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^{1*}K.B. Yernazarova, ¹Zh.T. Abdrassulova, ¹S.T. Tuleuhanov, ¹G.A. Tussupbekova,
²N.N. Salybekova, ²G. Isayev, ³H. Basim

¹Laboratory of Chronobiology and Ecological Physiology, Almaty, Kazakhstan

²Laboratory of Biotechnology, Turkestan, Kazakhstan

³Laboratory of Molecular Biology, Akdeniz University, Antalya, Turkey

*e-mail: kamilya_002@mail.ru

Biological features of the medicinal plant *Plantago major* L.

Abstract: Paper provides an overview of the therapeutic, morphological, anatomical and phytochemical features of *Plantago major* L. For studies, plant samples were collected four times from April to September 2018 in the Almaty region, with freshly harvested plants used for morphological and anatomical studies, and specially dried leaves used for phytochemical studies. Anatomical sections were produced using the classic blade method. Chemical composition, including vitamins B1 (thiamine chloride), B2 (riboflavin), B3 (pantothenic acid), B6 (pyridoxine), C (ascorbic acid), E (tocopherol), tannins was studied at the research laboratory for assessing the quality and safety of food products of the Almaty Technological University. Vitamins were determined according to the State Standard 31483-2012, with the help of the device “Kapel105-M” by the method of capillary electrophoresis (M-04-41-2005). Tannins were estimated by titration of margon-acid potassium using the classical method according to the State Standard 24027.2-80. As a result of a phytochemical study, large amount of vitamin C (ascorbic acid) was determined among vitamins, on average of 0.432 mg/100g. This means that preparations obtained from *Plantago major* L. can be used in the treatment of bleeding gums, against viral and other diseases. However, vitamin E was not detected in the plant. Amount of tannins was around 77.94 mg/100g; which indicates its high antimicrobial, hemostatic properties and prevents the replenishment of the gastrointestinal tract. The results of this study can be used as an additional material in the educational process in such courses as “Botany”, “Medicinal plants”, “Anatomy and morphology of plants”.

Key words: *Plantago major* L., xylem, phloem, ascorbic acid, tannins.

Introduction

Plantago (*P.*) *major* L. (large plantain) is a pharmacological plant, which belongs to the family *Plantaginaceae*. The area of its distribution is very wide. Plants belonging to the *Plantaginaceae* family grow around the globe, with about 260 species known. Large plantain can be found on the territory of all CIS countries. Despite the fact that it grows on the soil with different mechanical composition and moisture content, it is quite demanding for moisture and light, so it falls in the widespread and exposed areas of the shadowy regions. It is common in residential areas, open terraces, along the roads and pastures and never grows into large groups [1; 2]. There are 16 species in all regions of Kazakhstan. Among them there are large plantains (*P. major*), average plantain (*P. media*) and flat plantains (*P. lanceolata*). They grow on the banks of the river-

lake, in the mountains, deserts, in slopes where weeds are grown, along the road, in the vicinity. The height of the stem is about 20–70 cm. The leaves are oval, flat, with the whole edge, they grow around the roots. Occasionally, they are on the opposite of the stem, but sometimes tend to alternate. The tiny flowers are light brown, rarely light blue, in the form of grains. In the period from May to September it will bloom and give fruits and it is dioecious. The fruit bursts open when it is ripen. Seeds and leaves contain glycosides, carotene, phytoncid, and are therefore used in medicine [3].

Plantain is one of the most ancient medicinal plants. Even in China for medical purposes it was collected for more than 3,000 years ago. The ancient Greeks and Romans used plantain seeds for dysentery, inflammatory conditions of the stomach, intestines and other diseases. Hippocrates and Galen used it. Avicenna believed it was extremely good for ul-

cers. Leaves prevent bleeding, and when dried, promote healing of old and fresh ulcers. Avicenna used plantain leaves as a wound healing and hemostatic agent [4; 5].

The pharmacological value of the plantain carries a great deal of interest to many researchers and the work of both Kazakhstani and foreign scientists can be considered as a proof. In 2013 at the stomatology school in the University of Indiana, United States, Rayna E. studied *P. major* and determined its tumor and anti-inflammatory properties [6]. Levent A. at the University of Batman in Turkey has developed a new, simpler method for high-quality liquid chromatography for simultaneously identifying the retinol, retinol palmitate, beta-carotene, α -tocopherol and vitamin C from rat's serum treated with *P. major* and 7,12-dimethylbenzanthracene [7]. In 2015, Kazakh scientist Alimova U.S. identified the pharmacological and pharmaceutical aspects of construction of the suppositories with the added extracts of sweet flag and large plantain (*Acorus calamus* L. and *P. major*) [8]. This year, Dilbarhanov R.D. *et al.* studied antimicrobial activity of CO₂-extracts of sweet flag and large plantain [9].

In 2016, at the Rungta Dental Society of India, Sharma H. had studied the microbial effects of *P. major* [10]. This year at the Swedish University of Agricultural Sciences, Zubair M. has carried out a research to prove the application of a large plantain in traditional medicine [11]. In 2017, in Surabaya University of Indonesia there were investigated the effects of the precipitations and chemical compounds of *Plantago* on the proliferation of cytokines and cancer cells the inflammatory and antitumoral properties [12]. In June 2017 at the Plovdiv Medical University in Bulgaria, Lukova P. studied the medicinal properties of the *P. major* plant, including the fermented hydrolysis of polysaccharides extracted from the leaves [13]. In 2017 at the Malaysian International Islamic University, Adom M.B., has conducted an investigation to determine the chemical components and medical advantages of *P. major* [14]. In March 2018, in the Neurogenic Inflammation Research Center at the Mashhad Medical University in Iran Naji Ebrahim checked dextrorubicin-induced nephropathic protection in mice [15]. Same year, in Columbia at the University of Cartagena Karo D.S. studied the healing properties of anxiety and insomnia of a large plantain [16], in Mashhad Medical University in Iran Enezar H.N. studied the protective effect of *P. major* on liver inflammation caused by daxorubin [17].

The aim of the current research is to study morpho-anatomical and phytochemical characteristics of

P. major, growing in Almaty region, and assess its pharmacological value.

Materials and methods

Samples of the medicinal plants were collected four times from April to September 2018 in the Almaty region. Morpho-anatomical studies were carried out at the laboratory of collective use "Physical and chemical methods of research in biology" No. 216 of al-Farabi Kazakh National University. To carry out this experimental work, freshly harvested plants, their leaves and roots were used. Anatomical sections were prepared by the classical method, cutting off a thin plate with a razor blade. Photos of the plant were taken with a camera Canon EOS1200D 5472x3648 (20,0 million pixels) (Canon, Japan). Morphometric parameters of the plant were measured and anatomic images were examined with MT6300 microscope (Meiji Techno, Japan), eyepieces SWH10x F.N. 22 (O.D. 30 mm), software Vision Capture 2.1. Statistical processing of morphometric indicators was conducted in Microsoft Excel 2010.

Phytochemical parameters of *P. major* were studied on dried leaves. Collected leaves were dried by the shade method, under a well ventilated canopy, without sunlight [18]. The research was conducted at the Almaty Technological University on June 11-22, 2018 in the Research Laboratory for the Assessment of Quality and Safety of Food Products. Test conditions: temperature – 21±1°C, relative humidity – 81%. In the composition of *P. major* the water-soluble vitamins (B₁, B₂, B₃, B₆, C, E) were determined according to GOST 31483-2012, with the device Kapel 105-M (Lumex, Russia), using the method of capillary zone electrophoresis (M-04-41-2005) in accordance with the general provisions, stated in the TU 4215-023-20506233-98 regulatory document. According to the aforementioned regulatory document, the sample to be analyzed is introduced into a capillary pre-filled with electrolyte. After high voltage is applied to the ends of the capillary, the components of the mixture begin to move through the capillary at different speeds, depending on their structure, molecular weight and charge, and reach the detector located at the end of the capillary. The electrophoregram obtained is a sequence of peaks, each of which corresponds to a strictly defined substance. The Kapel 105-M system consists of the following main elements: quartz capillary; devices for filling the capillary with liquid, introducing the liquid sample into the capillary and supplying voltage from the high voltage source to the ends of the capillary (herein-

after referred to as the sample introduction device); high-voltage unit of positive, negative or switchable polarity, consisting of a high voltage source and a switching device; a detector to determine when the components of the sample reach the detection zone.

The Kapel 105-M system is available with a high-voltage switchable polarity unit, a photometric switch with a switchable wavelength, an automatic sample changing system and a water capillary cooling system. The vitamins were determined on capillary zone electrophoresis, using the wavelength exchange program by their absorption at 200 nm and 240 nm wavelengths [19]. The amount of tannins in medicinal plants has been estimated according to the state standard 24027.2-80 by the classical method, involving titration with manganese acid potassium [20]. All photos presented in the paper were taken by the authors.

Results and discussion

All of the plantain types are annual and perennial, some of them are weed. Leaves are often rarely seen and covered with roots. The flowers are small, two-sided, and actinomorphic, have upper-ended heads or germ-like flowerpots, often four-membered. Its tray has four blades or four blades. The crown of the flower is deciduous, has four blades, colored or dyed. The coenocarpal gynoeceum consists of two fertilized leaves that form two upper nodules [21; 22].

Plantains usually reproduce by the wind, but sometimes they come in contact with insects. The fruits are cenocarpus: a hawk with a cap. Seeds are small, with boar, small vertical seeds and nutritious endosperma; it falls off the covered foliage and spreads through the wind [23].

Large plantain (*P. major*) is a perennial herbaceous plant with the height of 10-50 (70) cm, one or more flower foliage and stem layers, 12 cm long plate, wide egg or ellipsoid, the side has a rarely uneven tooth (slightly thin), three to seven arched pipe tubes (Figure 1). The crusher is equal to the length of the leaf plate or slightly longer, rarely short. The pulp is lessened and the thin roots are pulled out from it [24].

The study of morphometric parameters of large plantains revealed changes in quantitative parameters (Table 1).



Figure 1 – Large plantain (*Plantago major* L.), growing on the territory of al-Farabi KazNU

Table 1 – Morphometric parameters of *Plantago major* L.

Parameters	April-June	July-September
Total length of the large plantain	17±3.02	40±3.10
Number of leaves	7±3.00	12±3.00
Length of the leaves, cm	1.4-4.80	4.1-21.30
Width of the leaves, cm	1-2.90	3.6-8.20
Number of leaf bands	5±1.00	8±1.00
Length of the root, cm	9±2.50	10±2.06
Number of roots	40±4.00	45±5.00
Number of fruits in a bush	-	234±20.00

Total length of the plant increased from April to June in the range of 17-20 cm, and from July to September – 40-44 cm. The number of leaves per plant within the first months ranged from 7 to 10, and from July to September it reached 12-15. During the general development of the leaf, the length of the leaves increased from 1.4 to 21.3 cm and width up to 1-8.2 cm. The number of leaf bands in the first sample increased from 5-6 to 8-9 at the end of the summer. The number of rhizomes reached 40-44, the length increased to 9-11.5 cm from April to June, and from July to September the number of rhizomes reached 45-50, the length reached 10-12 cm. Since the flowering period of *P. major* starts in June, the number of fruits has been tested since July and on one of these flowers there were 234-254 of them.

Plantago major L. has its own semiconductor, mechanical, separating and storing elements. These tissues combine all members of the plant into one system.

There are two types of conductive tissue – xylem (gr. Chylon – wood) and phloem (gr. Phloios – shell, sponges). They have structural and functional differences. The conductive elements of xylem consist of dead cells. They transfer water and dissolved substances from the roots to the leaves. Fluid in conductive elements preserves living protoplasts [25]. In the absorption zone, rhizodermatellular cells form the vascular bundles (Figure 2).

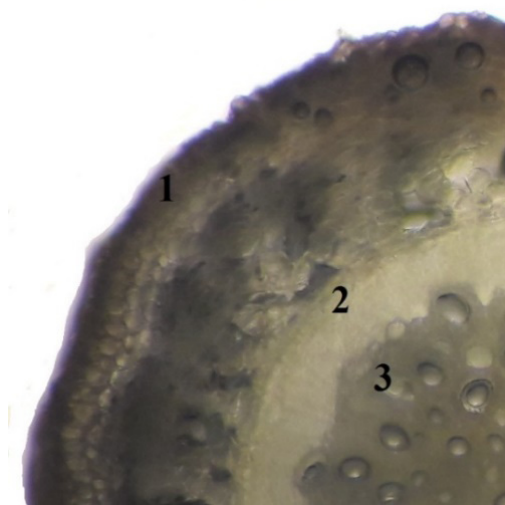


Figure 2 – Anatomical structure of *Plantago major* root, magnification x100.

Note: 1 – rhizoderm, 2 – phloem, 3 – xylem

The flowering shrub is directed upwards, decorated with thin, long cylindrical spikelets and dense small-sized flowers. There are four, 1.5-2.5 mm long, petals, in the flower receptacle; flowers in the crown are actinomorphic; cylinder tubes are formed on the underside. Four male parts are attached to the flower crown tube with twice as long purple pollen. Female parts have two nodes.

Anatomical structure of the large plantain leaf is specific to the herbaceous plants. Insular conductive bundles are located in the mesophyll. Xylem is located at the top of the leaf, and phloem is at the bottom. Xylem consists of round twisted tubules, and phloem of filter-shaped tubes with very large supportive cells. Leaf plate endurance is ensured at the expense of the subepidermal crown of collenchyma or sclerenchyma (Figure 3).



Figure 3 – Anatomical structure of *Plantago major* leaf, magnification x100.

Note: 1 – xylem, 2 – phloem, 3 – sclerenchyma, 4 – collenchyma

Drugs (fresh and canned juice, emulsion and ointment) with different pharmacological effects (anti-inflammatory, wound healing and antimicrobial) produced from plantain leaves, may be used in treatment of broad range of diseases, including stomach ulcers, laryngitis, hypotension, bronchial asthma and tuberculosis [26-28]; aqueous extracts show properties of lowering cholesterol and sclerosis [29; 30]. Galen's drugs (extract, juice) are used in the treatment of inflammatory diseases of mouth and throat, skin injuries, chronic gastric ulcers, burns, flegmonia, cholera, and purulent wounds [31]. Herbal preparations and fresh leaves have bacteriostatic effect on the pathogenic microbes of traumatic infections; the surface of the wound cleanses quickly from the purgation, inflammation stops, and the wound healing rapidly speeds up [32].

Quantitative indicators of vitamins and tannins are shown in table 2.

According to our results, the amount of vitamin C in the medicinal plant is the highest, with an average of 0.432 mg per 100 g of the raw material (Table 3).

Vitamin C (ascorbic acid) inhibits the formation of harmful substances during the biological oxidation, subsequently increasing the resistance of an organism (bones, tooth, liver, cardiovascular system) to infectious diseases. Insignificant amount of vitamin C leads to growing fatigue of the human body, blockage of saline shells and bleeding of gum. In case of long deprivation, due to increased gum disease, person is exposed to scurvy with further deterioration of the nervous system [33].

Table 2 – Vitamins and tannins content of *Plantago major* leaves

Indicators, unit of measurement	Precise results	Regulatory documents of research methods
Vitamins, mg/100 g:		
-B ₁	0.0137±0.0027	M-04-41-2005
-B ₂	0.0350±0.0147	
-B ₃	0.180±0.036	
-B ₆	0.0317±0.0063	
-C	0.432±0.148	
-E	Undefined	
-Tannin	77.94	State Standard 24027.2-80

Table 3 – Quantitative indicators of vitamins content in *Plantago plant* leaves

No.	Time, min	Component	Height	Start	End	Area	Concentration, mg/L	Concentration, mg/100g
1	4.587	B ₁ (thiaminechloride)	0.085	4.540	4.725	6.376	0.0025	0.0137±0.0027
2	6.032	B ₂ (riboflavin)	0.403	5.938	6.123	20.45	0.0064	0.0350±0.0147
3	7.265	B ₆ (pyridoxine)	0.857	7.207	7.403	25.84	0.0058	0,0317±0.0063
4	8.677	C (ascorbic acid)	0.574	8.513	8.847	43.28	0.079	0.432±0.148
5	10.142	B ₃ (pantothenic acid)	0.626	9.938	10.185	43.59	0.033	0.180±0.036

B₃ (pantothenic acid) is involved in biochemical reactions, such as high fat and amino acids biosynthesis and oxidation. According to our results, its content comprises 0.180 mg in average per 100 g of the raw material. The lack of pantothenic acid can lead to a large spectrum of abnormalities, including dermatitis, neuritis, pneumonia, etc. It is applied at such conditions as postoperative intestinal atony, as well as renal insufficiency, osteoarthritis in lesions of the skin and mucous membranes, photosystatosis in different types of arthritis, dry skin, hair loss, etc. [34].

B₂ (riboflavin) participates in significant oxidation processes. According to our results, its content comprises 0,0350 mg in average per 100 g of the raw material. It stimulates quick healing of traumas and maintains the eyesight. In case of insufficiency, lips become dried, and the body injuries are healed slowly. In addition, vitamin B₂ is used in treatment of burns, phototherapy, chronic hepoxo, insufficient carbohydrate feeding, or in the treatment of acute infectious diseases.

Vitamin B₆ (pyridoxine) is involved in amino acids exchange. According to our results, its content comprises 0,0317 mg in average per 100 g of the raw material. Vitamin deficiency slows down the growth

in children, causes blood loss and leads to dark facial spots in pregnant women [35]. During pyridoxine avitaminosis, the amino acids and proteins exchange is disrupted, blood formation gets worse, total amount decreases and anemia develops. Vitamin B₆ is involved in maintaining sodium and potassium in the body fluid. This, in turn, is of great importance to the nervous system.

B₁ (thiamine chloride) is necessary for the proper metabolism. According to our results, its content comprises 0.037 mg in average per 100 g of the raw material. The lack of this vitamin causes fatigue and problems with digestion process. Vitamin B₁ interacts with unsaturated fatty acids responsible for the development of urolithiasis and gallbladder pathologies. It helps to relieve skin inflammation, has good effect on the condition of mucous membranes. It is used against typhoid, neurodermatitis, pulse, burn injuries, scabies, and has a positive effect on the intellectual functions [36]. When vitamin B₁ is insufficient, amino acid metabolism results in significant issues.

Estimation of specific vitamins was performed using capillary zone electrophoresis, with the wavelength exchange program at 200 nm and 240 nm

wavelength. The wave diagram is characterized by the appearance of vitamins in a time frame indicated on Figure 4.

In addition to detecting some vitamins, we also aimed to detect the tannins, which are considered to be the only indication of the healing properties of the plant. Scientific data indicates that plant tannins not only serve as antioxidants, but also display anti-inflammatory, antibacterial, antitumoral and antifungal

activities. Tannins have a positive effect on gastrointestinal tract activity, fighting harmful microorganisms and stopping the bleeding. They can also be used for external hemorrhage and internal bleeding. Thus, tannins are used to treat various diseases in medicine [37]. According to our results, the amount of tannins in *P. major* medicinal plant constitutes in average as much as 77.94 mg of tannins per 100 g of raw material.

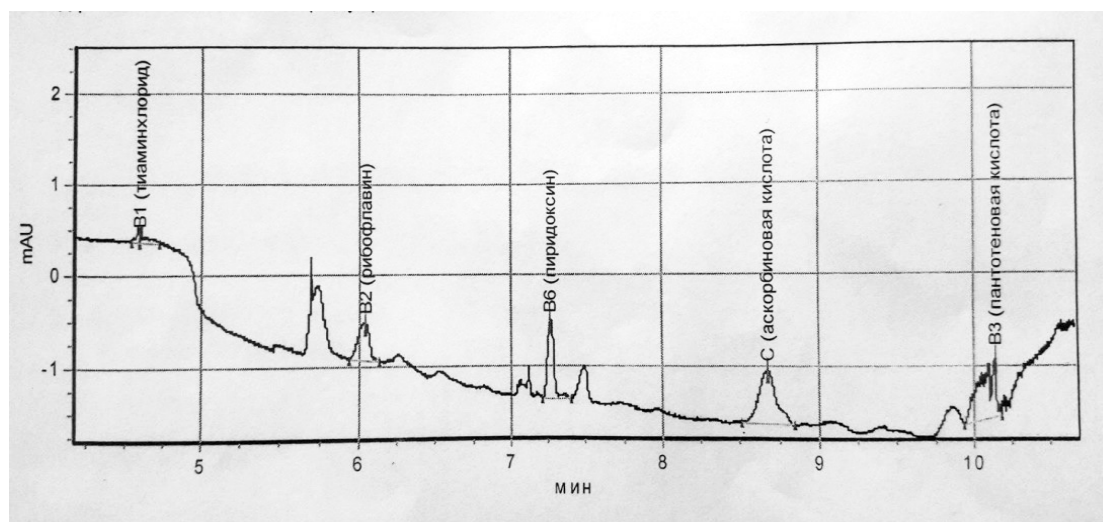


Figure 4 – The electrophoregram of the vitamin amount in leaves of the plant *Plantago major* L.

Note: min-off time (output of the vitamin indicator in the chromatography);

mAU – peak height (height indication of vitamin A peak)

Conclusion

Plantago major L. is a valuable object of research interest. Besides anatomical, morphological and phonological studies, research on its pharmacological effect was conducted. Latter was performed at the laboratory of collective use “Physical and chemical methods of research in biology” No.216 of al-Farabi Kazakh National University and commercially in the Research Laboratory for the Assessment of Quality and Safety of Food Products of Almaty Technological University in the period of April-September, 2018. The amount of vitamin B₁ (thiamine chloride), B₂ (riboflavin), B₃ (pantothenic acid), B₆ (pyridoxine), C (ascorbic acid) in *Plantago major* L. medicinal plant leaves was determined by the capillary zone electrophoresis, while such of tannins has been estimated according to the state standard 24027.2-80 by the classical method, involving titration with manganese acid potassium. The results show that vitamin C (ascorbic acid) can

be used to treat various infectious diseases, such as the inflammatory diseases of the colon and others. Vitamin E (tocopherol) was not found in the plant composition. The amount of tannins was 77.94 mg/100g of plant raw material, and this is a good indication that extracts from *Plantago major* L. plants may have high action to bacterial, bleeding and inflammatory processes in the gastrointestinal tract. Taking into consideration the importance of the identified vitamins and the therapeutic properties of the tannins, we conclude that *Plantago major* L. has also got a high phytochemical value.

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mine chloride), B (2) (riboflavin), B (3) (pantothenic acid), B (5) (nicotinic acid and nicotinamide), B (6) (pyridoxine), B (c) (folic acid), C (ascorbic acid) by capillary electrophoresis].

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