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COMPREHENSIVE STUDY OF PLANT MATERIAL *PULMONARIA MOLLIS* WULFEN EX HORNEM (BORAGINACEAE JUSS)

Studies of medicinal flora for the presence of new biologically important components and identification of potentially significant plant species, occupy a special niche in the development of drugs based on plant raw materials. In official medicine, medicinal plants are increasingly used and prioritized in the treatment and prevention of many diseases [1]. Long-term studies of medicinal plants have shown that extracts from them have low toxicity and exhibit therapeutic properties, and the variety of biologically active substances provides a wide range of pharmacological actions of plant preparations. Based on literature data, *Pulmonaria mollis* is rich in polyphenolic compounds, glycosides (rosmarinic acid), naphthaquinones, terpenes, heterocyclic compounds – allantoin, and many of them have pronounced antioxidant, antibacterial and anti-inflammatory activity. A comprehensive study was conducted on the aboveground part of *Pulmonaria mollis* Wulfen ex Hornem., growing in the Alakol District of the Zhetysu Region, Republic of Kazakhstan. The content of biogenic elements (Ca, Mg, Fe, Cu), and quantitative content of tannins, flavonoids, and free organic acids was determined. The compositional profile of amino acids was established, and quercetin, isorhamnetin, rutin, gallic, and ferulic acids were identified. The complex of biologically active substances in *Pulmonaria mollis* (quercetin, rutin, gallic acid) may be of interest for research in the prevention of oxidative stress and cardiovascular pathology.

Key words: *Pulmonaria mollis*, amino acid composition, macro and micronutrients, polyphenols, phytochemicals, capillary electrophoresis, phytopreparation.

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Pulmonaria mollis Wulfen ex Hornem (Boraginaceae juss) өсімдік шикізатын кешенді зерттеу

Дәрілік флораны жаңа биологиялық маңызды компоненттердің болуын зерттеу және өсімдіктердің ықтимал маңызды түрлерін анықтау өсімдік шикізатына негізделген препараттарды жасауда ерекше орын алады. Ресми медицинада дәрілік өсімдіктер көбірек қолданылады және көптеген ауруларды емдеу мен алдын-алуда басымдыққа ие болады [1]. Дәрілік өсімдіктердің ұзақ мерзімді зерттеулері олардан алынған сығындылардың уыттылығы төмен және емдік қасиеттері бар екенін көрсетті, ал биологиялық белсенді заттардың алуан түрлілігі өсімдік препараттарының фармакологиялық әсерінің кең спектрін қамтамасыз етеді. Әдеби деректерге сүйенсек, *Pulmonaria mollis* Wulfen ex Hornem (жұмсақ медуница) полифенолды қосылыстарға, гликолиздерге, гетероциклді қосылыстарға – аллантоиндерге бай және олардың көпшілігінде айқын антиоксиданттық, бактерияға қарсы және қабынуға қарсы белсенділік бар. Қазақстан Республикасы, Жетісу облысының Алакөл ауданында өсетін *Pulmonaria mollis* Wulfen ex Hornem (жұмсақ медуница) өсімдігінің жер үсті бөлігіне кешенді зерттеу жұмыстары жүргізілді. Биогендік

гендік элементтердің (Ca, Mg, Fe, Cu) құрамы, таниндердің, флавоноидтардың, бос органикалық қышқылдардың сандық құрамы анықталды. Аминқышқылдарының композициялық профилі анықталып, кверцетин, изорамнетин, рутин, галл, ферул қышқылдары анықталды. *Pulmonaria mollis* құрамындағы биологиялық белсенді заттар кешені (кверцетин, рутин, галл қышқылы) тотығу стрессінің және жүрек-қан тамырлары ауруларының алдын-алу саласындағы зерттеулерге қызығушылық тудыруы мүмкін.

Түйін сөздер: *Pulmonaria mollis*, аминқышқылдарының құрамы, макро және микроэлементтер, полифенолдар, фитохимиялық заттар, капиллярлық электрофорез, фитопрепараттар.

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Комплексное изучение растительного сырья *Pulmonaria mollis* Wulfen ex Hornem (Boraginaceae juss)

Изучение лекарственных растений на наличие новых биологически важных компонентов и выявление потенциально значимых видов растений занимает особую нишу в разработке лекарственных препаратов на основе растительного сырья. В официальной медицине лекарственные растения все чаще используются и занимают приоритетное место в лечении и профилактике многих заболеваний [1]. Многолетние исследования лекарственных растений показали, что экстракты из них обладают низкой токсичностью и проявляют терапевтические свойства, а разнообразие биологически активных веществ обеспечивает широкий спектр фармакологического действия растительных препаратов. На основе литературных данных, *Pulmonaria mollis* богата полифенольными соединениями, гликозидами (розмариновая кислота), нафтахинонами, терпенами, гетероциклическими соединениями – аллантоином, и многие из них обладают выраженной антиоксидантной, антибактериальной и противовоспалительной активностью. Проведено комплексное исследование надземной части *Pulmonaria mollis* Wulfen ex Hornem., произрастающей в Алакольском районе Жетысуской области Республики Казахстан. Определено содержание биогенных элементов (Ca, Mg, Fe, Cu), количественное содержание дубильных веществ, флавоноидов и свободных органических кислот. Установлен композиционный профиль аминокислот, идентифицированы кверцетин, изорамнетин, рутин, галловая и феруловая кислоты. Комплекс биологически активных веществ в *Pulmonaria mollis* (кверцетин, рутин, галловая кислота), может представлять интерес для исследовании в области профилактики оксидативного стресса и сердечно-сосудистых патологии.

Ключевые слова: *Pulmonaria mollis*, аминокислотный состав, макро и микроэлементы, полифенолы, фитохимические вещества, капиллярный электрофарез, фитопрепарат.

Introduction

This work is part of the research aimed at studying the biological activity of total preparations obtained from the aboveground part of *Pulmonaria mollis* Wulfen ex Hornem., conducted at the Department of Biodiversity and Bioresources of Al-Farabi Kazakh National University since 2022, with the participation of staff from the Laboratory of Plant Resources of the Institute of Botany and Phytointroduction [1].

The genus of perennial plants *Pulmonaria L.* comprises 18 species, distributed in the Mediterranean, Europe, the Caucasus, as well as in Central and Southeast Asia [2]. In the flora of Kazakhstan, one species, *Pulmonaria mollis* Wulfen ex Hornem., is found, growing along forest edges, meadow slopes, and forest valleys [3, 4].

Species of the genus *Pulmonaria*, particularly *P. officinalis L.* and *P. obscura* (Dumort.), are utilized in folk medicine for treating pulmonary diseases as soothing and binding agents for the respiratory tract. Among the Siberian peoples, *Pulmonaria* is widely used as a wound-healing, hemostatic, and antiseptic remedy [5, 6]. Extracts from the aboveground part of *P. mollis* exhibit antioxidant, anticoagulant, anti-anemic, fibrinolytic, hemostatic, diuretic, and cholagogue activities [7, 8]. Additionally, the underground part possesses contraceptive properties [9]. Aqueous extract from the aboveground part of *P. obscura* demonstrates anticonvulsant effects

[10]. *P. mollis* is recommended for use as an immunomodulatory agent. In some regions of Siberia and England, the leaves of *P. mollis* are harvested in early spring and consumed as a vitamin supplement in salads [11, 12].

Many species of *Pulmonaria* are utilized for decorative purposes in creating shaded areas of landscape design (*P. obscura, P. officinalis L., P. angustifolia L., P. longifolia* (T. Bastard) ex Boreau, *P. mollis, P. rubra* Schott*., P. saccharata* Mill, among others) [13, 14].

The aboveground part of *Pulmonaria* species accumulates polyphenols (phenolic acids, flavonoids), pyrrolizidine alkaloids, primarily derivatives of N-oxides [15, 16]. Some authors suggest that the content of a complex of trace elements (Fe, Cu, Mn) positively influences the treatment of anemia [5]. Aqueous solutions contain phenolic compounds (gallic, caffeic, ferulic acids, catechin, dihydroquercetin, kaempferol), notably lithospermic acid, which exhibits contraceptive activity [15, 9]. Additionally, an essential oil has been isolated, predominantly comprising saturated acids and their esters [17]. Allantoin has been discovered in the underground part, the accumulation of which depends on the nitrogen content in the soil [18].

To comprehensively investigate the prospective medicinal plant *P. mollis*, growing in the Alakol District of the Zhetysu Region, studies were conducted on the aboveground part, collected during the flowering phase, to determine the content of biogenic elements, the compositional profile of amino acids, and some biologically active substances (BAS).

Materials and Methods

The object of the study was a wild-growing medicinal plant commonly used in folk medicine. The material for comprehensive investigation was the air-dried aboveground part of *Pulmonaria mollis* Wulfen ex Hornem., collected during the flowering phase in late April 2022 in the territory of the Alakol District of the Zhetysu Region, where the lungwort grew along the edges of a deciduous forest at an altitude of 1044 meters above sea level (Fig. 1).

Pulmonaria mollis, known as Zhumsaq balshatyr" in Kazakh, or Soft Lungwort, is a perennial herbaceous plant reaching heights of up to 35 cm. It features straight, thinly ribbed stems covered throughout with soft glandular hairs and small-scale-like leaves at the base. The basal leaves are large, elongated-elliptic, gradually narrowed at the base into a long-winged petiole, almost equal to the lamina. Stem leaves are ovate-lanceolate, sharp, with semi-stem-embracing bases, reduced and sessile. The flowers, after fading, droop in small multicolored clusters gathered at the ends of the stems in a shield-like manner. The pedicels are short, later lengthening, and sparsely covered with short white erect hairs. The calyx is bell-shaped, covered along the veins with short semi-pressed bristles and glandular fuzz, with triangular-lanceolate teeth, twice shorter than the undivided part of the calyx. The corolla is pink before pollination, then violet-blue, funnel-shaped, with a short tube drawn upwards and a longer flare, one-third cut into semi-circular lobes. The nutlets are globose-ovoid, shiny, slightly obtuse at the apex, with small depressions at the base. It blooms from May to June. In Kazakhstan, it grows along forest edges, wooded meadow slopes, and forest valleys [3].

The aboveground parts of the plants were cut and dried using an air-shaded method, spreading the cut material in a single layer and periodically mixing it. The resulting air-dried raw material was subsequently ground.

The content of macro- and micronutrients was determined using atomic absorption spectroscopy on a spectrometer with software and electrothermal atomization "KVANT-Z.ETA-T" (Russia). Atomic absorption measurements of biogenic elements were carried out according to regulatory documentation (GOST 30178-96) [19]. Samples were prepared using the wet ashing method with nitric acid and hydrogen peroxide [20, 21]. Ground plant material (2 g) was placed in quartz crucibles with a tightly closed lid, to which 0.75 ml of 30% H_2O_2 and 3 ml of 67% HNO_3 (osch) were added. The mixture was treated with ultrasound for 8-10 minutes. Then, 0.5 ml of 30% H_2O_2 was added, the crucibles were covered, and placed in a drying cabinet for 24 hours at 60°C. After cooling, 9 ml of distilled water was added, and the mixture was heated again in the drying cabinet for another 24 hours at the same temperature. The mixture was then transferred to test tubes and centrifuged for 10-15 minutes (6000 rpm), and 2 ml of the resulting solutions were transferred to microtest tubes and supplemented with an internal standard.

Figure 1 – *Pulmonaria mollis* Wulfen ex Hornem. in its natural habitat in the territory of the Alakol District of Zhetysu Region

Amino acid analysis was conducted after preliminary hydrolysis of the plant material with hydrochloric acid (1:1) at 105°C for 14-16 hours. The acid hydrolysate was evaporated to minimum volume using a rotary evaporator and then converted into phenyl isothiocyanate derivatives. Subsequently, they were separated and quantitatively determined using capillary electrophoresis method "Kapell 105 M" (Russia). Detection was carried out in the UV range at 254 nm.

According to standard methodologies [22, 23], certain biologically active substances (BAS) were quantitatively determined in the aboveground part of *P. mollis*.

Results and Discussion

Analysis of the collection funds of the Institute of Botany and Phyto Introduction (Herbarium AA) allowed to identify 18 specimens, the majority of which were collected in the territory of southeastern and eastern Kazakhstan (Fig. 2).

The analysis of macro- and micronutrients showed a high content of calcium (1004.3 mg/100g) and magnesium (215 mg/100g) (Table 1). Our obtained results regarding the content of Ca and Mg in the aboveground part of *P. mollis* are consistent with existing literature data for the studied species [24].

The iron content in the study is much lower may be due to soil conditions. The amount of iron in the plant depends on its constant in the soil its acidity (pH). In Zhetysu region, Alkol district where P.mollis were collected soil cover is represented by sierozems. For sierozem is characterised by high content of carbonates, pH>7 (alkaline reaction) because of which iron forms insoluble compounds and become difficult to absorb for plants. Thus, Table 1 shows the results where Fe content in the study (8.84 mg/100 g) is much lower than the literature data (40.53 mg/100 g).

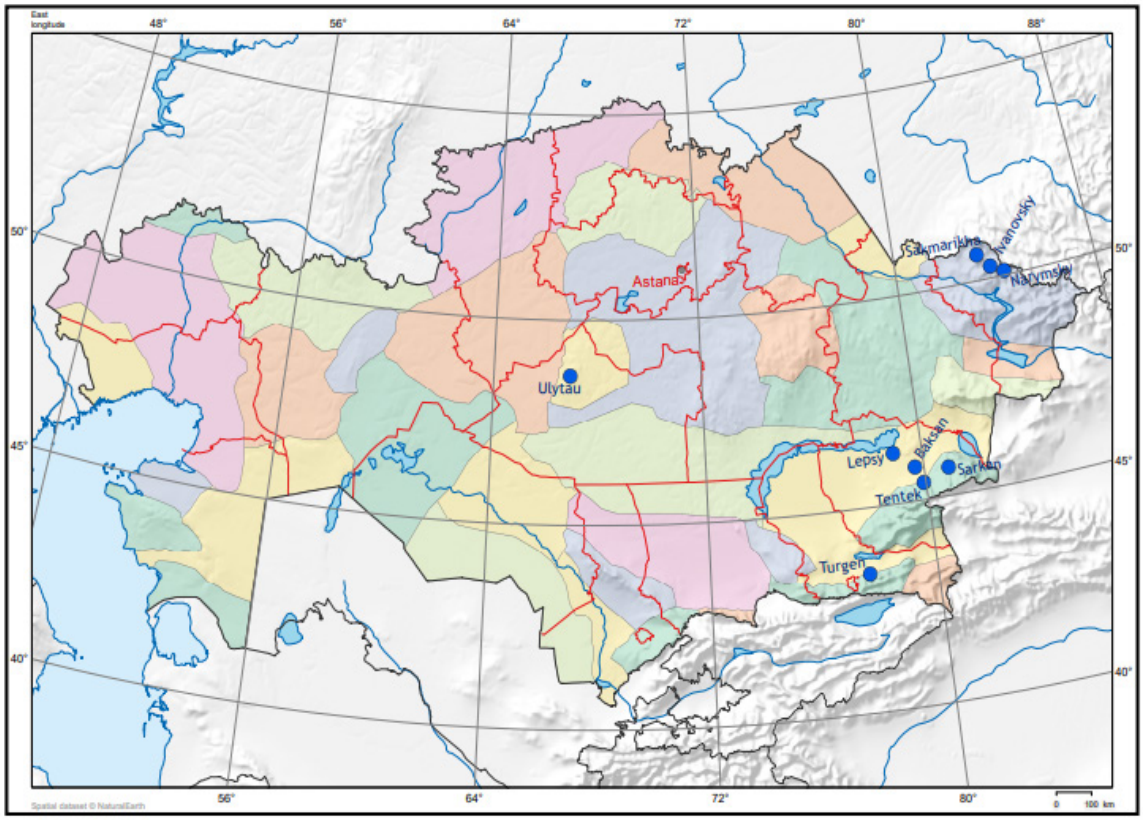

Figure 2 – Distribution of *Pulmonaria mollis* in the territory of Kazakhstan according to data from Herbarium AA.

Table 1 – Content of Ca, Fe, Mg, and Cu in the aboveground part of *Pulmonaria mollis* Wulfen ex Hornem. (mg/100 g)

Element	Experimental Data (mg/100 g)	Literature Data [24, 25] (mg/100 g)	
Ca (Calcium)	1004.3	426.5	786.4
Fe (Iron)	8.84	8.33	40.53
Mg (Magnesium)	214.9	27.03	169.4
Cu (Copper)	1.59	0.696	1.43

The high calcium and magnesium content can be due to several factors:

Species of the borage family, in particular the soft medusa, grow in 'ecotopes with different degrees of expression of ecological factors' – high mountains, increased level of solar radiation. In addition, early spring species are characterised by Ca accumulation and prefer alkaline moist soils.

For medunica species, the calcium and magnesium content varies between 0.3-1.5% (in our case 1%).

Trichomes of the P.mollis plant itself tends to accumulate macronutrients, at the expense of which calcium and magnesium contents can be high in the plant.

The determination of amino acid content in the aboveground part of the Kazakhstani species P. mollis was conducted for the first time, resulting in the identification of 14 amino acids (Fig. 3).

Based on the obtained data, 14 amino acids were found in the aboveground mass of *Pulmonaria mollis*, including 7 essential ones (valine, isoleucine, leucine, methionine, lysine, threonine, phenylalanine) with high contents of valine, threonine, and leucine. Among the 7 non-essential amino acids (alanine, glycine, serine, tyrosine, arginine, histidine, proline), proline, arginine, and alanine predominated. Histidine, tyrosine, and methionine were found in the lowest amounts.

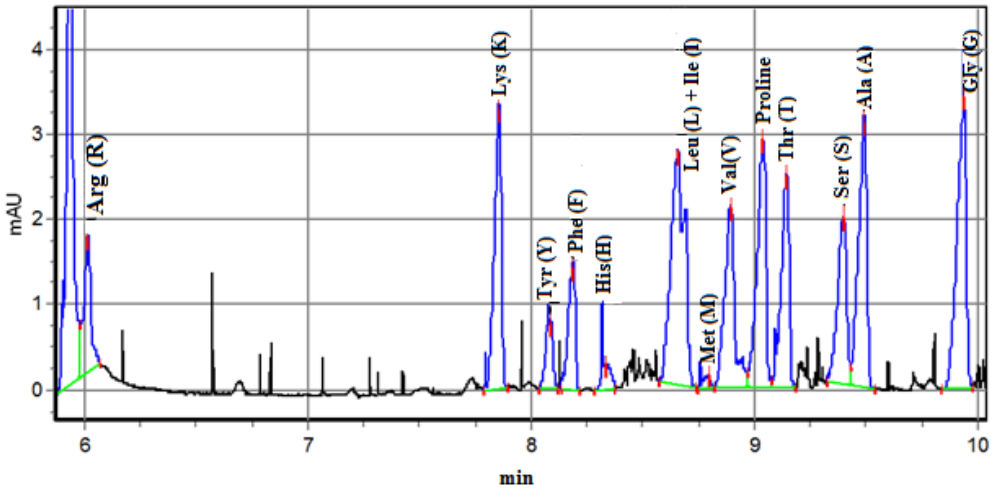

Figure 3 – Chromatogram of amino acids in the aboveground part of *Pulmonaria mollis* Wulfen ex Hornem

Based on the amino acid content, they can be arranged in the following sequence: proline > valine > leucine > threonine > arginine > lysine > alanine > glycine > phenylalanine > isoleucine > serine > tyrosine > histidine > methionine, indicating a predominance of proline, valine, and leucine (968, 870, 850 mg/100 g). In the study by Kruglov et al. (2012), the compositional profile of amino acids in the leaves of three species of lungwort (*P. mollis, P. obscura, P. officinalis*) was determined, where in *Pulmonaria mollis*, the content of glutamine, leucine, and asparagine acid predominated compared to our data.

Qualitative analysis for the content of major groups of biologically active substances (BAS) was conducted for water-alcohol extracts using TLC (thin-layer chromatography) and HPLC (high-performance liquid chromatography) methods. The presence of condensed tannins (Folin-Ciocalteu assay, green-black coloration), phenolic acids (DzPNA), nitrogen-containing compounds, sterols, and terpenes (vanillin in HCl) was established using two-dimensional and one-dimensional paper chromatography (TLC) in various solvent systems. Quercetin, isoramnetin, rutin, gallic acid, and ferulic acid were identified.

Quantitative determination was conducted for free organic acids, flavonoids, tannins (condensed substances), as well as vitamin C. The data are presented in Table 1.

Table 2 – Quantitative content of some biologically active substances in the aboveground part of *Pulmonaria mollis* Wulfen ex Hornem

The indicators of the quality of the raw material and the main groups of biologically active substances.	The content in the raw material.		
	Soft lungwort (*P.mollis*)	Unclear lungwort (*P.mollis* (27)	Narrow-leaved lungwort (*P.mollis*) (27)
Free organic acids, %	4,48	4,54	5,45
Flavonoids, %	0,83	0,52	2,17
Tannins (condensed substances), %	13,42	21,72	31,52
Vitamin C, mg %	6,42	2,4	14,88

Conclusion

As a result of comprehensive research on the plant material of *P. mollis*, the content of Ca, Mg, Fe, and Cu was determined, with a noticeable predominance of Ca and Mg, which is consistent with the literature. Among the detected trace elements, iron (Fe) predominates [24,25] .

Fourteen amino acids were identified with high levels of proline, valine, and leucine. The content of tannins amounted to 13.4%, flavonoids 0.82%, and free organic acids 4.48%. The potential use of *Pulmonaria mollis* requires further research (in vitro in vivo).

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