antifungal, antimicrobial, antimalarial, and antitumor properties of these plants.

**Keywords:** Anatomy, *Hedysarum theinum*, *Hedysarum neglectum*, Morphology, Phytochemistry

## Introduction

The family *Fabaceae* consists of about 20000 species belonging to some 751 genera. *Hedysarum* L. is the second largest genus in the tribe Hedysareae (*Fabaceae* Lindl.). The flora of Kazakhstan has 38 species of the genus *Hedysarum*. One important species of the genus *Hedysarum* is *Hedysarum theinum* Krasnob. And *Hedysarum neglectum* Ledeb., which grows in Kazakhstan. The species is vegetatively immobile and reproduces only by seeds. *H. theinum* is resistant to disease and has a fairly high seed production. The introduction assessment indicates a high plasticity and a high degree of adaptation of the species (Zinner *et al*., 2021). However, in the Republic of Altai and Northern Kazakhstan, average seed production is noted, which is sufficient for the regular renewal of populations and for maintaining the stability of their age structure (Karnaukhova *et al*., 2021). Based on the analysis, the relationship between morphological variability of productivity traits and genetic variability of electrophoretic polypeptide spectra of seeds of valuable medicinal species *H. theinum* (Erst *et al*., 2014).

The adaptation of the species *H. theinum* by anatomical features of leaf blade structure to high-gradient habitats of

South Siberia was traced; it was revealed that heliomorphic features are intensified (Karnaukhova *et al*., 2021; Karnaukhova, 2016). *H. theinum* is protected and included in the red data book of the Altai Republic as a rare or declining species (Artemov, 2018).

Representatives of the genus *Hedysarum* are promising medicinal and high-protein fodder plants with high trypsin-inhibiting activity in leaves. Trypsin inhibitory activity is one of the immunity factors in plants that ensures the presence of general non-specific systemic resistance. In leaves of *H. theinum* values of trypsin-inhibitory activity reaches a maximum only in favorable conditions during the flowering phase of seasonal development of plants in the Altai Republic (Zhmud *et al*., 2020).

A study of the antidepressant effects of species of the genus *Hedysarum* of *H. theinum* roots, *H. ignorecum* Ledeb. Roots, *H. alpinum* L. herbs showed a pronounced anti-anxiety effect of the medicinal plant extracts and a complete absence of a depressive effect. This substantiates the possibility of using these drugs for the prevention and correction of mental disorders associated with aggression, increased levels of anxiety, and cognitive disorders (Fedorova *et al*., 2021).

The use of one of the valuable medicinal plants, *H. theinum* is of particular interest to public health. *Hedysarum* finds widespread use in folk medicine, hardly used in official medicine due to its practically unexplored phytochemical composition. Although, modern medicine recognizes that this plant has medicinal properties for many diseases due to the specific combination of valuable biologically active compounds (Vdovitchenko *et al*., 2007).

*H. theinum*-red root, of the family *Fabaceae* is a mountainous-Altai-Middle Asian-Mongolian endemic, classified as category 2a as a globally declining species. The plant is perennial, with thickened roots, bare stems, stipules large, fused, leaves elliptic, 4-8 paired, corolla pinkish-purple, and seeds ovoid. Plants of the Western Altai vary in size, with stems 45-70 cm high and smaller leaves about 2 cm long and 0.6-1.0 cm wide, while plants of the Southern Altai are twice as long as those of the Western Altai, with a stem thickness of 1 cm and 11-13 nodes and larger leaves, 4 cm long and 1 cm wide.

*H. neglectum* is a forage, medicinal plant, which is found in the Kazakhstan Altai (Sazanova *et al*., 2017). *H. neglectum* in its ground part contains Sugar, vitamins and provitamins, and tannins; the underground part of the plant contains such compounds as alkaloids, isoflavonoids, saponins, tannins, etc., (Nekratova and Shilova, 2015).

Outside Kazakhstan, *H. theinum* is distributed in the Russian part of the Western and Central Altai and in the mountain ranges of western Mongolia. It belongs to the mesopsychrophyte ecological group. Occurs in humid conditions of mountain systems, the altitude of which reaches 1700-2100 m above sea level. It prefers moist, humus-rich soils (Kubentayev *et al*., 2019).

*H. theinum* is also very similar to *H. neglectum*, with which it is often mixed, thus differing in structure and chemical composition of the root. It has shorter and denser multifarious inflorescences and is also distinguished by shorter pedicels, longer bracts, bracts reaching the apex of the calyx teeth, filiform teeth of the larger calyx, larger flowers with a pod (Kubentayev *et al*., 2018). *H. theinum* is a relic of the glacial epoch, isolated from the older boreal forest *H. alpinum*. Within Kazakhstan, it occurs in the Southwestern and Southern Altai ranges, Southwestern Altai: I vanovsky (Orazov *et al*., 2022).

The purpose of the work is to identify the anatomical features and determine the phytochemical composition of the components of two species of *Hedysarum*.

## Materials and Methods

Samples of plants of *H. theinum* and *H. neglectum* of *the Fabaceae* family were collected from natural populations of Kazakhstan Altai in natural habitats in the territory of Western Altai, in the valley of Big Poperechka River, Ivanovo ridge (Fig. 1), during the period from May 2020 to August 2021. In order to study the current state of natural habitats in 2020-2021, itinerary and reconnaissance field surveys were conducted across the Western Altai in Kazakhstan Altai, forming a number of small ridges, depressions, and river valleys. The 15 plant samples were collected for anatomical examination and recorded, and 15 samples were collected for phytochemical analysis, dried, and crushed, Table 1 Presented GPS coordinates of two *Hedysarum* species.


**Fig. 1:** Point of collection of *Hedysarum*, *A-Hedysarum theinum, B-Hedysarum neglectum*

**Table 1:** Information on the material collected

| Name of species | Coordinates [°] longitude | Latitude | Height above sea level | Gathering point |
|---|---|---|---|---|
| *Hedysarum theinum* Krasnob | 50° 20' 10" N | 83°53'14" E | 1470 | East Kazakhstan region, vicinity of Ridder town, Ivanovsky ridge, valley of the Big Poperechka River |
| *Hedysarum theinum* Krasnob | 50°19' 59" N | 83°52'44" E | 1812 | East Kazakhstan region, vicinity of Ridder town, Ivanov ridge, on a rocky mountainside |
| *Hedysarum neglectum* Lindl | 50°18' 50" N | 83°52'42" E | 1938 | East Kazakhstan region, vicinity of Ridder town, Ivanov ridge, on a motley grassy mountainside |

Studies were conducted using semi-stationary methods (Childibayeva *et al*., 2022). Under field conditions, we described the identified populations with *H. theinum* and *H. neglectum,* established floristic composition (Kubentayev *et al*., 2021), abundance according to Drude (Sumbembayev *et al*., 2022), collected herbarium and collected samples of above- and below-ground plants for further anatomical and phytochemical. Latin names of plants are given according to POWO (2019), and the phylogenetic plant classification system is indicated according to Takhtajan (2009).

Raw material samples for the microscopic study were freshly fixed in Strasburger-Fleming's solution (96% ethyl alcohol: Glycerol: Water in a 1:1:1 ratio) (Dyuskalieva *et al*., 2014; Yazid *et al*., 2020). Temporary preparations were prepared from transverse sections, measured, and followed by microphotography with an inverted microscope MX 700, Austria. Anatomical sections of the samples of *Hedysarum theinum* and *H. neglectum* were made using the microtome MZP-01 "Technom". The thickness of the anatomical sections was 10-15 µm. Temporary preparations were enclosed in glycerin. Microphotographs of the anatomical sections were taken on an MX 700 microscope with a CAM V400/1.3M video camera. Measurements of micro preparations were carried out on a aMCX100 microscope with a 519CU 5.0M CMOS Camera.

Statistical processing of research materials was performed according to the method of G.F. Lakin (Izbastina *et al*., 2020). Phytochemical analysis of two *Hedysarum* species.

Elemental analysis of the above-ground and underground parts of plants was performed by gas chromatography with mass spectrometric detection (Agilent 7890A/5975C). Quantification of organic substances was carried out by hydrodistillation followed by volume weight analysis of the product (Özek *et al*., 2021; Bauyrzhan *et al*., 2022), also using EXCEL-2010 statistical software package.

The dynamics of the accumulation of biologically active substances is important for any medicinal plant since it allows us to estimate the optimal time for harvesting raw materials. During expedition research samples of above-ground and underground organs of *H. theinum* and *H. neglectum* were taken, after which phytochemical analysis was carried out.

For the phytochemical analysis, dry parts of *Hedysarum* (above-ground part and root) were ground as a powder in a thresher. Weighed 20 mg of dry powder and prepared an extract for analysis.

Determination of organic compounds in the $CO_2$ extract.

Sample preparation and analysis methods: 4 extracts were taken and analyzed by gas chromatography with mass spectrometric detection (7890A/5975C).

Analysis conditions: Sample volume 1.0 µL, sample entry temperature 250°C, no flow splitting. Separation was carried out using a DB-35MS chromatographic capillary column with a length of 30 m, an inner diameter of 0.25 mm, and a film thickness of 0.25 µm at a constant carrier gas speed (helium) of 1 mL/min. The chromatography temperature was programmed from 40 °C (0 min exposure time) at a heating rate of 5°C/min to 150°C (3 min exposure time) followed by a heating rate of 5°C/min to 280°C (1 min exposure time). Detection was performed in SCAN mode m/z 34-850. Agilent MSD ChemStation software (version 1701EA) was used to control the gas chromatography system, and record and process the results and data. Data processing included the determination of retention times, peak areas as well as the processing of the spectral information obtained with a mass spectrometric detector. Wiley $7^{th}$ edition and NIST'02 libraries were used to decipher the obtained mass spectra (the total number of spectra in the libraries is more than 550 thousand).

## Results and Discussion

The study of morphological parameters of *H. theinum* and *H. neglectum* species allowed us to reveal their adaptation to growing conditions, there are some morphological, physiological, and biochemical features, even for closely related species of the genus *Hedysarum* growing in certain soil and climatic conditions.

The characteristic structure of *H. theinum*: Greenish stems, plant height averages 60-70 cm, the species is similar to *H. neglectum* in the coloring of flowers, but differs in height, *H. theinum* is 30-40 cm higher, also in structure and size of leaves, when cutting the root of *H. theinum* is brown-red, with a pleasant smell, while *H. neglectum* is white, but odorless.

Life forms in the community are represented by shrubs, bushes, semi-shrubs, and herbaceous forms. The distribution of plants vertically occurs in 2 tiers, (Figs. 2-3).

**DOI: 10.3844/ojbsci.2023.323.335**

**Fig. 2:** Coenopopulation of *H. theinum*

**Fig. 3:** Coenopopulation of *H. neglectum*

Adil Kusmangazinov *et al*. / OnLine Journal of Biological Sciences 2023, 23 (3): 323.335
**DOI: 10.3844/ojbsci.2023.323.335**

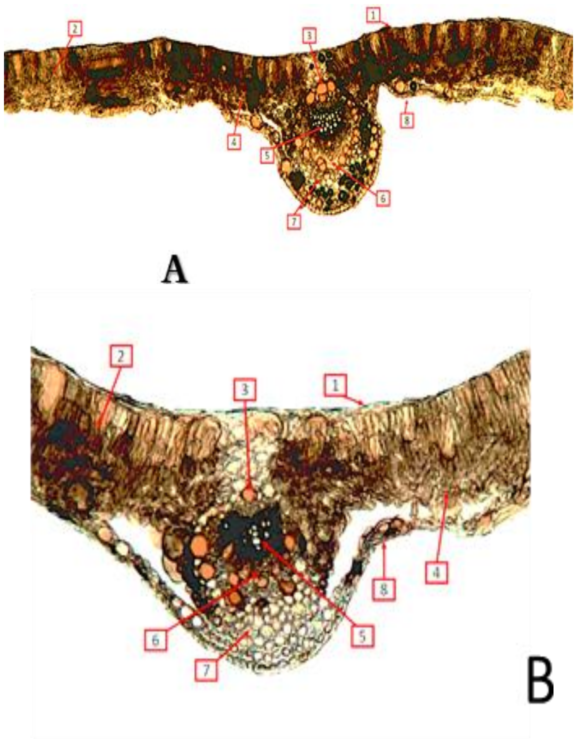



**Fig. 4:** Cross-section of the leaves

Evaluation of anatomical parameters *H. theinum* and *H. neglectum*. Anatomical research allows a deeper understanding of the plant under the influence of various factors and in the combination of external and internal structures. The significance of studying the morphogenetic patterns of leaf evolution considers that the adaptation of plants to different environmental conditions is often associated with changes in leaf structure occurring at the morphological and anatomical levels. The study of the leaf anatomical structure as the most plastic organ of plants deserves great attention since the peculiarities of the structure of different leaf tissues allow both tracing conservative structures and revealing adaptive traits that have evolved in plants during adaptation to modern conditions.

The anatomical structure of the leaf of *H. theinum.* The leaf is covered on both sides by the epidermis. The cells of the upper epidermis are cutinized, consisting of densely compressed cells, rounded in shape. Beneath the upper epidermis, barrel-shaped idioblasts and multilobate cells are revealed closer to the lower epidermis. The mesophyll is divided into columnar and spongy. The palisade parenchyma is located below the upper epidermis, and the cells of the spongy mesophyll are located above the lower epidermis. The middle vein is strongly convex downwards, the main conductive bundle consists of the xylem and phloem, and the bundle is collaterally closed. Numerous inclusion cells with tannins are found in the bundle lining and above the lower epidermis, (Fig. 4A) Table 2.

A-Anatomical structure of the leaf blade of *Hedysarum theinum*. 1-Upper epidermis; 2- Columnar mesophyll; 3-Inclusions; 4-Spongy mesophyll; 5- Xylem; 6-Phloem; 7-Parenchyma; 8-Lower epidermis. B-Anatomical structure of the leaf blade of *Hedysarum neglectum,* 1-Upper epidermis; 2-Columnar mesophyll; 3-Inclusions; 4-Spongy mesophyll; 5-Xylem; 6-Phloem; 7-Parenchyma; 8-Lower epidermis.

The anatomical structure of the leaf of *H. neglectum.* Leaf dorsoventrally, palisade parenchyma located beneath the upper epidermis, spongy parenchyma located under palisade parenchyma, (Fig. 4B), Table 2. The leaf is covered on both sides with cells of the covering tissue by the epidermis, the cells of the upper epidermis differ in their smaller size from those of the lower epidermis, the lower epidermis shows large, colorless cells, presumably motor cells. Epidermal cells on both sides are strongly cutinized, consisting of small, tightly packed, colorless cells. Columnar mesophyll consists of two rows of palisade parenchyma, cells elongated rectangular, tightly adjoining each other. The cells of the spongy mesophyll are more loosely connected, with large intercellular spaces. The main vein contains a large conductive bundle, consisting of a top-directed xylem and a down-directed phloem. The bundle lining is clearly visible, the cells having inclusions, in the form of tannins. Most of the tannins accumulated in the lining cells surrounding the conductive bundles. Above the conductive bundle, colorless cells of the parenchyma, which divides the columnar mesophyll and is located below the upper epidermis, are clearly visible. Sclerenchyma is well developed beneath the phloem, which adjoins the lower epidermis.

In comparing the leaves of the two species, the changes were reflected in the transition from the typically dorsiventral leaf structure of *H. theinum* to the isolateral-palisade mesophyll structure of *H. neglectum*. In both species, barrel-shaped idioblasts and multilobed cells were found.

A-Anatomical structure of the stem of *Hedysarum theinum.* 1-Epidermis; 2-Primary cortical parenchyma; 3-Collenchyma; 4-Fibers; 5-Inclusions; 6-Phloem; 7-Cambium; 8-Sclerenchyma; 9-Xylem; 10-Pith. B-Anatomical structure of the stem of *Hedysarum neglectum.* 1-Epidermis; 2-Primary cortex parenchyma; 3-Collenchyma; 4-Bast fibres; 5-Inclusions; 6-Phloem; 7-Bundle cambium; 8-Xylem; 9-Parenchyma; 10-Cavity.

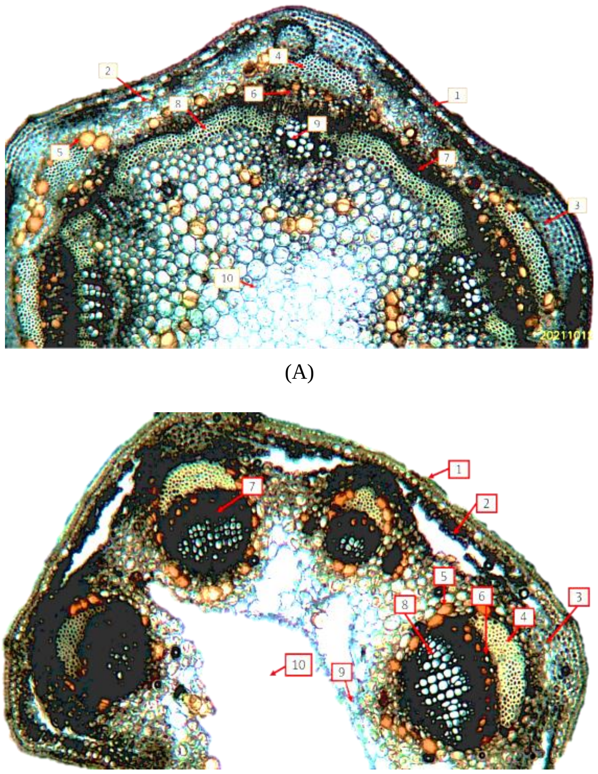
The anatomical structure of the stem of *H. theinum.* The stem is covered with epidermal cells. The epidermal cells are strongly cutinized. Parenchyma, chlorenchyma, collenchyma, and sclerenchyma cells are found in the primary cortex. Above the central cylinder, there are multifarious cells with inclusions exclusively of tannins. In the central cylinder, the conductive bundles are arranged in a circle, bast fibers are well-developed above the phloem and a continuous layer of cambium can be clearly distinguished between the xylem and phloem. The main difference in the stem of H. theinum is that there is a continuous sclerenchymal ring, which is underneath the cambium layer, (Fig. 5A). The continuous sclerenchyma ring consists of numerous rows of sclerenchyma cells, i.e., it forms a thick ring. In the center of the stem are the medullary cells, which consist of thin-walled, transparent cells, they are multifaceted and rounded.

**Table 2:** Morphometric data of the leaf blade of the two species *Hedysarum*

| Species | Thickness of the upper epidermis, μm | Thickness of the lower epidermis, μm | The thickness of the mesophyll, μm | | Length of vascular bundle, μm | |
|---|---|---|---|---|---|---|
| | | | Columnar | Spongy | Xylem | Phloem |
| *Hedysarum theinum* | 23.68±4.3 | 28.59±1.5 | 144.19±2.3 | 120.14±9.6 | 78.85±11.7 | 51.64±18.9 |
| *Hedysarum neglectum* | 16.48±3.4 | 18.57±0.1 | 127.40±6.5 | 131.20±8.7 | 62.33±23.3 | 49.27±15.9 |
| Average value | 20,08 | 23,58 | 135,80 | 125,67 | 70,59 | 50,45 |
| Standard deviation | 5,09 | 7,09 | 11,87 | 7,82 | 11,68 | 1,68 |
| Coefficient of variability | 0,25 | 0,30 | 0,09 | 0,06 | 0,17 | 0,03 |

**Table 3:** Morphometric data of the stem of the two species *Hedysarum*

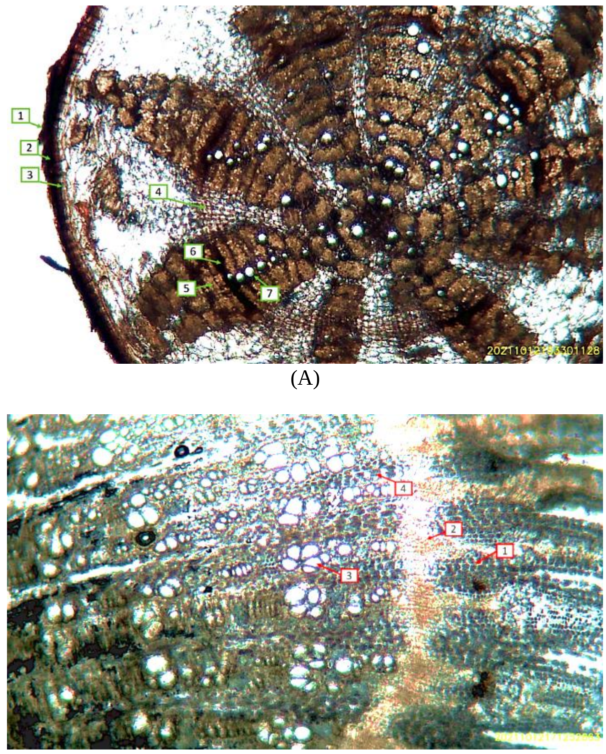
| Species | The thickness Stem, μm | of the cuticle, μm | Thickness of epidermis, μm | Length of vascular bundle, μm | | The thickness of the bast fibre, μm |
|---|---|---|---|---|---|---|
| | | | | Xylem | Phloem | |
| *Hedysarum theinum* | 650.68±14.3 | 19.59±1.5 | 27.37±1.1 | 107.55±12.5 | 38.85±18.9 | 48.59±0.94 |
| *Hedysarum neglectum* | 587.48±13.4 | 17.57±0.17 | 22.87±3.5 | 95.33±13.3 | 29.71±10.7 | 54.15±0.30 |
| Average value | 619,08 | 18,58 | 25,12 | 101,44 | 34,28 | 51,37 |
| Standard deviation | 44,69 | 1,43 | 3,18 | 8,64 | 6,46 | 3,93 |
| Coefficient of variability | 0,07 | 0,08 | 0,13 | 0,09 | 0,19 | 0,08 |



(A)

(B)

**Fig. 5:** Cross-section of the stem



(A)

(B)

**Fig. 6:** Cross-section of the root

The anatomical structure of the stem of *H. neglectum*. A cross-section of the stem is covered with the epidermis, whose cells are strongly cuticular, consisting of small, rounded cells. The central cylinder occupies more space than the primary cortex. The fascicles are circular, collaterally open with the cambium, the cambium being conspicuously wispy only. Above the phloem, the bast fibers are well-developed. Xylem consists of numerous rows of vessels. Tannins are found mainly in the bundle wrappers and in the phloem. Pith collapsed, stem hollow, (Fig. 5B), Table 3.

A-Anatomical structure of the root of *Hedysarum theinum*. 1-Phellema; 2-Phellogen; 3-Phelloderm; 4-Ray; 5-Phloem; 6-Cambium; 7-Xylem. B-Anatomical structure of the root of *Hedysarum neglectum*. 1-Phloem; 2-Cambium; 3-Xylem. 4-Ray.

Figure 6 shows the anatomical structure of the root of *H. theinum*. Unlike the annually renewed above-ground organs, the root grows annually and as a result, changes its anatomical structure. The root in the cross-section is covered by the periderm of the secondary covering tissue, which is formed from the phylogenic formation tissue.

The plant is perennial, in this respect, the primary bark is destroyed and the secondary bark is formed. In the cortex zone, the pith or parenchyma rays are well expressed. The central cylinder contains the xylem and medullary rays. The cambium is between the xylem and phloem. The sclerenchyma is well developed. There are xylem vessels in the center.

Figure 6 shows the anatomical structure of the root of *H. neglectum*. The root on the section is covered by the periderm of the secondary covering tissue, which consists of living phellogen cells, phelloderm, and dead phelloderm cells. The secondary cortex is expressed beneath the periderm and there are xylem and medullary rays in the central cylinder. The cambium is between the xylem and phloem. The sclerenchyma is well developed. There are large xylem vessels in the center. Large 3-5 xylem vessels are concentrated and alternate with smaller xylem vessels. Root morphometric data of two *Hedysarum* species showed that periderm thickness was *H. theinum* 43.54±13.3 µm, *H. neglectum* 51.41±18.4 µm, root diameter in *H. theinum* 680.68±15.3 µm, *H. neglectum* 697.48±21.7 µm. Cambium thickness in *H. theinum* 86.59±10.5, in *H. neglectum* 83.57±9.17.

Although a systematic study of the chemical components of *Hedysarum* has been carried out to provide taxonomic evidence for the genus and to support the pharmacological uses of several species within the genus, little data is available on the chemical components of *H. theinum* and *H. neglectum*. Therefore, we present the results of a detailed chemical analysis of extracts from the underground and above-ground parts of the two species from the Kazakhstan Altai Mountains. The results can be used for a variety of human and animal health and nutritional applications. The analysis from the root extract of the two species showed that *H. theinum* has 29 compounds out of a total of 40 components. Although only 15 compounds are peculiar to both species. In *H. neglectum*, the methylpyruvate is six times greater than in *H. theinum*. Only *H. theinum* detected levoglucosenone which is a highly functionalized chiral compound, levoglucosenone is used as a building block in organic synthesis to produce a wide range of natural and non-natural compounds. The lactone y-oxybutyric acid (GHB) depressant used as a psychoactive agent predominates in *H. neglectum*, also this plant has almost twice as many disaccharides consisting of two monosaccharides: Glucose and fructose than in *H. theinum*. In *H. theinum* the phenol butanone is detected, which has a sweet, berry, and floral taste, butanone is undetectable in *H. neglectum*. Also, in *H. theinum* the glycoside ethyl α-d-glucoside is present in a multiplicative amount of 45.23%, and a negligible content of only 0.8% is detected in *H. neglectum*. In *H. theinum*, the highest quantity of vitamin E was found in 11.36% and the plant steroid gamma-sitosterol in 9.2%, but these components were absent in *H. neglectum*. Phytol, an acyclic chemical compound whose basis is made up of isoprene residues, is also a part of *H. theinum*, in the other species that have not been found. In both species, squalene, which participates in metabolism and in the synthesis of steroids and cholesterol, belonging to the group of carotenoids, was found. A comparative phytochemical analysis of the root of the studied species is shown in Table 4, (Figs. 7-8).

**Table 4:** Percentage of compounds, % of root of two *Hedysarum* species

| No | Compound | *Hedysarum theinum* | *Hedysarum neglectum* |
|---|---|---|---|
| 1 | Propanoic acid, 2-oxo-, methyl ester | 1,01 | 6,5 |
| 2 | Formamide, N-methoxy- | - | 0,6 |
| 3 | Levoglucosenone | 0,49 | - |
| 4 | 2-Furanmethanol | - | 1,0 |
| 5 | Methane, trimethoxy- | 0,19 | - |
| 6 | 2-Propanol, 1,1-dimethoxy- | - | 1,3 |
| 7 | 2-Cyclopenten-1-one, 2-hydroxy- | 0,26 | - |
| 8 | 1,2-Cyclopentanedione | - | 2,1 |
| 9 | 2-Hydroxy-gamma-butyrolactone | 0,23 | 6,0 |
| 10 | 2,5-Dimethyl-4-hydroxy-3(2H)-furanone | 0,22 | - |
| 11 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 0,57 | 1,7 |
| 12 | 2-Propanone, 1-(acetyloxy)- | - | 0,8 |
| 13 | Cyclopropyl carbinol | 0,24 | 1,4 |
| 14 | Benzeneethanol, 4-hydroxy- | 0,54 | - |
| 15 | 4-Cyclopentene-1,3-dione | - | 1,3 |
| 16 | Sucrose | 5,29 | 9,1 |
| 17 | 2-Butanone, 4-(4-hydroxyphenyl)- | 3,07 | - |
| 18 | Octadecanoic acid | 1,50 | - |
| 19 | Ethyl α-d-glucopyranoside | 45,23 | 0,8 |
| 20 | Benzenepropanol, 4-hydroxy-α-methyl-, (R)- | 1,95 | - |
| 21 | 3-O-Methyl-d-glucose | 2,88 | 4,5 |
| 22 | 2-Methoxy-4-vinylphenol | - | 1,4 |
| 23 | Hexadecanoic acid, ethyl ester | 1,17 | 4,5 |
| 24 | Dibutyl phthalate | 5,26 | 9,4 |
| 25 | 1-Hexadecanol | - | 6,0 |
| 26 | Ethyl Oleate | 0,20 | 1,3 |
| 27 | 2-(3,4-Dimethoxyphenyl)-6-methyl-3,4-chromanediol | - | 0,6 |

**Table 4:** Continue

| | | | |
|---|---|---|---|
| 28 | 9,12-Octadecadienoic acid, ethyl ester | 1,55 | 14,2 |
| 29 | 9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)- | 1,40 | 14,4 |
| 30 | 5,9-Dodecadien-2-one, 6,10-dimethyl-, (E, E))- | - | 0,3 |
| 31 | 2-Propenoic acid, 3-(3,4,5-trimethoxyphenyl)-, methyl ester | 0,43 | 3,3 |
| 32 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 4,50 | - |
| 33 | S-Indacene-1,7-dione, 2,3,5,6-tetrahydro-3,3,4,5,5,8-hexamethyl- | 0,19 | 1,8 |
| 34 | 2(5H)-Furanone | - | 0,7 |
| 35 | Ethyl tetracosanoate | 0,35 | 1,5 |
| 36 | Squalene | 0,68 | 0,6 |
| 37 | Docosanoic acid, ethyl ester | - | 1,4 |
| 38 | Vitamin E | 11,36 | - |
| 39 | γ-Sitosterol | 9,20 | - |
| 40 | Medicarpin | - | 1,7 |

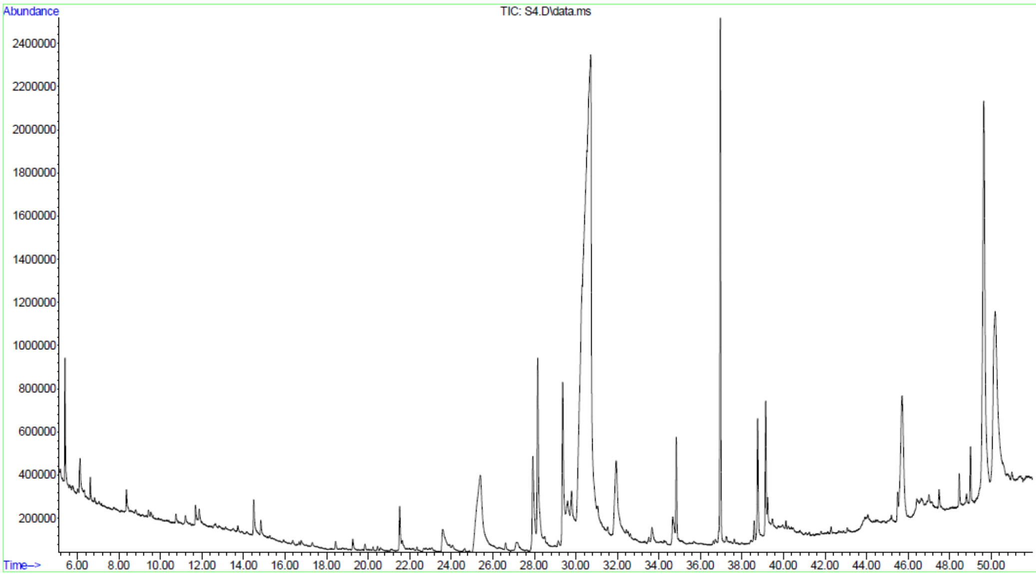



**Fig. 7:** Chromatogram of root extract of *Hedysarum theinum*

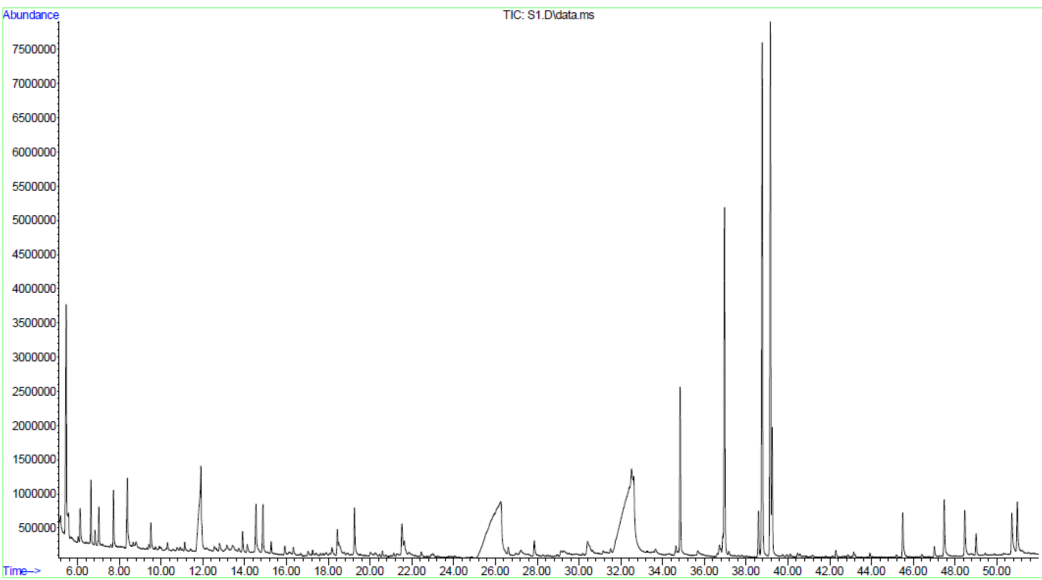



**Fig. 8:** Chromatogram of root extract of the *Hedysarum neglectum*

**Table 5:** Percentage of compounds, % of the above-ground organs of the two *Hedysarum* species

| No. | Compound | *Hedysarum theinum* | *Hedysarum neglectum* |
|---|---|---|---|
| 1. | Propanoic acid, 2-oxo-, methyl ester | 0,87 | 0,95 |
| 2. | Pyrimidine, 2-methyl- | 0,53 | - |
| 3. | N-(2-Methylbutylidene) isobutylami | 0,66 | 0,25 |
| 4. | 9,12-Octadecadienoic acid, ethyl ester | - | 2,33 |
| 5. | 1-Butanamine, 2-methyl-N-(2-methylbutylidene)- | 0,48 | 0,16 |
| 6. | 1-Propanamine, 2-methyl-N-(2-methylpropylidene)- | 0,47 | 0,39 |
| 7. | Ethanol, 2-(9,12-octadecadienyloxy)-, (Z, Z)- | - | 0,29 |
| 8. | Pyrazine, 2,5-dimethyl- | 0,16 | 0,18 |
| 9. | 1-Butanamine, 2-methyl-N-(2-methylbutylidene)- | 0,31 | 0,16 |
| 10. | Hexanoic acid, 2-tetrahydrofurylmethyl ester | 0,45 | 0,32 |
| 11. | Fumaric acid, ethyl 2-methylallyl ester | - | 0,29 |
| 12. | Methylamine, N-(1-butylpentylidene)- | 0,24 | - |
| 13. | Butanoic acid, 4-hydroxy- | 0,17 | 0,28 |
| 14. | Heptacosane | - | 1,19 |
| 15. | N-Butyl-tert-butylamine | 0,22 | - |
| 16. | Butyl 9,12,15-octadecatrienoate | - | 0,29 |
| 17. | 2-Hydroxy-gamma-butyrolactone | 0,14 | 0,21 |
| 18. | cis-p-mentha-1(7),8-dien-2-ol | 0,33 | - |
| 19. | Diisooctyl phthalate | - | 0,22 |
| 20. | Cyclohexanol, 2,6-dimethyl- | 0,17 | - |
| 21. | Phytol, acetate | - | 0,46 |
| 22. | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 0,31 | 0,66 |
| 23. | Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)- | - | 0,69 |
| 24. | 1-Butanol, 3-methyl-, acetate | 1,68 | - |
| 25. | 1-Propanone, 1-phenyl- | 0,27 | 0,22 |
| 26. | Benzofuran, 2,3-dihydro- | 0,30 | 0,32 |
| 27. | Acetamide, 2-(5-ethyl-4H-1,2,4-triazol-3-ylthio)-N-(5-methyl-3-isoxazolyl)- | - | 0,51 |
| 28. | 1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl- | 0,45 | 0,30 |
| 29. | 1H-Pyrrole-2,5-dione, 3-ethenyl-4-methyl- | 0,16 | - |
| 30. | 2-Methoxy-4-vinylphenol | 0,25 | 0,36 |
| 31. | 2-Ethylbutyric acid, eicosyl ester | 0,26 | - |
| 32. | N-Phenethyl-2-methylbutylidenimine | 0,19 | - |
| 33. | Benzene, (1-nitroethyl)- | 0,46 | - |
| 34. | 2-Piperidinemethanamine | - | 0,17 |
| 35. | (Hexahydropyrrolizin-3-ylidene)-acetaldehyde | 0,20 | 0,22 |
| 36. | trans-β-Ionone | 0,53 | 0,42 |
| 37. | 3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0] hept-1-yl)- | 0,42 | 0,38 |
| 38. | Nanofin | - | 0,14 |
| 39. | 1,3-Benzenediol, 5-pentyl- | 0,23 | 0,36 |
| 40. | Sucrose | 1,85 | 4,10 |
| 41. | 2,5-Dimethyl-4-hydroxy-3(2H)-furanone | - | 0,29 |
| 42. | 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- | 1,47 | 1,40 |
| 43. | 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- | 0,33 | - |
| 44. | Pyrazine, methyl- | - | 0,28 |
| 45. | 3',5'-Dimethoxyacetophenone | 0,23 | 0,53 |
| 46. | 3-Methyl-4-phenyl-1H-pyrrole | 0,51 | 0,54 |
| 47. | Benzeneacetaldehyde | - | 0,17 |
| 48. | 4-Hydroxy-β-ionone | 0,31 | - |
| 49. | 2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]]- | 0,53 | 4,44 |
| 50. | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 6,05 | 4,09 |
| 51. | α-D-Glucopyranoside, methyl | 5,81 | - |
| 52. | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 2,90 | 4,09 |
| 53. | 1,2-Cyclopentanedione | - | 0,24 |
| 54. | 2-Pentadecanone, 6,10,14-trimethyl- | 0,58 | 0,80 |
| 55. | Hexadecanoic acid, ethyl ester | 6,61 | 12,21 |
| 56. | 1-(1'-pyrrolidinyl)-2-propanone | - | 0,26 |
| 57. | 9,10-Dimethyltricyclo [4.2.1.1(2,5)]decane-9,10-diol | 22,49 | 15,61 |
| 58. | 4-Cyclopentene-1,3-dione | - | 0,14 |
| 59. | Dibutyl phthalate | 8,75 | 10,50 |
| 60. | Oxazolidine, 2,2-diethyl-3-methyl- | - | 0,21 |
| 61. | Phytol | 15,28 | 13,96 |
| 62. | Ethyl Oleate | 0,28 | 0,44 |
| 63. | Octadecanoic acid, ethyl ester | 1,52 | - |
| 64. | 9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)- | 2,54 | 4,20 |
| 65. | 9,12,15-Octadecatrienoic acid, (Z, Z, Z)- | 1,46 | - |
| 66. | Retinol, acetate | 0,39 | 0,37 |
| 67. | 4,8,12,16-Tetramethylheptadecan-4-olide | 0,22 | 0,26 |
| 68. | Phthalic acid, di(2-propylpentyl) ester | 0,30 | - |
| 69. | Phytol, acetate | 0,33 | 9,27 |
| 70. | Squalene | 0,61 | 1,00 |
| 71. | Vitamin E | 5,47 | 0,37 |
| 72. | 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol | 0,51 | - |
| 73. | Tetratetracontane | 0,57 | - |
| 74. | 1-Butanol, 3-methyl-, formate | - | 1,80 |

In the above-ground (stem, leaf, fruit) organs of the two *Hedysarum* species studied the total number of components was 74, of which 55 compounds are found in *H. theinum* and 57 in *H. neglectum*. In both species, 37 components were found. in *H. neglectum* 11 components have significant contents, in *H. theinum* 9 components contain significant percentages, the highest 22.49% correspond to monoterpenes (9,10-dimethyltricyclo (4. 2.1.1 (2.5)) decane-9,10-diol) and 15.61% in *H. neglectum*. in both the species the highest phytol content 13.96-15.28, Table 5, (Figs 9-10).

Detected and identified: A group of phytosteroids, a number of aromatic and heterocyclic compounds, and vitamins. For example, retinyl acetate is a natural form of vitamin A, which is the acetate ester of retinol. It has potential antitumor and chemopreventive activity and the antioxidant properties of Vitamin E are also due to the ability of the mobile hydroxyl of the chromane core of its molecule to interact directly with free oxygen radicals and free radicals of unsaturated fatty acids. A comparison of the phytochemical composition of the two studied species reveals significant differences in quantity and quality. The data obtained can be used for the species identification of *H. theinum* and *H. neglectum*, which has so far been difficult due to the lack of clear systematic morphological characters.

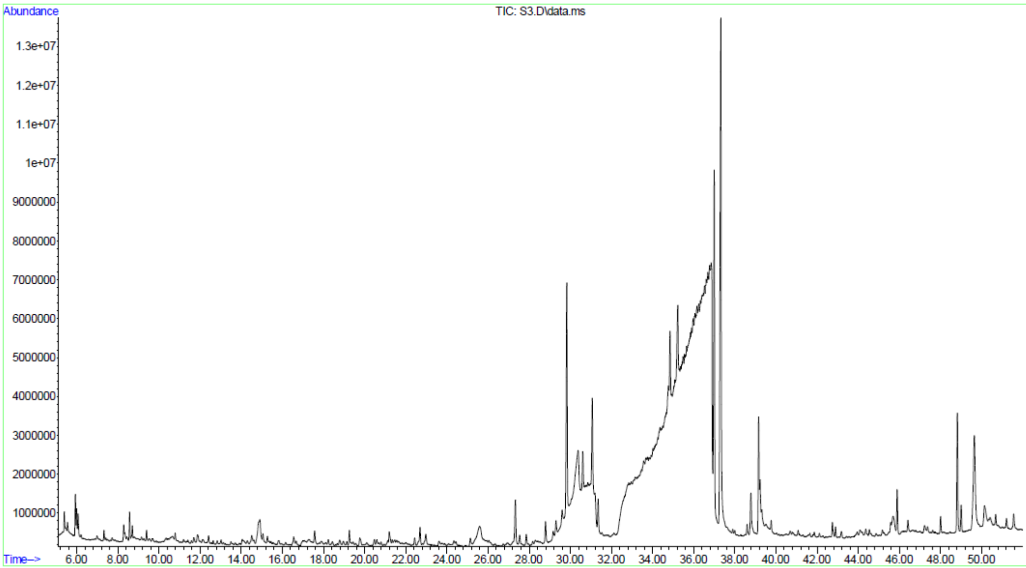



**Fig. 9:** Chromatogram of the extract of the aboveground parts of *Hedysarum theinum*

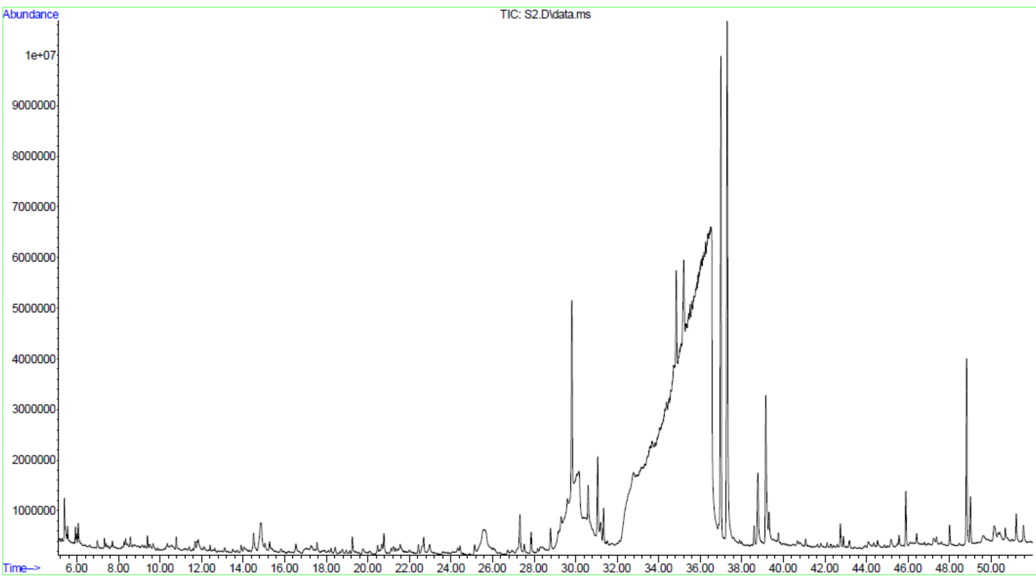



**Fig. 10:** Chromatogram of the extract of the aboveground parts of the *Hedysarum neglectum*

In Kazakhstan, the genus *Hedysarum* consists of 38 species, without a description of *H. theinum*, as the species *H. theinum* was described later in 1985 by Krasnoborov, so it is not included in the flora of Kazakhstan published in 1966. Thus, the total number of Kazakhstan species of *Hedysarum* is about 20% of the total genus. *H. theinum* is endemic to the Altai Mountains of Kazakhstan. According to the last classification, the species is a ubiquitous shrinking species, belongs to category-2 vulnerable, declining ranges at risk of extinction due to human activities, and ranks narrowly local endemics or sub-endemics of the Altai. The priority of studying, preservation, and rational use of plant biodiversity is a current trend in our country. In Kazakhstan, during the years of independence, several strategies related to the problems of ecology and environmental protection have been developed. At the present time, there are widely presented research concerning the complex study of individual species of promising plants. Especially widely represented are directions related to the study of phytochemical composition. The results obtained contribute to the development and improvement of the method of phytochemical analysis of plant raw materials *H. theinum* collected from natural populations of Eastern Kazakhstan. According to Kotukhov's study, the stem height of *H. theinum* reaches up to 140 cm and 0,7 cm thickness with 12-14 knots and large leaves, 5 cm length and up to 1,3 cm width. Western Altai plants are smaller in size: 45-70 cm tall and with smaller leaves: About 2 cm long and 0.6-1.0 cm wide.

*H. theinum* is similar to *H. neglectum*, with which it is often mixed and from which it differs in root structure and chemical composition, short, dense multifarious inflorescences. Also with shorter pedicels, longer bracts, bracteoles reaching the apex of calyx teeth, filiform teeth of the larger calyx, larger flowers with a boat-like flower rounded on the lower front margin, and fruit with a broad margin. The experimental data obtained on the general phytochemical screening of the studied phytopreparations showed that the key type of biologically active substances is condensed tannins. The presence of other phenolic compounds: Xanthones, and flavonoids was also shown. The phytochemistry of the roots of *H. theinum* and *H. neglectum* have been shown to have only minor concentrations of the xanthone mangiferin. Phytosteroids, derivatives of pyran, pyridazine, morphine, phthalic acid, azulene, porphyrin, and its analogs and some other compounds were found in plants of genus *Hedysarum* for the first time. The key group of biologically active substances of *H. theinum* and *H. neglectum* condensed tannins was determined.

The reasons for the rarity of *H. theinum* are related to biotic and anthropogenic factors. Among the biotic factors, the leading one is the ecological factor, while the main anthropogenic factor is the collection of *H. theinum* root by local inhabitants for traditional medicine. Therefore, there are currently recommendations to include the species in the red data book.

## Conclusion

In the anatomical structure of the stem, *H. theinum* has a thick sclerenchymal ring under the cambium, which is absent in *H. neglectum*. The leaf thickness of *H. neglectum* is greater than that of *H.theinum*. Although the leaf length of *H. theinum* is longer than that of *H. neglectum*.

Phytosteroids, a number of aromatic and heterocyclic compounds, vitamins, and a number of other compounds have been found in plants of the genus *Hedysarum*. The key group of biologically active substances of *H. theinum* and *H. neglectum* was determined. The most important differences in chemical composition (fatty acids, oxycinnamic acids, flavonoids, etc.) which allow species identification of *H. theinum* and *H. neglectum* have been established. Flavonoids are natural coloring agents, food antioxidants, tannins, they have antimicrobial action.

The work revealed that the above-ground organs of *H. theinum* accumulates trace amounts of phytol 15.28%, dimethyltricyclodecanediol-22.49%, vitamin E-5.47%, compared with *H. neglectum* shows that the plant is medicinal, has anti-inflammatory, antithrombotic and vasodilating properties. Accordingly, the presence of vitamin E in *H. neglectum* is only 0.37%.

The underground part of *H. theinum*, that is, the root, contains the highest amount of ethyl α-d glucopyranoside -45.23%, which in *H. neglectum* is only 0.8%. An identification probability of 90-92% showed in the roots that *H. theinum* contains 11.36% tocopherol, which is not found in the other species. There is also squalene, which inhibits the growth of cancer cells, removes heavy metals, mitigates the side effects of radio-chemotherapy, prevents diabetes, has antioxidant properties, normalizes blood pressure, reduces the risk of heart attacks and strokes, and strengthens the immune system. In this study, the results showed that *H. theinum* is indeed a very valuable medicinal plant that should be cherished and multiplied for the production of dietary supplements and medicines.

## Acknowledgment

Acknowledgments to the committee on science of the ministry of science and higher education of the Republic of Kazakhstan for their support in conducting and financing this research.

## Funding Information

This research was carried out and funded by the Committee on Science of the ministry of science and higher education of the Republic of Kazakhstan, grant AP09561631 "Research of botanical and molecular genetic features of *Hedysarum theinum* Krasnob. (*Fabaceae* Lindl.) for the conservation of biodiversity".

## Author's Contributions

**Adil Kusmangazinov:** Conceptualization, written originally drafted preparation.

**Meruyert Sakenovna Kurmanbayeva:** Written reviewed and edited, supervision, project administration.

**Irina Zharkova:** Visualization and methodology.

**Dina Karabalayeva:** Data management, formal analysis.

**Raushan Kaparbay:** Resource investigation.

**Tursynbek Kaiyrbekov:** Software validation.

All authors read and approved the final manuscript.

## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

## References

Artemov, I. A. (2018). Flora of Belukha Nature Park (Altai Republic). *Vestnik Tomskogo gosudarstvennogo universiteta. Biologiya [Tomsk State University Journal of Biology]*, *42*, 69-101. https://doi.org/10.17223/19988591/42/4

Bauyrzhan, Turalin, Natalya, Kurbatova, Zarina, Inelova, Andrey, Kupriyanov, Meruert, Kurmanbayeva, Karime, Abidkulova, & Aliya, Bazargaliyeva. (2022). Phytochemical study of *Crambe tatarica* Sebeok, a promising source of medicinal plant extracts. *Pakistan Journal of Botany*, *54*(2). https://doi.org/10.30848/PJB2022-2(38)

Childibayeva, A., Ametov, A., Kurbatova, N. V., Akhmetova, A., Tynybekov, B. M., & Mukanova, G. A. (2022). Structural Characteristics of *Rosa Iliensis* Chrshan. Under Conditions of the Floodplains of the Rivers Ili and Sharyn. *Journal of Ecological Engineering*, *23*(1), 296-304. https://doi.org/10.12911/22998993/143943

Dyuskalieva, G. O., Kurmanbayeva, M. S., Kalieva, A. N., Almerekova, S. S., & Kairova, M. Z. (2014). Anatomic-morphological and biochemical characteristics of Patrinia intermedia (Horn.) Roem. et Shult in conditions of the South-East of Kazakhstan. *World Applied Sciences Journal*, *29*(12), 1473-1483. https://doi.org/10.5829/idosi.wasj.2014.29.12.13972

Erst, A. A., Zheleznichenko, T. V., Novikova, T. I., Dorogina, O. V., & Banaev, E. V. (2014). Ecological and geographic variability of *Hedysarum theinum* and features of its propagation *in vitro*. *Contemporary Problems of Ecology*, *7*, 67-71. https://doi.org/10.1134/S1995425514010053

Fedorova, Y. S., Kulpin, P. V., Kotova, T. V., Denisova, S. V., & Beregovykh, G. V. (2021, October). Study of the antidepressant properties of some plants. In *AIP Conference Proceedings* (Vol. *2419*, No. 1, p. 050001). AIP Publishing LLC. https://doi.org/10.1063/5.0069670

Izbastina, K., Kurmanbayeva, M., Bazargaliyeva, A., Ablaikhanova, N., Inelova, Z., Moldakaryzova, A., ... & Turuspekov, Y. (2020). Morphological, anatomical structure and molecular phylogenetics of Anthemis trotzkiana Claus. *Pak. J. Bot*, *52*(3), 935-947. https://doi.org/10.30848/PJB2020-3(39)

Karnaukhova, N. A. (2016). Anatomo-morphological features of the leaves of *Hedysarum theinum* (*Fabaceae*) in Western Altai. *Contemporary Problems of Ecology*, *9*, 349-354. https://doi.org/10.1134/S1995425516030057

Karnaukhova, N. A., Selyutina, I. J., & Syeva, S. Y. (2021). Reproductive biology of *Hedysarum theinum* (*Fabaceae*). *Botanicheskii Zhurnal*, *106*(6), 556-566. http://botjournal.ru/wp-content/uploads/2021/05/106-6-4_Karnaukhova.pdf

Kubentayev, S. A., Kotukhov, Y. A., Danilova, A. N., Suleimenov, A. N., & Sumbembayev, A. A. (2019). Phytocoenotic structure and stocks of main medical plants in southern part of Altai Mountain System (East Kazakhstan). *Journal of Computational and Theoretical Nanoscience*, *16*(7), 2822-2834. https://doi.org/10.1166/jctn.2019.8136

Kubentayev, S. A., Zhumagul, M. Z., Kurmanbayeva, M. S., Alibekov, D. T., Kotukhov, J. A., Sitpayeva, G. T., ... & Izbastina, K. S. (2021). Current state of populations of *Rhodiola rosea* L.(*Crassulaceae*) in East Kazakhstan. *Botanical Studies*, *62*, 1-20. https://doi.org/10.1186/s40529-021-00327-4

Kubentayev, S. A., Suleimenov, A. N., Kotukhov, J. A., Danilova, A. N., & Sumbembayev, A. A. (2018). Phytocenotic characteristics and stocks of the main medicinal plants of the South-Western Altai (East Kazakhstan). *Eurasian Journal of BioSciences*, *12*(2).

Nekratova, A. N., & Shilova, I. V. (2015). Ecological and geographical confinement of rare medicinal plants of forest flora of the Kuznetski Alatau in need of protection.

Orazov, A., Myrzagaliyeva, A., Mukhitdinov, N., & Tustubayeva, S. (2022). Callus induction with 6-BAP and IBA as a way to preserve Prunus ledebouriana (Rosaceae) and endemic plant of Altai and Tarbagatai, East Kazakhstan. *Biodiversitas Journal of Biological Diversity*, *23*(6). https://doi.org/10.13057/biodiv/d230645

Özek, G., Chidibayeva, A., Ametov, A., Nurmahanova, A., & Özek, T. (2021). Chemical Composition of Flower Volatiles and Seeds Fatty Acids of *Rosa iliensis* Chrshan, an Endemic Species from Kazakhstan. *Records of Natural Products*, *16*(3). https://doi.org/10.25135/rnp.271.2105.2083

POWO. (2019). Plants of the world online. Facilitated by the Royal Botanic Gardens, Kew. http://www.plantsoftheworldonline.org

Sazanova, A. L., Kuznetsova, I. G., Safronova, V. I., Belimov, A., Popova, Z. P., Tikhomirova, N. Y., & Osledkin, Y. S. (2017). Study of the genetic diversity of microsymbionts isolated from *Hedysarum* gmelinii subsp. setigerum, growing in the Baikal Lake region. *Selskokhoziaistvennaia Biol*, *52*(5), 1004-1011. https://doi.org/0.15389/agrobiology.2017.5.1004rus

Sumbembayev, A., Tergenbaeva, Z., Kudabayeva, G., Tashmetova, R., Genievskaya, Y., & Szlachetko, D. (2022). Assessment of state of Dactylorhiza fuchsii (Orchidaceae) populations from the Altai mountains of Kazakhstan. *Biodiversitas Journal of Biological Diversity*, *23*(9). https://doi.org/10.13057/biodiv/d230903

Takhtajan, A. (Ed.). (2009). *Flowering plants*. Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-1-4020-9609-9

Vdovitchenko, M. Y., Kuzovkina, I. N., Paetz, C., & Schneider, B. (2007). Formation of phenolic compounds in the roots of *Hedysarum theinum* cultured *in vitro*. *Russian Journal of Plant Physiology*, *54*, 536-544. https://doi.org/10.1134/S1021443707040164

Yazid, F., Hasanah, N. B., Rosmalena, H. M., & Prasasty, V. D. (2020). Antidiabetic and antioxidant potential of Vernonia amygdalina leaf extract in alloxan-induced Sprague-Dawley rats. https://doi.org/10.3844/ojbsci.2020.190.200

Zhmud, E. V., Zinner, N. S., & Dorogina, O. V. (2020). Trypsin inhibitor activity in representatives of the genus *Hedysarum* (*Fabaceae*): The polyvariance of seasonal dynamics in Southern Siberia. *Труды по прикладной ботанике, генетике и селекции*. https://doi.org/10.30901/2227-8834-2020-3-25-31

Zinner, N. S., Nekratova, A. N., Shchukina, A. V., & Kovaleva, A. L. (2021). Biological features of high altitude rare medicinal plant species *Hedysarum theinum* Krasnob. In Western Siberia cultivation. https://doi.org/10.3897/abs.7.e67122