

**MINISTRY OF HEALTH OF UKRAINE
NATIONAL UNIVERSITY OF PHARMACY**

faculty for foreign citizens' education

Department Pharmacognosy

QUALIFICATION WORK

on the topic «**PHARMACOGNOSTIC STUDY OF THE *PLANTAGO MAJOR*
PURPLE VARIETY**»

Prepared by: higher education graduate of group
(ΦM18(5,0д)engl-03)

specialty 226 Pharmacy, industrial pharmacy
educational program Pharmacy

Adam KOURITII

Supervisor: professor of higher education institution of
department Pharmacognosy, Doctor of Pharm. n.,
professor Tetiana GONTOVA

Reviewer: head of higher education institution of
department Medicinal Chemistry, Doctor of Pharm. n.,
professor Lina PEREKHODA

Kharkiv – 2023 year

ANNOTATION

In the qualification work, a pharmacognostic study of the leaves of *Plantago major* variety Purple was performed. Polysaccharides, amino acids, organic acids, phenolic compounds, minerals were identified in the leaves, and their content was determined. Morphological-anatomical features and indicators of the quality of raw materials, the content of extractive substances were determined.

The qualification work is laid out on 56 pages. The main volume of the work is 48 pages. The work is illustrated with 11 tables and 10 figures.

Key words: pharmacognostic analysis, rosette leaves, *Plantago major* variety Purple.

АННОТАЦІЯ

У кваліфікаційній роботі виконано фармакогностичне вивчення листя подорожника великого сорту Пурпурний. В листках ідентифіковано полісахариди, амінокислоти, органічні кислоти, фенольні сполуки, мінерали, визначено їх вміст. Визначено морфолого-анатомічні ознаки та показники якості сировини, вміст екстрактивних речовин.

Кваліфікаційна робота викладена на 56 сторінках. Основний об'єм роботи - 48 сторінки. Робота проілюстрована 11 таблицями і 10 рисунками.

Ключові слова: фармакогностичний аналіз, листья розетки, подорожник великий сорту Пурпурний.

CONTENT

LIST OF CONVENTIONAL DESIGNATIONS	5
INTRODUCTION	6
CHAPTER 1. PHARMACOGNOSTIC STUDY OF SPECIES OF THE PLANTAGO GENUS (Literature Review)	9
1.1. Botanical characteristics of the genus and varieties	9
1.2. Chemical composition of raw materials	14
1.3. Use in medicine	16
CHAPTER 2. STUDY OF GROUPS OF BIOLOGICALLY ACTIVE SUBSTANCES IN RAW MATERIALS OF THE <i>PLANTAGO MAJOR</i> PURPLE VARIETY.	21
2.1. Research objects, reagents and devices.....	21
2.2. Monosaccharide composition of polysaccharides	27
2.3. Identification of amino acids	28
2.4. Qualitative analysis of ascorbic acid and organic acids	29
2.5. Qualitative composition of phenolic compounds	31
Conclusions to chapter 2.....	32
CHAPTER 3. STUDY OF THE QUANTITATIVE CONTENT OF THE MAIN BIOLOGICALLY ACTIVE SUBSTANCES IN THE RAW MATERIALS OF THE <i>PLANTAGO MAJOR</i> PURPLE VARIETY	34
3.1. Study of polysaccharides	34
3.2. Study of amino acids by gas-liquid chromatography	35
3.3. Quantitative determination of the amount of organic acids	36
3.4. Determination of the quantitative content of a number of phenolic compounds groups	37
3.5. Mineral composition of grass	38
Conclusions to chapter 3.....	40

CHAPTER 4. STUDY OF MORPHOLOGICAL AND ANATOMICAL CHARACTERS AND NUMERICAL QUALITY INDICATORS OF RAW MATERIALS OF THE <i>PLANTAGO MAJOR</i> PURPLE VARIETY	41
4.1. Morphological characteristics of raw materials	41
4.2. Anatomical characteristics of raw materials	42
4.3. Determination of numerical indicators of the quality of raw materials and the content of extractive substances	45
4.4. Sieve analysis	46
Conclusions to chapter 4	46
GENERAL CONCLUSIONS	48
LIST OF SOURCER USED	50
APPENDICES	57

LIST OF CONVENTIONAL ABBREVIATIONS

- AES – atomic emission spectrometry
- BAS – biologically active substances
- WSP – water-soluble polysaccharides
- GLC – gas-liquid chromatography
- SPhU – State Pharmacopoeia of Ukraine
- SPh XI – State Pharmacopoeia XI edition
- EPh – European Pharmacopoeia
- PC – pectin compounds
- PCh – paper chromatography
- TLC – thin layer chromatography
- SS – standard sample

INTRODUCTION

Actuality of theme. Expansion of the range of modern effective and safe medicines can be achieved by introducing new original medicines based on natural raw materials to the pharmaceutical industry. Modern science has investigated the pharmacological properties of many plants, but today there is still a large amount of research in the field of phytotherapy. Species of the genus *Plantago* are well-known and interesting to study and create medicinal preparations based on them

Species of the genus *Plantago* L. are found in different plant communities and exhibit ecological diversity. Plantains are quite widely represented in nature and play a specific role in various plant communities. In addition, most plants of this genus are widely used in folk and official medicine as anti-inflammatory, expectorant, wound healing, hemostatic and antiulcer agents. In recent years, varieties of *Plantago major* have become widespread. They are easily propagated, cultivated and can provide raw materials for pharmaceutical production. Therefore, the pharmacognostic study of *Plantago major* plant material of the Purple variety and the expansion of the directions of use is an urgent task of pharmacy.

The purpose of the work is the pharmacognostic study of *Plantago major* of the Purple variety and the determination of prospects for using the raw material as a source of BAS complex for the development of herbal preparations.

To solve the set goal, it was necessary to complete the task:

- conduct a literature search on the systematic position, botanical characteristics, issues of cultivation, chemical composition, medicinal use of species of the genus *Plantago* L;
- to investigate the chemical composition and quantitative content of the main groups of biologically active substances in plant raw materials;
- to investigate the morphological and anatomical structure of raw materials and to determine diagnostic signs;

- study the main numerical indicators of raw materials and the content of extractive substances depending on the type of extractant.

The object of the study is the grass *Plantago major* of the Purple variety, harvested on the plots of the botanical garden named Hryshka (Kyiv, 2021).

The subject of research is the study of the chemical composition of the herb *Plantago major* of the Purple variety; establishment of diagnostic macro- and microscopic signs, numerical indicators of raw materials.

Research methods. Macro- and microscopic (study of macro- and micropreparations of raw materials); physical (determination of loss in mass during drying, total ash, solubility); chemical (qualitative reactions of BAS identification); physical and chemical (paper chromatography, thin-layer chromatography, spectrophotometry in the UV and visible parts of the spectrum, atomic emission spectrometry); statistical processing of the results of the experiment "Microsoft Office Excell 2003", in accordance with the requirements of the State Pharmacopoeia of Ukraine.

Practical significance of the obtained results. The analysis of the results obtained during the experiment showed the perspective of using the herb *Plantago major* of the Purple variety for obtaining BAS complexes.

Scientific novelty of the obtained results. For the first time, a comprehensive pharmacognostic study of the herb *Plantago major* of the Purple variety was conducted.

For the first time, complexes of polysaccharides and pectin substances were obtained from the grass *Plantago major* of the Purple variety in the flowering phase, their content, total ash content, and mass loss during drying were determined. The component composition of sugars of water-soluble polysaccharides was studied.

For the first time in the herb *Plantago major* of the Purple variety, the amino acid composition and their content were determined by gas chromatography.

For the first time, hydroxycinnamic acids (chlorogenic, neochlorogenic, ferulic), flavonoids (quercetin, kaempfeol, rutin, hyperoside, luteolin) were discovered in the herb. Quantitative content of total flavonoids, total hydroxycinnamic acids, total polyphenols was studied.

For the first time, ascorbic, tartaric, and citric acids were identified, the content of ascorbic acid and the amount of organic acids were determined.

The mineral composition of the raw material was studied. The content of heavy metals was determined.

For the first time in the herb *Plantago major* of the Purple variety, the loss in mass during drying, the content of total ash, the content of extractive substances when using different extractants was determined.

For the first time, the morphological and anatomical diagnostic features of the herb *Plantago major* of the Purple variety were investigated.

Approbation of the results and publication. The results of scientific research are presented at the X International Scientific-Practical Conference «Modern achievements of pharmaceutical technology», on May 10-11, 2023, Kharkiv, National University of Pharmacy.

Gontova T., Kouritii A. The pharmacognostic study of the herb *Plantago major* Purple variety. X International Scientific-Practical Conference «Modern achievements of pharmaceutical technology» dedicated to the 60th anniversary of the birth of Doctor of Pharmaceutical Sciences, Professor Gladukh Ievgenii Volodymyrovych, which will take place on May 10-11, 2023, Kharkiv. 2023 – P. 190.

Structure and scope of qualification work. The qualification work is laid out on 56 pages of typewritten text, 48 pages of the main text, consists of an introduction, 4 chapters, general conclusions and a list of literary sources, appendices. The work is illustrated with 10 figures, 11 tables. The list of references includes 63 sources.

CHAPTER 1

PHARMACOGNOSTIC STUDY OF SPECIES OF THE PLANTAGO GENUS (Literature Review)

1.1 Botanical characteristics of the genus and varieties

The systematic position of *Plantago* is as follows:

Kingdom: Plantae (Plants), Type/Department: Angiosperms (Angiosperms)

Class: Magnoliopsida (Magnoliopsida, dicots), Order/Order: Lamiales (Lamiales), Family: Plantaginaceae (Plantain), Genus: *Plantago* (Plantain) [60, 63].

Studies of *Plantago* pollen have shown that as early as 4,000 years ago, this plant has been used for various purposes. The plant was spread throughout the world by Europeans; the Indians called it the "white man's footprint", as it appeared wherever Europeans went.

It should be noted the high plasticity of species and their ability to develop various ecotopes. The combination of different types of behavior in a changing environment, as well as a high degree of lability, is a necessary mechanism for the adaptation of species, which determines the possibility of conservation and long-term existence. Among the species of the *Plantago* genus, a great ecological diversity has been revealed. Among them there are ecologically highly specialized species and species with a high value of the tolerance index, i.e. widely specialized species capable of adapting to a different combination of environmental conditions [18].

The area of distribution is diverse from mountainous terrain to deserts, marshy places, species are found in North and South America, New Zealand, Australia, North Africa, and the Mediterranean. The plantain can be found near homes, along roads, in gardens, fields, meadows, pastures, along river banks, in clogged wet places, in steppes, and mountainous terrain [21].

About 200 species are known worldwide [60, 63]. Among the species of the *Plantago* genus are woody plants, perennial and annual herbs. About 18 species grow in Ukraine and they are grasses.

Among plant species, population studies cover quite widely such species of the *Plantago* genus as *P. major* L. and *P. lanceolata* L. These studies, which began in the 1920s and 1930s, were continued in the United States. Over the past 20 years, the program of Dutch researchers has been intensively developing, including the study of soil and phytocenotic aspects: morphology, physiology and biochemistry of plants; taxonomy and intraspecific variability of characters; ecology and genetics of populations. These species are well studied from a chemical point of view [62].

The following characteristics can be attributed to the general characteristics of species of the plantain genus (Table 1.1).

Table_1.1

**General morphological characteristics of species of the *Plantago* genus,
common in Ukraine**

Plant Organ	Characteristic
1	2
Underground organs	short rhizome with numerous adventitious roots forming a adventitious system
Leaves	long-petiolar leaves with a simple leaf blade and arcuate venation, collected in a basal rosette
Stems	leafless, erect, flower-bearing arrows
Inflorescences	Flowering stems with spike at the top

Contin. of Table 1.1

1	2
Flowers	Greenish small actinomorphic flowers are bisexual, the calyx is four-lobed or four-lobed, the corolla is tubular, short, membranous; stamens 4 or rarely 1-2, attached to the tube, gynoecium coenocarpous, pistil 1 with a two-lobed receptacle, ovary superior
Fruits	an egg-shaped, two-nested capsule with numerous, small, brown, dark-brown seeds. Seeds with fleshy endosperm. Most types of wind-swept

Plantago major L. (Fig. 1.1) is the most widespread species in Ukraine. It is distinguished by the following morphological features. The plant is perennial, up to 50 cm tall. The root system is fibrous with a short rhizome. The stem is shortened. The leaves are broadly elliptic or egg-shaped, the venation is arcuate (veins 5-6), the edge is solid. The inflorescence is a spike. The flowers are small, with a greenish-white or pink perianth. The fruit is an egg-shaped multi-seeded capsule.



Fig. 1.1 *Plantago major* L.

In addition to *Plantago major* L., the official species in Europe and Ukraine are *Plantago psyllium* L. and *Plantago lanceolata* L. [3]. Medicinal plant raw

materials are leaves, grass, roots of *Plantago major* L. and *Plantago lanceolata* L. and seeds of *Plantago psyllium* L., leaves - *Plantaginis majoris* folium and seeds - *Plantaginis majoris* seeds are used for the manufacture of medicines. Leaves and grass are harvested during flowering, seeds after ripening [15, 16, 17, 34].

The selection of species is aimed at increasing the yield and quality of products, resistance to diseases, pests and adverse changes in the external environment (drought resistance, winter resistance, resistance to lodging), creation of varieties suitable for mechanized cultivation at all stages of growth.

Among the species of the genus *Plantago* there are also modern varieties [4, 10]. *Plantago major* is more often used to create varieties. On the basis of this species, decorative varieties have been bred that differ in size, leaf color, and flower color (Fig. 1.2, Table 1.2).



Fig. 1.2 Varieties of plantain

1 – Purple, 2 – Purple Perversion, 3 – Variegata, 4 – Rosularis, 5 –Poltava.

Table 1.2

Morphological features of Plantago varieties

The name of the variety	Morphological features
Plantain variety Poltavs'kyi	medium-ripe variety; a perennial herb up to 40 cm tall with a short rhizome and secondary fibrous roots; with green, elongated-lanceolate leaves, up to 30-40 cm long; flowering stems are reddish, up to 40 cm tall; flowers are small green, bisexual, with an actinomorphic tubular corolla [63].
Plantain variety Variegata	a perennial grass with a short rhizome and secondary fibrous roots; with large long-petioled leaves, up to 40 cm long; leaf blade is green with white stripes; flowering stem is upright, up to 40 cm tall; flowers are small green, bisexual, with an actinomorphic tubular corolla.
Plantain variety Rosularis	a medium-sized perennial grass up to 20-30 cm tall with a short rhizome and secondary fibrous roots; leaves are green, long-petioled, ovate-elongated or wide-oval; peduncles in the shape of a rosette resemble heads of cabbage (hence the name "rose-shaped"). Seeds are not formed.
Plantain variety Purple (synonym Atropurpurea)	a medium-sized, winter-hardy decorative leafy perennial grass, up to 20-30 cm tall; long-petioled, wide-oval leaves of purple color; peduncles are green, which later acquires a red-brown color.
Plantain variety Purple Perversion	a medium-sized perennial herb up to 20-30 cm tall with decorative "corrugated" purple leaves with a wide oval blade and a wavy edge. At the place of the flower stalks rosettes are formed, as in the Rosularis variety.

1.2 Chemical composition of raw materials

Plantago major and other species of the genus contain different groups of biologically active substances. Polysaccharides were found in plantain leaves; mucus; flavonoids (apigenin, luteolin, baicalin and scutellarin); iridoids (aucubin - during hydrolysis it splits into glucose and aukubigenin, catalpol, aukuboside); vitamins (K, ascorbic acid, provitamin A); phenols and their derivatives (tyrosol); phenolic acids (vanillic, p-hydroxybenzoic, ferulic, p-coumaric, salicylic, benzoic, cinnamic, chlorogenic, neochlorogenic, methyl and ethyl esters of cinnamic acid, etc.); bitterness, tannins; steroidal saponins; traces of alkaloids, mineral salts [1, 2, 9, 23, 24, 33, 45]. The content of tannins - 4-5.7% [61].

Raw plantain contains triterpenic acids (sitosterolic, oleanolic, and ursolic) and monoterpenoids (linalool), which exhibit anti-inflammatory, cytotoxic, and antioxidant effects [56].

Free organic acids (citric, malic, tartaric, malonic, amber) were found in the grass of such species as *Plantago major* and *Plantago lanceolata* [13, 28, 45]. Studying the composition of organic acids of leaves *P. major*, fumaric, lilac, vanillic, p-hydroxybenzoic, ferulic, p-coumaric, gentisic, salicylic, benzoic, cinnamic acids [9, 33]. Alkaloids - indicanin, plantagonin - were isolated from the raw materials of *Plantago major* [56]. Also, the raw materials of species of the genus *Plantago* contain fatty acids, namely palmitic, stearic, oleic, linolenic [56].

The quantitative content of volatile compounds in the organs of *Plantago major* and *Plantago lanceolata* was determined. The highest content of volatile compounds was in flowers up to 0.04%. When studying the qualitative composition of *Plantago major* leaves, squalene (14%), β -ionone (8%), hexadecane (6.8%), linalool (5.4%), phenyl acetaldehyde (2.2%) were found. β -ionone (5.6%), squalene (5.1%), eugenol (4.1%) were found in the leaves of *Plantago lanceolata* [23, 33].

Aucubin is considered to be the main iridoid glycoside in the leaves of *P. major*, but its content varies greatly during the growing season; its highest content is noted in summer samples of raw materials (1.3%). The known lability of this compounds leads to the fact that aucubin may be completely absent in the raw materials used (the effect conditions of harvesting, storage, processing, etc.).

Polysaccharides obtained from plantain leaves are mainly pectin-like polysaccharides, which consist mainly of galacturonic acid together with galactose, arabinose and rhamnose [41, 58]. In the seeds of *Plantago major*, polysaccharides are represented by xylose (40%), arabinose (13%), galacturonic acid (17%), glucuronic acid (16%), and in the leaf by arabinose (10%), glucose (22%), galactose (8%), xylose (2%), galacturonic acid (55%). Polysaccharides of *Plantago lanceolata* leaves contain galacturonic acid (71%) and arabinose (29%) [11, 26]. The seeds and seed husks of *Plantago ovata* are high in polysaccharides, which are recognized by the FDA as dietary fiber [59, 31].

Plantain seeds are also rich in various biologically active substances. Thus, succinic acid was discovered from organic acids, aucubin from iridoids, as well as mucus, steroids, and saponins; tannins; fatty oil, which includes oleic, linoleic, linolenic, elaidic acids, triglycerides of oleic and linoleic acids, higher fatty acids *P. major* seeds contain free carbohydrates Glc, Frc, Xyl, Rha, Sac, and Plt [19, 37].

The outer walls of the seeds contains polysaccharides that swell in the presence of water and form highly viscous slimy solutions. The water-soluble polysaccharides of the seeds were found to be acid arabinoxylans Xyl:Ara:GalUA 61:13:24 and Xyl:Ara:Gal:GalUA 78:13:3:6 [50, 53].

Fatty acids, free and after hydrolysis of triglycerides, were isolated from seeds *P. major*. It has been established that about 65% of fatty acids are unsaturated (oleic 18:1 - 37.4%, linoleic 18:2 - 25.3%) [11].

1.3 Use in medicine

The use of plantain was mentioned by Dioscorides in *De Materia Medica* around the 1st century BC. AD, who used it for dog bites. *P. major* is described in the writings of an Islamic author of the 12th–13th centuries. Ibn El Beitar, who translated Greek medical texts. Plantain is even mentioned by Shakespeare in *Romeo and Juliet*. Simon Paulli describes *P. major* in *Flora Danica* (1648) as a very effective wound healing agent. Ethnopharmacological studies show that this plant has been used in almost all countries of the world for a wide variety of diseases [22, 37].

Species of the genus plantain are used in folk medicine and functional nutrition [20, 40, 42, 48, 52]. Recent studies have shown that plantain polysaccharides improve bowel function, exhibit hypoglycemic and immunomodulatory effects. The main types of raw materials are seeds and leaves, but there is information about the use of underground organs and grass.

Any part of the plant stops nosebleeds, hematemesis, treats epilepsy. Juice plantain in a warm form, introduced into the ear helps with ear pain. A decoction of the plant and the juice of the roots, when rinsed, it heals inflammation of the oral mucosa, toothache, cures all severe diseases of the oral mucosa [29, 31, 49]. Ingestion of psyllium seeds stops bleeding from internal organs [17, 36].

Condensed (in the Sun) psyllium juice, when taken internally treats tuberculosis, weight loss, hematemesis, lung ulcers, increased blood pressure, shortness of breath from the predominance of blood, epilepsy [29, 52]. Condensed juice strengthens the liver, kidneys and spleen hot natures, opens their blockages, soothes thirst normalizes the digestion of food. It stops the bleeding from all internal organs, treats gonorrhoea [52, 54].

Ingestion of its leaves, seeds opens blockage of the liver, kidneys, bladder, treats intestinal ulcers [39]. Plantain leaves boiled with salt, lentils and vinegar stop bloody diarrhea. If you drink plantain juice, condensed wine (musallas) treats pain

in the kidneys and urinary bubble [51]. Ingestion, its condensed juice and enema decoction seed cures intestinal ulcers [27, 31, 39].

Psyllium seeds are used as a laxative in the treatment of stomach ulcers. The seeds are also used for treatment of male and female infertility, diabetes. Leaves are considered a diuretic, antitumor agent [11; 12]. Plantain flowers are used for diarrhea. Plantain roots, in the form of extracts, are used in the treatment of fevers, tuberculosis of the lungs, as an antitussive crushed leaves mixed with the same amount of sugar and left warm place for 3 weeks. The juice allocated at the same time is consumed 3-4 times a day. Art. spoons for cancer of the lungs and stomach [29, 31].

Plantain lanceolate is used in the treatment malaria, enuresis. Its juice, mixed with honey, is used externally in the treatment of erysipelas [57]. A tincture of leaves in vodka is used for toothache.

Plantago major seeds showed pronounced antiproliferative activity against MCF-7, MDA-MB-231, HeLaS3, A549 and KB cell lines, as well as the highest inhibition of TNF- α , IL-1 β , IL-6 and IFN- γ production. The roots showed antiproliferative and inhibitory effects of cytokines. Seeds are the main source for the development of antitumor and anti-inflammatory drugs [24].

Plantago ovata (psyllium) is widely used in the food industry and medical practice. Metamucil and Mucilin are psyllium products that support blood glucose levels, lower cholesterol levels, and have a laxative effect [43, 46, 55]. In traditional Chinese medicine, psyllium is used for disorders of vital energy, pain, diuresis, and rheumatism, and in Brazilian medicine for wound healing [54]. In Indian medicine, plantain leaves and seeds are used as an anti-inflammatory and antibacterial agent [43].

Among plantain polysaccharides, pectins predominate, their molecular weight is usually lower than in seeds. Polysaccharides are usually resistant to digestion in the gastrointestinal tract and can reach the colon [53]. Thanks to this, they contribute to digestion and the work of the gastrointestinal tract, have

prebiotic potential and an adaptive effect on the condition of the pancreas, regulate metabolic processes in many systems and organs [27, 58]. When consumed, plantain polysaccharides are used by the intestinal microbiota, which contributes to the selective stimulation of the growth and activity of the intestinal microbiota [26, 27, 47]. A study of polysaccharides from the leaves of *P. major* showed that they are powerful immunomodulators [15].

Preparations of polysaccharides from species of the genus *Plantago* L. are known as expectorants, wound-healing, emollients, pain relievers and laxatives [16, 50].

Psyllium is used as a folk remedy for diarrhea, as psyllium husk can increase the amount of normal stool and decrease the amount of loose stool in patients with diarrhea [16, 58].

The drug "Mukofalk (orange)" granules (Dr. Falk Pharma GmbH., Germany) is used for chronic constipation; irritable bowel syndrome.

The preparations "Plantaglucid", "Plant juice" are used for hypoacid gastritis, peptic ulcer disease of the stomach and duodenum.

Preparations "Cough plantain syrup" by Dr. Theiss (Naturwaren, Germany), "Herbion Plantain Syrup" (KRKA, Slovenia), "Bronchial" (Dr. Muller Pharma, Czech Republic), "Plantain Syrup Dr. Tysa" (Germany), "Pectoral" (Mepha Ltd., Switzerland, "Tusavit" (Montavit GmbH, Austria, "Evkabal cough syrup" (Esparma, Germany) are used in the treatment of cough in infectious and inflammatory diseases of the upper respiratory tract (laryngitis, pharyngitis, tracheitis, bronchitis).

Infusion of leaves of *Plantago lanceolate*, *Plantago medium* is used as an antiseptic, anti-inflammatory, analgesic, wound-healing, expectorant. Galenic preparations, extracts from raw plantains have hemostatic, bactericidal, wound-healing, anti-inflammatory, hypolipidemic, analgesic, expectorant, secretory, diuretic properties [43].

It is known that plantain leaves have a hemostatic effect and are used to stop bleeding of various etiologies, namely external, hemorrhoidal, uterine, intestinal, gastric, nasal, pulmonary, and varicose ulcers. This effect is associated with the content of vitamin K1, C and flavonoids, which belong to the derivatives of benzo- γ -pyrone (phenylchroman) [2, 3, 9]. These BARs affect capillary walls and reduce their permeability. Thanks to vitamin K1, herbal preparations are used to reduce the cholesterol content in the blood plasma.

A decoction of plantain leaves or grass is prescribed for bronchitis, whooping cough and other diseases of the respiratory organs, accompanied by the release of thick secretions, with reduced stomach acidity, acute gastrointestinal diseases, namely gastritis, enteritis, enterocolitis. Decoction of leaves is used for stomatitis and gingivitis [30, 69].

An infusion of *Plantago media* leaves is drunk as a "blood purifier" for urticaria, heartburn, flatulence, and inflammation of the bladder [9, 53].

Plantago major leaf extract exhibits immunomodulating, hypoglycemic and antiviral effects, and the liquid extract has hepatoprotective and anti-inflammatory effects [16].

Antimicrobial activity of extracts from raw materials of *Plantago major*, *Plantago lanceolata* against strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus sp* has been proven [3].

Herbal preparations from plantain are used as anti-inflammatory, wound-healing agents in the treatment of acute pancreatitis, cystitis, pyelonephritis, skin diseases, as bactericidal - in infectious processes of the stomach, intestines, kidneys, bladder [15, 53, 57].

Plantain extract containing aucubin reveals a neuroprotective mechanism of hypoglycemic action in a rat model of diabetic encephalopathy.

Scientists associate the antioxidant, anti-inflammatory, and cytotoxic effects of *Plantago altissima* and *Plantago lanceolata* phytopreparations with the

accumulation of hydroxycinnamic acids and (chlorogenic, neochlorogenic) and flavonoids (apigenin, luteolin, luteolin-7-O-glucoside) [9].

Experimental studies have shown that the use of psyllium extract on the background of a high-fat diet leads to a significant decrease in body mass index, weightretroperitoneal and parauterine adipose tissue, smalldecrease in blood glucose concentration, prevents the development of oxidative stress [61].

Experimental studies have shown that the aqueous extract of the collection, consisting of *Mentha spicata* and *Plantago major*, has an anxiolytic and hypnogenic effect [48, 50].

Extracts of *Plantago asiatica* L. have a pronounced nephroprotective effect, prevent the development of nephrotic syndrome [51].

Therefore, species of the genus *Plantago* L. have a diverse composition of biologically active substances, the wide distribution of wild species, breeding and cultivation of varieties of plantain determines the prospects of their pharmacognostic study.

CHAPTER 2

STUDY OF GROUPS OF BIOLOGICALLY ACTIVE SUBSTANCES IN RAW MATERIALS OF THE *PLANTAGO MAJOR* PURPLE VARIETY

2.1 Research objects, reagents and devices

1. The objects of the study are the grass *Plantago major* Purple variety. The raw materials were harvested at the experimental site of the botanical garden named after Hryshka (Kyiv, 2021) [5]. Preparation and drying of raw materials, obtaining extracts (water, water-alcohol), and pharmacognostic analysis were carried out in accordance with the requirements of SPhU and SPh XI [5, 6, 7, 8]. To carry out the experiment, the raw material was crushed to 3-5 mm (shredder brand CO 124A).

2. Water and water-alcohol extracts were obtained for preliminary chemical analysis of raw materials. Aqueous extracts were obtained from 10.0 g of air-dry raw material, 100 ml of purified water was added, and heated on a boiling water heater in a reflux flask for 1 hour. The extraction was carried out three times with new volumes of purified water. The obtained extracts were combined, filtered, evaporated under vacuum to the volume (1:1). Amino acids, polysaccharides, flavonoids, hydroxycinnamic acids, tannins, organic acids, ascorbic acid were found in aqueous extracts. Alcoholic extracts were prepared as described above using 40% and 70% ethanol. Chloroform (1:1) was added to the combined alcohol extracts to remove lipophilic compounds. The supernatant was heated on a water heater until traces of chloroform were removed. It was cooled, filtered, washed with ethyl acetate. The combined fractions of the ethyl acetate extract and the aqueous residue were separately evaporated under vacuum and used for the identification of flavonoids and hydroxycinnamic acids [34].

3. To identify individual substances, TLC methods were used on "Silufol" plates and PC using "Filtrak" paper (FN-3). During the experiment, we used 3

solvents, reagents that met all SPhU requirements [38, 47]. Chromatography was performed in an upward stream of solvents with one- or two-fold acceleration at $T = 25\text{ }^{\circ}\text{C}$.

To perform TLC analysis, solvent systems were used, which were prepared in the ratio of components:

- N^o. 1 n-butanol – pyridine – purified water (6:4:3) for determination of carbohydrates;
- N^o. 2 ethyl acetate – acetic acid – formic acid – purified water (100:11:11:25) to determine organic acids;
- N^o. 3 acetic acid 15% for the determination of flavonoids, ascorbic acid, hydroxycinnamic acids;
- N^o. 4 5 n-butanol – acetic acid – purified water (4:1:5); for the determination of flavonoids, hydroxycinnamic acids [47].

Substances were detected on chromatograms in visible light, by color or by fluorescence in UV light before and after treatment with appropriate reagents (3% solution of iron(III) chloride; 10% alcoholic solution of sodium hydroxide; 0.04% aqueous solution of sodium 2,6-dichlorophenolindophenolate, ammonium hydroxide vapors).

4. UV absorption spectra were taken on a SF-46 spectrophotometer in a cuvette with a layer thickness of 10 mm at the appropriate wavelengths [30].

5. The qualitative composition of polysaccharides was studied after acid hydrolysis using qualitative reactions [11, 38, 47].

To obtain water-soluble polysaccharides, 5 g (exactly weighed) of crushed raw materials were placed in a flask with a capacity of 250 ml with a ground stopper, and 100 ml of purified water was added. The flask was connected to a reflux condenser, boiled for 30 min, cooled, and filtered. 96% ethanol (three times the volume) was added to 5 ml of the extract.

Observation: loose white flakes were formed, which fell into the sediment. The precipitate was filtered, put into a test tube, 5 ml of diluted hydrochloric acid

was added, hydrolyzed by boiling for 1 hour. The hydrolyzate was cooled, 10 ml of Fehling's reagent was added, and boiled for 5 min.

The result of the reaction is the formation of a brown sediment (the presence of monosaccharides).

Identification of sugars was carried out by the method of paper chromatography in system N° 1 and N° 4.

Standard samples of glucose, fructose, galactose, rhamnose, arabinose, glucuronic and galacturonic acids were used for identification. Chromatograms were treated with aniline phthalate reagent and heated in an oven at $T=100-105\text{ }^{\circ}\text{C}$ for 5 minutes.

The quantitative content of the sum of water-soluble polysaccharides and pectin substances was determined by the gravimetric method according to the method described in the monograph "Big-leaved plantain" SPhU [6-8].

6. *Identification and determination of ascorbic acid content.* Ascorbic acid was detected by TLC methods on Sorbfil plates in system No. 2 and PC in system No. 3, followed by treatment with a 0.04% aqueous solution of sodium 2,6-dichlorophenolindophenolate. The content of ascorbic acid was determined by the volumetric titration method [38, 47].

Organic acids. Organic acids were determined in aqueous extracts using PC in system No. 2 compared to authentic samples. Chromatograms were treated with a reagent of 0.03% solution of bromocresol green methanol [38, 47]. Quantitative determination of the amount of organic acids was carried out according to the SPhU method in terms of malic acid.

8. *Amino acids.* For the study of amino acids, aqueous extracts were used, the developer was a 0.2% alcoholic solution of ninhydrin, followed by heating in a water bath for 5 min at $T=70-80\text{ }^{\circ}\text{C}$ [38, 47]. When the extracts were cooled, a blue color was observed, which confirms the presence of amino acids.

For chromatography, PC, solvent system No. 5 was used, after processing the chromatogram was heated to $T=105\text{ }^{\circ}\text{C}$ for 3 min. Detection of substances on

chromatograms was carried out by the color of the spots and the value of R_f. The gas chromatography method was used to determine the amino acid composition and content of components. Chromatography conditions are selected [38, 47].

9. *Phenolic compounds*. Phenolic compounds were detected using qualitative test-tube reactions (Table 2.1.)

Table 2.1

Results of detection of compounds of phenolic nature in the herb

Plantago major Purple variety

Group of BAS	Conditions of the experiment	Result
1	2	3
Phenolic compounds	Add to 1 ml of aqueous extract. 5 ml of ammonia solution and 2 ml of 10% sodium phosphate-molybdenum solution in hydrochloric acid.	Dark blue color
Flavonoids are flavone derivatives	Cyanidine reaction. Add to 1 ml of extract. 2 drops of concentrated hydrochloric acid and 0.05 g of magnesium metal powder.	Red color
Flavonoid glycosides and aglycones	Cyanidine reaction according to Briant. After carrying out the cyanidin reaction, 0.5 ml of n-octanol was added to the colored solution and shaken.	The color of the lower layer is crimson (flavonoid glycosides), the color of the upper layer is pink (aglycones)
Hydroxycinnamic acids	Reaction with a 3% solution of iron(III) chloride with a 50% alcohol extract (1:5)	Green-gray color

Contin. of Table 2.1

1	2	3
Flavonoids	Reaction with 10% alkali solutions. Add 2 drops of 10% water-alcohol solution of potassium hydroxide to 1 ml of alcohol extract.	Yellow precipitate (flavones, chalcones, aurones)
Tanning substances pyrocatechin derivatives	3 drops of 1% iron(III) chloride solution were added to 0.5 ml of the aqueous extract.	Green color
Condensed tannins	2 drops of a 1% iron-ammonium alum solution were added to 2.0 ml of the aqueous extract.	Black-green precipitate

Identification of hydroxycinnamic acids was carried out by the PC method in solvent systems No. 3 and 4. Detection of substances on chromatograms was carried out by fluorescence in UV light before and after treatment with ammonia vapors and diazo reagent, as well as by comparative values of R_f with standard samples.

Identification of flavonoids was carried out by the PC method in systems No. 3 and 4. Chromatograms were studied in UV light before and after treatment with ammonia vapors, 2% KOH (alcohol solution), $AlCl_3$ solution. The R_f value results are the average values of five determinations [38, 47].

To obtain UV absorption spectra, a complexing reagent - aluminum chloride was used. UV absorption spectra for the determination of flavonoids were recorded on SF-46 in the range from 200 to 700 nm. The content of the sum of hydroxycinnamic acids was determined by the SF method in the region of 325 nm, the content was calculated in terms of chlorogenic acid [38, 47, 8].

Sample preparation for the quantitative determination of flavonoids, hydroxycinnamic acids in raw materials was carried out according to the standardized methods of SPhU [6-8].

Spectrophotometric determination of anthocyanins was carried out according to the method of SPhU 2.0 monograph "Blueberry fruits dried" in terms of cyanidin-3-O-glucoside chloride [7, 8].

The quantitative content of the sum of polyphenolic substances in raw materials was determined by the SF method [12].

10. *Mineral composition of raw materials.* Determination of the qualitative composition and quantitative content of minerals in raw materials was carried out on the DSF-8 device using the method of atomic emission spectrometry. The content of heavy metals was determined according to SPhU methods [ДФУ].

11. *Macro- and microscopic analysis* of raw materials was carried out according to the SPhU 2.8.23 method [7, 14]. Morphological studies of raw materials were carried out using a magnifying glass and a BM binocular microscope. The anatomical structure of the epidermis of leaves and flowers was studied on preparations from the surface, stems - on transverse and longitudinal sections. Canon digital camera, MBI-6 and Biolam (LOMO) microscopes for working in direct and reflected light were used to record the results of the experiment. The photos were partly processed using the Adobe Photoshop CS2 9.0 program.

12. *Pharmacognostic studies* were carried out using the analysis methods of SPhU 2.8 "Methods of pharmacognosy" and SPh XI edition [5, 6-8]. The used methods of pharmacognostic research are presented in Table 2.2.

Table 2.2

Methods of pharmacognostic research of raw materials

Pharmacognostic research	Methods	Sources of literature
1	2	3
Raw material sampling and sample preparation	SPhU monograph 2.8.20 "Medicinal plant raw materials: sampling and sample preparation"	12

Continuation of Table 2.2

Determination of the dry residue of the extract	SPhU monograph 2.8.16 "Determination of the dry residue of extracts"	12, 13
Determination of the content of foreign impurities in raw materials	SPhU 2.8.2 "Foreign impurities in medicinal plant raw materials".	12, 13
Determination of total ash in accordance with the requirements of the monograph	SPhU 2.4.16 "Common ash"	12, 13
The content of extractive substances in accordance with the requirements of the monograph	SPh XI edition	6, 12

13. The results were processed using the method of mathematical statistics in accordance with the requirements of the SPhU monograph [7].

2.2 Monosaccharide composition of polysaccharides

As seen from the literature review (Chapter 1) Plantain has been used in medicine for thousands of years for diabetes, obesity, constipation, diarrhea, which is due to a complex of biologically active substances, including polysaccharides. The function and mechanism of psyllium polysaccharides have been investigated and gradually elucidated.

Preliminary studies of raw material samples with Fehling's reagent confirmed the presence of polysaccharides in the large purple plantain grass. Identification of monosaccharides was carried out after hydrolysis of polysaccharides by the PC method (Chapter 2).

The results are presented in fig. 2.1.

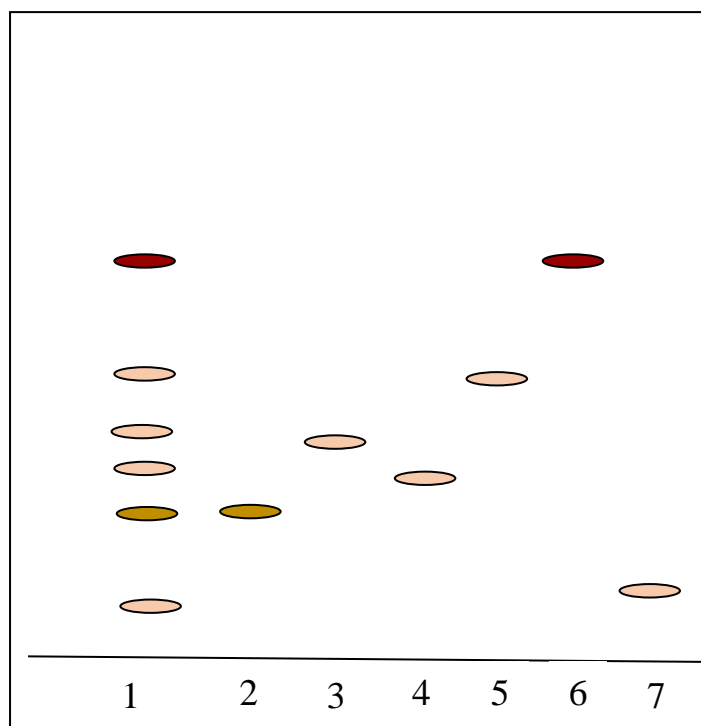


Рис. 2.1 Scheme of the chromatogram of monosaccharides after hydrolysis of polysaccharides grass *Plantago major* Purple variety

1 – extract, 2 – glucose, 3 – galactose, 4 – fructose, 5 – arabinose, 6 – rhamnose, 7 – mixture of galacturonic and glucuronic acids

According to the results of chromatography were detected glucose ($R_f=0.39$), fructose ($R_f=0.51$), galactose ($R_f=0.44$), rhamnose ($R_f=0.59$), arabinose ($R_f=0.50$), glucuronic ($R_f=0.22$) and galacturonic acid ($R_f=0.15$).

2.3 Identification of amino acids

Amino acids are found in all parts of the body and play several roles in the human body [1, 40]. They take part in the synthesis of proteins and precursors in the formation of secondary molecules of metabolism [42].

The therapeutic use of amino acids is important for official medicine. The most well-known areas of therapeutic application of amino acids are the treatment of brain metabolism and neurotransmission imbalances, immune system disorders,

cardiovascular and gastrointestinal health [40], treatment of liver diseases, fatigue, skeletal muscle damage, cancer prevention, burns, injuries and sepsis, urinary diseases and diabetes.

According to the results of the test tube reaction, grass *Plantago major* Purple variety contains free amino acids. Using paper chromatography, amino acids were detected in aqueous extracts (Fig. 2.3).

8 free amino acids were identified, of which 4 were replaceable and 5 were essential.

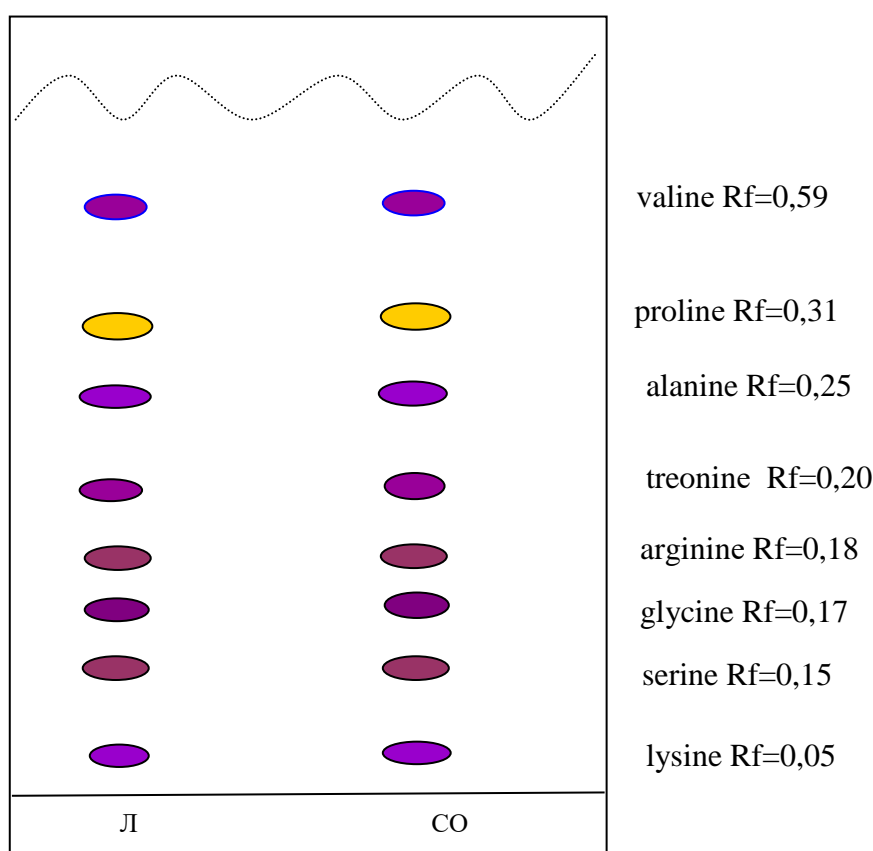


Fig. 2.2 Scheme of the chromatogram of free amino acids grass *Plantago major* Purple variety

2.4 Qualitative analysis of ascorbic acid and organic acids

Organic acids are promising chemicals because they are building blocks for the production of other valuable products used in the chemical, food, and pharmaceutical industries [28]. L-ascorbic acid (vitamin C) plays an important role

in the vital activity of the human body. Due to the presence in the L-ascorbic acid molecule of the enediol group $C(OH) = C(OH)$, it has strongly pronounced reducing properties; participates in the regulation of redox processes, carbohydrate metabolism, blood clotting, tissue regeneration, the formation of steroid hormones. Plant organic acids are active metabolites, are involved in the synthesis amino acids, detoxification of heavy metals in plants. Ascorbic acid, lemon, apple are important for normal functioning of the human body, take part in metabolic processes, regulate the activity of the digestive system, have bactericidal, antioxidant action, help to reduce the inflammatory response, accelerate tissue regeneration, activate cellular respiration, protein synthesis [45].

The qualitative composition of ascorbic acid and organic acids was determined by the paper chromatography method [5, 7, 13]. Chromatograms were set in the system ethyl acetate – acetic acid – formic acid – purified water (100:11:11:25), treated with the reagent 0.04% sodium 2,6-dichlorophenolindophenolate. A white spot on a pink background was observed ($R_f=0.68$), which confirms the presence of these substances (Fig. 2.4).

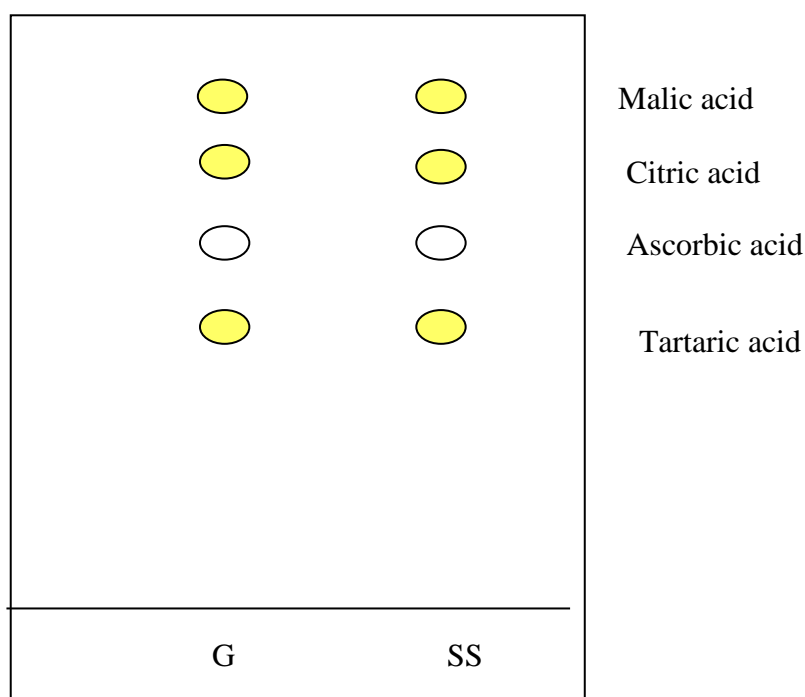


Fig. 2.4 Scheme of the chromatogram of organic acids:

G - is grass, SS - is a standard sample

Organic acids in the water extracts of the plantain grass of the large variety Purple were detected by Rf and the color of the spots in yellow color after processing the chromatograms with a 0.03% solution of bromocresol green methanol (Fig. 2.4).

As a result of the experiment, malic (Rf=0.63), citric (Rf=0.47) and tartaric (Rf=0.38) acids were identified in the raw material.

2.5. Qualitative composition of phenolic compounds

In recent years, many studies in the field of plant physiology and biochemistry are associated with the diversity and biological action of phenolic compounds - substances of secondary metabolism formed in plants [46].

According to the literature, phenolic compounds of species of the genus *Plantago* L. show anti-inflammatory, antispasmodic, hemostatic, antioxidant, hypoazotemic, hypoglycemic, antimicrobial, diuretic, antisclerotic, hepatoprotective, P-vitamin effects [9, 12, 33 49, 56]. Flavonoids also include anthocyanins, which are water-soluble pigments that color fruits, leaves, petals in a variety of shades from pink to violet [32]. Anthocyanins are found as glycosides in vacuoles. It is confirmed that anthocyanins are stress metabolites whose biosynthesis is activated during effects on plants of unfavorable environmental factors that anthocyanins can serve as markers of the level of plant adaptation to stressful conditions. The antioxidant effect of phenolic compounds on plant and animal organisms is especially widely studied.

According to the results of test-tube reactions, the presence of compounds of phenolic nature in plantain grass of the large variety Purple was confirmed (Table 2.1). The next stage was the study of the qualitative composition of hydroxycinnamic acids, flavonoids using PC in systems 3 and 4 and TLC in system 5 (Table 2.3).

Table 2.3

**Results of chromatographic analysis of phenolic substances in extracts
from plantain grass of the large variety Purple**

№ zone	Substance	R _f		Coloration in UV light after treatment with ammonia vapors
		Literary sources	PC analysis	
Hydroxycinnamic acids				
1	Ferulic acid	0,88	0,86	Emerald blue
2	Neochlorogenic acid	0,62	0,61	Emerald blue
3	Chlorogenic acid	0,63	0,63	Emerald blue
Flavonoids				
4	Kaempferol	0,77	0,76	Yellow
5	Quercetin	0,73	0,71	Yellow
6	Hyperoside	0,66	0,65	Dark yellow
7	Routine	0,50	0,51	Dark yellow
8	Luteolin	0,43	0,42	Dark yellow

Analysis of chromatograms showed that the extracts of the grass contain ferulic, chlorogenic and neochlorogenic acids, which had a bright blue glow in UV light, and when treated with ammonia vapors, the spots acquired an emerald-violet color. Flavonoids are represented by aglycones (kaempferol, quercetin) and glycosides (rutin, luteolin, hyperoside).

Conclusions to chapter 2

1. Based on the results of the preliminary analysis of the large purple plantain grass, polysaccharides, amino acids, organic acids and compounds of phenolic nature were found.
2. Polysaccharides of the purple plantain grass are represented by glucose, fructose, galactose, rhamnose, arabinose, glucuronic and galacturonic acids. Of the free amino acids, valine, proline, alanine, threonine, arginine, glycine, serine and lysine were found.

3. Malic, tartaric, citric and ascorbic acids have been identified as organic acids.
4. Phenolic compounds are represented by chlorogenic, neochlorogenic, ferulic acids, quercetin, kaempfeol, rutin, hyperoside, luteolin.

CHAPTER 3

**STUDY OF THE QUANTITATIVE CONTENT OF THE MAIN
BIOLOGICALLY ACTIVE SUBSTANCES IN THE RAW MATERIALS OF
THE *PLANTAGO MAJOR* PURPLE VARIETY**

3.1 Study of polysaccharides

The analysis of literary scientific sources showed that polysaccharides and pectins are responsible for a number of pharmacological activities of species of the genus *Plantago* (Chapter 1). Fractions of polysaccharides and pectin substances from grass *Plantago major* Purple variety were obtained by well-known methods and their indicators of complexes were determined, namely total ash, total protein content, loss in mass during drying [7, 8, 9, 11, 16, 25, 38, 47]. The results are given in the table. 3.1.

Table 3.1

**Indicators of polysaccharide fractions from the herb *Plantago major* Purple
variety (m=5, $\bar{X} \pm \Delta X$)**

Indexes	Herb	
	Polysaccharides	Pectin substances
The content of the fraction ¹	10,88 ± 0,49	6,76±0,46
Loss in mass during drying ²	6,08±0,44	7,45±0,53
Total protein ²	8,79±0,41	-
Total ash ²	5,31±0,58	5,17±0,37

Notes: 1 – based on absolutely dry raw materials,

2 – based on the completely dry residue

The content of the polysaccharide fraction was 10,88%, and pectin content was 6,76%, which is 1.6 times less. The protein content in the grass was 6,08%.

The content of total ash in the polysaccharide and pectin fractions was almost the same and amounted to 5,31% and 5,17%, respectively. This variety of plantain is promising for further study from the point of view of the content of polysaccharides and pectin substances.

3.2 Study of amino acids by gas-liquid chromatography

Preliminary analysis of grass *Plantago major* Purple variety revealed free amino acids (Chapter 2). The qualitative composition and quantitative content of amino acids after hydrolysis of the grass extract was determined by gas-liquid chromatography. The results are shown in Table 3.2.

Table 3.2

Amino acid composition of the herb *Plantago major* Purple variety

№	Amino acid name	Contents *, %
1	2	3
Essential amino acids		
1	Valine	0,96
2	Leucine	0,59
3	Isoleucine	0,58
4	Threonine	0,46
5	Methionine	0,17
6	Lysine	0,46
7	Phenylalanine	0,54
8	Arginine	0,98
Substitute amino acids		
9	Alanine	1,05
10	Glycine	0,84

Contin. of Table 3.2

11	Serine	0,56
12	Tyrosine	0,17
13	Histidine	0,19
14	Aspartic acid	0,65
15	Glutamic acid	1,18
16	The sum of essential amino acids	4,74
17	The sum of substituted amino acids	4,64
18	The total amount of amino acids	9,38

Note. * – in terms of completely dry raw materials

The content of the sum of substitutable and non-substitutable acids was almost the same in the hydrolysates of plantain grass extracts. The total content of amino acids was 9.38%. Of the essential acids, arginine 0.98% and valine 0.96% accumulated in larger quantities. Phenylalanine, leucine and isoleucine accumulated in almost the same amount. Of the substitute acids, alanine 1.05%, glutamic acid 1.18% and glycine 0.84% accumulated in larger quantities.

3.3 Quantitative determination of the amount of organic acids

According to previous studies (Chapter 2), the herb *Plantago major* Purple variety contains malic, citric, tartaric and ascorbic acids as organic acids.

Analysis of the literature showed that organic acids accumulate in the raw materials of all plants and exhibit antimicrobial, anti-inflammatory, antipyretic and other effects [28]. Ascorbic acid takes part in metabolic processes, which is associated with antioxidant activity, which is enhanced in combination with flavonoid substances [9]. Therefore, at the next stage, the content of ascorbic acid and the amount of organic acids in the herb *Plantago major* Purple variety was

determined. Quantitative content of ascorbic acid and amounts of free organic acids were determined by chemical titration. The results are given in the table. 3.3.

Table 3.3

Quantitative content of ascorbic acid and the amount of free organic acids in the herb *Plantago major* Purple variety

Quantitative content, y % ($\bar{x} \pm \Delta \bar{x}$), %, $\mu=6$	
Ascorbic acid	Amounts of free organic acids
0,34 ± 0,07	0,56 ± 0,04

The analysis of experimental data showed that the content of the sum of organic acids was 1.65 times higher in the grass than ascorbic acid.

3.4 Determination of the quantitative content of a number of phenolic compounds groups

Phenolic substances (PCs) are found in various plant organs and perform appropriate functions [35, 46]. They contribute to the coloring of flowers, leaves and fruits, attract pollinators and seed dispersers. They perform a protective function against the action of ultraviolet radiation, eating by herbivores and disease-causing organisms. PCs in plants play an important role in growth control and have antioxidant, structural, attractant, signaling and protective functions [4, 9, 32, 49].

According to preliminary data, hydroxycinnamic acids, flavonoids and tannins were found in the herb *Plantago major* Purple variety. The red color of the leaves indicates that the cytoplasm of the cells contains anthocyanins.

Therefore, we determined the amount of hydroxycinnamic acids by spectrophotometry in terms of chlorogenic acid (wavelength 325 nm), the amount of flavonoids in terms of rutin (wavelength 410 nm), the amount of anthocyanins

in terms of cyanidin-3-O-glucoside chloride (wavelength 528 nm), and polyphenolic substances in terms of gallic acid (wavelength 270 nm) [38, 47, 6-8].

The results of the study are presented in table. 3.4.

Table 3.4

The results of determining the quantitative content of the main groups of biologically active substances

A group of biologically active substances	Content, y %*
The amount of hydroxycinnamic acids	3,05 ± 0,05
The amount of flavonoids	0,87 ± 0,03
The amount of anthocyanins	1,32±0,05
The amount of polyphenolic substances	2,02 ± 0,03

Note. n =5, «*» – in conversion to absolutely dry raw materials

The amount of hydroxycinnamic acids was 3,05 %, the amount of flavonoids was 0,87 %, sum of anthocyanins was 1,32 %, the amount of polyphenolic substances was 2,02 %.

Therefore, the raw material of *Plantago major* Purple variety is promising for the development of complexes of biologically active substances.

3.5 Mineral composition of grass

Minerals are a mandatory component of dew raw materials. They are important in the biochemical processes of plants. They are important in mineral nutrition and plant growth and development. The root system of plants absorbs minerals in the form of ions from the soil, which are Ca, K, Na, Zn, Mn and Fe. Minerals are included in the composition of phytopreparations and affect their pharmacological activity [61]. But it can also be toxic minerals, such as arsenic and

copper, cobalt, strontium and mercury. Therefore, modern pharmacy has requirements for the content of heavy metals in raw materials.

Therefore, as part of the pharmacognostic analysis, the content of macro- and microelements in the herb *Plantago major* Purple variety was investigated. The data of the experiment are given in table 3.5.

Table 3.5

Study of the mineral composition of raw materials

Chemical element	Quantitative content (мг/г)
Macroelements	
Mg	9,21
Ca	11,56
P	2,43
Si	10,17
K	14,20
Na	9,85
Microelements	
Al	1,04
Mn	1,46
Fe	1,27
Cu	0,09
Sr	0,001
Zn	1,08

Note. Hg, As, Cd, Co, Pb, Ni, Mo < 0,03

Analysis of mineral glass herb *Plantago major* Purple variety revealed 19 elements. Of the macroelements, K, Ca, and Si accumulated in significant amounts (14.20, 11.56, and 10.17 mg/g, respectively). Of the trace elements, Fe, Mn, Al, and Zn were accumulated in significant amounts (1,27, 1,46, 1,04, and 1,08 mg/g, respectively). The content of heavy metals met the requirements of the SFU for the quality of raw materials.

Conclusions to chapter 3

1. The content of Polysaccharides and Pectins in the herb *Plantago major* Purple variety was 10.8% and 6.76%, respectively. For both complexes, the loss in mass during drying, the content of total protein, and the content of total ash were determined.
2. The component composition of 15 amino acids and their content, as well as the total content of amino acids (9.38%), the content of the sum of substituted (464 %) and essential (4,74 %) amino acids, was determined by the method of gas chromatography.
3. The content of organic acids and vitamin C in the herb *Plantago major* Purple variety was (0,34 % i 0,56 % in accordance).
4. Phenolic compounds accumulated in the herb *Plantago major* Purple variety in the following ratios: sum of oxycinnamic acids (3.05%), sum of flavonoids (0.87%), sum of anthocyanins (1.32%) and sum of polyphenols (2.02%).
5. The mineral composition of the raw material is represented by 19 minerals, the content of heavy metals in the raw material met the requirements of the SPhU.

CHAPTER 4
STUDY OF MORPHOLOGICAL AND ANATOMICAL CHARACTERS
AND NUMERICAL QUALITY INDICATORS OF RAW MATERIALS OF
THE *PLANTAGO MAJOR* PURPLE VARIETY

4.1 Morphological characteristics of raw materials

The raw material of *Plantago major* Purple variety is an herb harvested in the summer during the flowering phase (Fig. 4.1).



1



2



3



4

Fig. 4.1 Morphological features of *Plantago major* Purple variety herb.

1 – spring basal rosette of leaves, 2 – summer rosette of leaves, 3 – rosette of leaves with flowering shoots, 4 – flowering shoots.

The grass is represented by large leaves with long strong petioles. The leaf blade is wide-oval, the edge is solid, the veins protrude from the lower side. The venation is arcuate. Leaves in the phase of rosette formation (Fig. 4.1.1) are grayish-green with a brownish tint, in the phase of flowering - burgundy. The leaf blade is 25 to 35 cm long and 8 to 15 cm wide. The flowering stem is unbranched, leafless, erect, up to 25-35 cm long. The spike inflorescence is located at the top, the flowers are small, whitish-brown, sessile or on a short peduncle, bisexual, corolla actinomorphic tubular, calyx 4-parted, stamens 4, they are attached to the tube of the corolla. The gynoecium is coenocarpous with a two-lobed receptacle, the ovary is in the upper position. The fruit is a small box with small brown seeds.

Dried raw materials of *Plantago major* Purple variety are represented by whole or cut fragments of leaves, stems, and flowers. Veins are clearly visible on the surface of the leaf plate. Petioles are rough, fibrous. The raw material may contain fruits - fruitcases with small shiny seeds.

4.2 Anatomical characteristics of raw materials

Considerable attention is paid to the study of the anatomical structure of species of the genus *Plantago*. Species have general anatomical features and individual ones [44].

We studied the anatomical features of the leaves of *Plantago major* Purple variety herb harvested in summer. The upper epidermis of the leaf consists of parenchymal, large, sinuous-walled, thin-walled cells (Fig. 4.2.1). Stomata are frequent, large, oval, randomly arranged. There are four white proboscis cells, they do not differ from epidermal cells. The type of stomatal apparatus is anomocytic. Stomata with 2 near stomatal cells closing perpendicularly are rare. The type of stomatal apparatus is diacytic. The cuticle layer is thin, folded type. The epidermis on the lower side of the leaf consists of parenchyma cells of various sizes, with weakly wavy or even membranes, which have weak thickenings of secondary cell

membranes and are pierced by straight pores (Fig. 4.2.2). On the surface of the epidermis there are trichomes with rosettes of cells at the base. Rosettes consist of 5-7 radially arranged large cells. The epidermis above the veins (Fig. 4.3.3) is formed by long, straight-walled cells with slightly thickened membranes. There are also rare trichomes along the veins (Fig. 4.3).

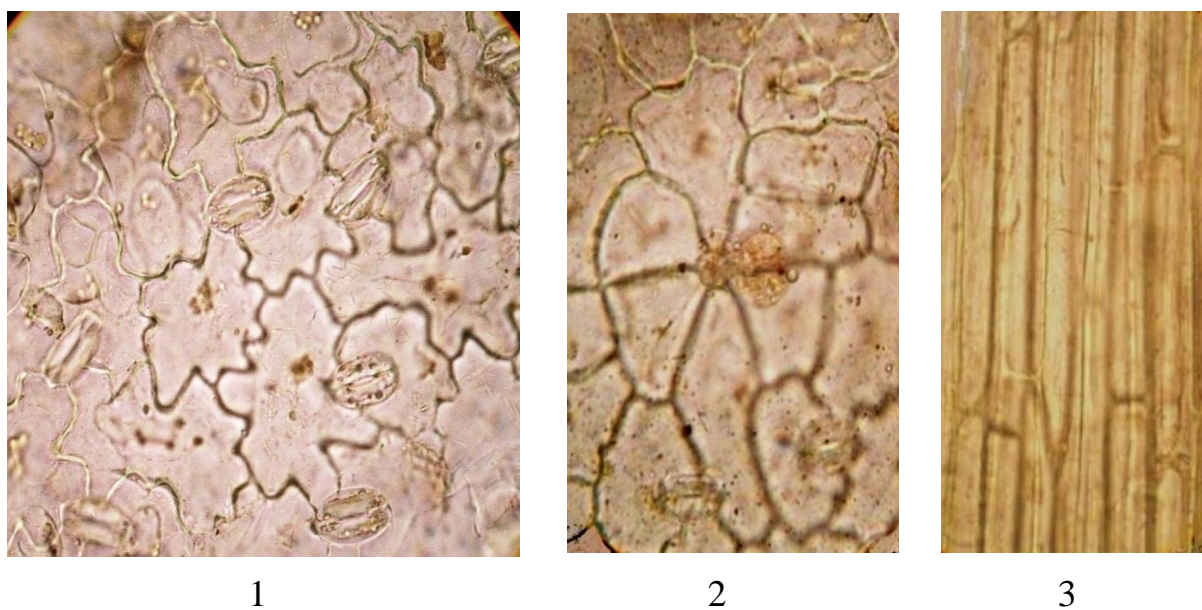


Fig. 4.2 Microscopic signs of *Plantago major* Purple variety herb.

1 – upper epidermis, 2 – lower epidermis, 3 – epidermis above the vein.

There are two types of trichomes - covering and glandular (Fig. 4.3). Hairs are simple, 1-3-celled, cone-shaped, thick-walled, with an expanded rounded thickened basal cell (Fig. 4.3. 1, 2), long multicellular hairs are rarely found along the veins, with a 5-11-celled radial rosette around a dome-shaped basal cell (Fig. 4.3.4, a, c, 4.3.5). Glandular hairs have a one-cell elongated stalk and a two-cell oval head with a dark secretion (Fig. 4.3.3).

The petiole of the leaf (Fig. 4.4) in the basal part is semi-lunar in shape, with wing-shaped outgrowths strongly protruding upwards with thin tops. The adaxial side of the leaf is strongly concave, while the abaxial side is strongly convex, slightly wavy in outline under the fascicles. Under the epidermis there are several layers of

angularly and lacunose collenchyma. The bundles are small, the main one in the central part of the petiole and three on the sides. The parenchyma is homogeneous.

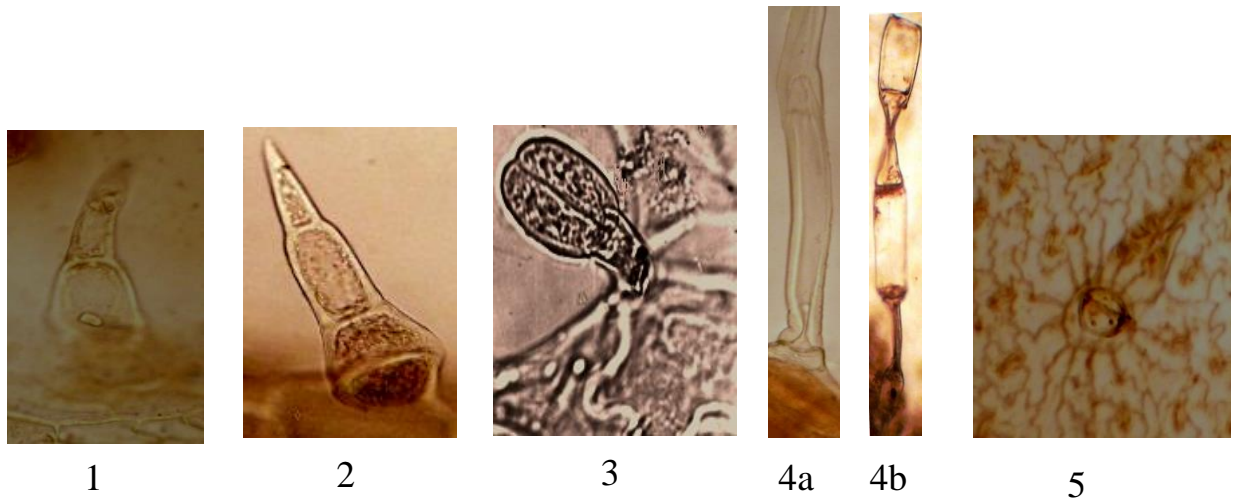


Fig. 4.3 Trichomes *Plantago major* Purple variety herb.

1 – 2-cell covering hair, 2 – 3-cell covering hair, 3 – glandular hair, 4 – long multicellular hair: a – base, b – body, 5 – rosette.

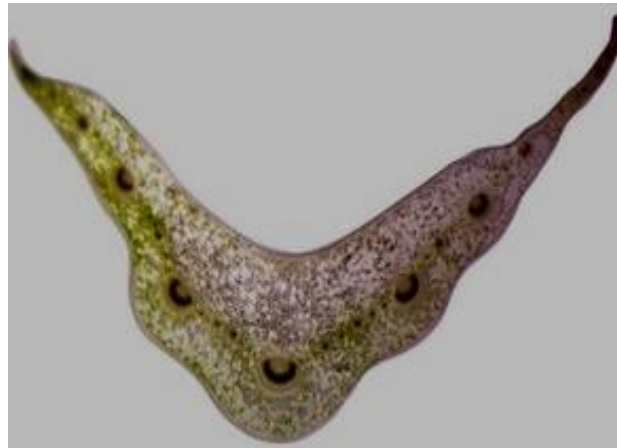


Fig 4.4 Petiole *Plantago major* Purple variety herb

Thus, the conducted morphological and anatomical studies of *Plantago major* Purple variety herb made it possible to identify the diagnostic signs of the leaves, which can be used in the future in the identification of the variety, the development of documentation for the standardization of raw materials.

4.3 Determination of numerical indicators of the quality of raw materials and the content of extractive substances

Determination of the quality of the raw material *Plantago major* Purple variety herb was carried out according to the following indicators: extraneous impurities, loss in mass during drying, total ash in accordance with the requirements of the SPhU methods 2.8.2. [7, 8]. The content of extractive substances was also investigated, depending on the type of extractant that was used. The methods are described in Chapter 2. The results are presented in the table. 4.1.

Table 4.1

Quality indicators of raw materials of *Plantago major* Purple variety herb and content of extractive substances in extracts

Indexes	Content in grass, in %
Brown particles	2,68
Organic impurities	0,07
Mineral impurities	0,02
Loss in mass during drying	9,03
Total ash	3,47
Water-extractable substances	37,37
Substances extracted with 40% ethanol	35,36

According to the results of the experiment, the content of browned particles was 2.68%, organic impurities - 0.07%, mineral impurities - 0.02%. The mass loss during drying was 9.03%, and the total ash content was 3.47%.

In the comparative analysis of the content of extractive substances in the extracts from the raw materials of *Plantago major* Purple variety herb using two solvents, it was found that 37.37% is extracted with water, and slightly less - 35.36% with 40% ethanol.

4.4 Sieve analysis

To conduct a sieve analysis of the leaves of *Plantago major* Purple variety herb, the methodology of the SPhU monograph 2.9.12 "Sieve analysis" was used. The raw material was crushed into particles of different sizes, sifted through a 355 sieve [7, 8]. The results of the experiment are given in table 4.2.

Table 4.2

Sieve analysis of *Plantago major* Purple variety herb

Type of raw material	m, g small fraction	m₁, g large fraction
Grass	9,74	3,54

According to the results of the experiment, it was proved that the degree of grinding of plantain grass met the requirements of SPhU.

Conclusions to chapter 4

1. For the first time, the morphological and anatomical characteristics of the leaves of *Plantago major* Purple variety herb were highlighted.

The leaves are large with long strong petioles; the leaf blade – wide-oval, grayish-green with a brownish tint, in the phase of flowering – burgundy, the edge is solid, the veins protrude from the lower side; the venation is arcuate. The flowering stem is spike inflorescence unbranched, leafless, erect, the flowers – small, whitish-brown, sessile or on a short peduncle, bisexual, corolla actinomorphic tubular; the fruit is a small box with small brown seeds.

The upper epidermis of the leaf consists of parenchymal, large, sinuous-walled, thin-walled cells and lower ones - parenchyma cells of various sizes, with weakly wavy or even membranes, which have weak thickenings of secondary cell membranes and are pierced by straight pores. The types of stomatal apparatus are anomocytic and diacytic. There are two types of trichomes - covering and glandular. covering hairs are simple, 1–3-celled, cone-shaped, thick-walled, with

an expanded rounded thickened basal cell, long multicellular hairs are rarely found along the veins, with a 5–11-celled radial rosette around a dome-shaped basal cell. Glandular hairs have a one-cell elongated stalk and a two-cell oval head with a dark secretion. The petiole in the basal part is semi-lunar in shape, with wing-shaped outgrowths strongly protruding upwards with thin tops. The adaxial side of the leaf is strongly concave, while the abaxial side is strongly convex. Under the epidermis there are several layers of angularly and lacunose collenchyma. The bundles are small, the main one in the central part of the petiole and three on the sides.

2. The indicators of the quality of raw materials (browned particles, organic impurities, mineral impurities) were determined, a sieve analysis of small and large fractions was carried out, and the content of extractive substances in the herb *Plantago major* Purple variety herb was determined, depending on the type of solvent.

GENERAL CONCLUSIONS

1. An analysis of literary sources on the study of the systematics of the genus *Plantago*, the issues of breeding varieties based on plantain, the chemical composition of raw plantains, and their use in folk and official medicine was carried out.
2. Complexes of polysaccharides and pectin substances were obtained from the herb *Plantago major* Purple variety herb in the flowering phase, their content, component composition and numerical quality indicators were determined. The composition of polysaccharides is represented by glucose, fructose, galactose, rhamnose, arabinose, glucuronic and galacturonic acid. The content of Polysaccharides and Pectins in the herb *Plantago major* Purple variety was 10.8% and 6.76%, respectively. For both complexes, the loss in mass during drying, the content of total protein, and the content of total ash were determined.
3. For the first time, the qualitative composition and quantitative content of 15 amino acids in the herb *Plantago major* Purple variety herb was studied by gas chromatography. Among organic acids, malic, citric, tartaric and ascorbic acids were identified. The amount of organic acids and ascorbic acid in the grass was determined (0,34 % i 0,56 % in accordance).
4. Hydroxycinnamic acids, namely chlorogenic, neochlorogenic, ferulic, flavonoids (quercetin, kaempfeol, rutin, hyperoside, luteolin) were identified for the first time in the raw materials of *Plantago major* Purple variety herb. Quantitative content of the amount of flavonoids in the herb was (0,87%), the amount of hydroxycinnamic acids (3,05%), the amount of anthocyanins (1,32%), the amount of polyphenols (2,02%).
5. The mineral composition of raw materials and the content of heavy metals were studied for the first time.
6. For the first time, an analysis of the macro- and microscopic features of the leaves of *Plantago major* Purple variety herb was carried out, the indicators of the

quality of raw materials and the content of extractive substances were determined when using water and 40% ethanol.

LIST OF REFERENCES

1. Амінокислотний склад рослинної сировини *Plantago media* L. та *Plantago altissima* L. флори України. Т. В. Хортецькая, О. В. Мазулін, Г. П. Смойловська та ін. *Запорозж. мед. журн.* 2012. № 3. С. 132 – 134.
2. Вивчення вмісту вітаміну К у листі перспективних видів роду *Plantago* L. флори України у вегетаційний період. Т. В.Хортецькая, О. В. Мазулін, Г. П. Смойловська, Г. В. Мазулін. *Актуальні питання фармац. та мед. науки та практики.* 2012. № 3. С. 26 – 28.
3. Герасимова І. В. Перспективи застосування подорожника великого у медичній та фармацевтичній практиці. *Sciencerise.* 2015. №11, (4). С. 20-24.
4. Гонтова Т.М., Макачук В.В. Перспективи фармакогностичного вивчення сортів подорожника великого. «Теоретичні та практичні аспекти дослідження лікарських рослин» : матеріали V Міжнародної науково-практичної internet-конференції (м. Харків, 23-25 листопада 2022 р.) – Харків: НФаУ, 2022. С. 56-57.
5. Государственная фармакопея СССР: Вып. 2. Общие методы анализа. Лекарственное растительное сырье МЗ СССР. Изд.11-е, доп. М.: Медицина, 1990. 400 с.
6. Державна Фармакопея України: в 3 т. / ДП «Український науковий фармакопейний центр якості лікарських засобів». 2-е вид. Х.: ДП «Український науковий фармакопейний центр якості лікарських засобів», 2014. Т. 3. 732 с.
7. Державна Фармакопея України / ДП «Український науковий фармакопейний центр якості лікарських засобів». 2-ге вид. Харків : ДП «Український науковий фармакопейний центр якості лікарських засобів», 2015. Т. 1. 1110 с. 135.
8. Державна Фармакопея України / ДП «Український науковий фармакопейний центр якості лікарських засобів». 2-е вид. Допов. 1. Харків :

Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів», 2016. 360 с.

9. Маційчук О. П. Дослідження фенольного складу листків та квітів подорожника великого та подорожника ланцетолистого. *Укр. мед. альманах*. 2012. № 3. С. 119 – 121.

10. Належна практика культивування та збирання вихідної сировини рослинного походження : Настанова СТ-Н МОЗУ 42–4.5 : 2012. Київ, 2012. 14 с.

11. Оленников Д.Н., Кащенко Н.И. Полисахариды. Современное состояние изученности: экспериментально-наукOMETрическое исследование. *Химия растительного сырья*. 2014. № 1. С. 5-26.

12. Питання введення до ДФУ національної монографії «Подорожника великого листа». Е. Е. Котова, А. Г. Котов, О. Г. Вовк и др. *Фармаком*. 2010. № 2. С. 5 – 13.

13. Полухина Т.С., Рамазанова Д.М., Атаева Н.А. Количественное определение суммы органических кислот в листьях подорожника большого (*Plantago major* L.). *World science: problems and innovations* 2017. P. 266-268.

14. Стандартизация сырья подсолнечника однолетнего по макро- и микроскопическим признакам для разработки проекта монографии Государственной Фармакопеи Украины. О. А. Соколова, А. Г. Котов, Т. Н. Гонтовая, Э. Э. Котова. *Вестник фармации*. 2018. № 1 (79). С. 6–11.

15. Acetylation Modification Improves Immunoregulatory Effect of Polysaccharide from Seeds of *Plantago asiatica* L. L. Jiang, S. Nie, D. Huang, Z. Fu, M. Xie. *Journal of Chemistry*. 2018. P. 1-10.

16. Akbar J S. Handbook of 200 Medicinal Plants: A Comprehensive Review of Their Traditional Medical Uses and Scientific Justifications. *Springer International Publishing, Cham*. 2020. P. 1455-1463.

17. Anti-Inflammatory Property of *Plantago major* Leaf Extract Reduces the Inflammatory Reaction in Experimental Acetaminophen-Induced Liver Injury. Hussan F., et al., *Evid. Based Complement. Alternat. Med.* 2015. P. 347861.
18. Atlas of Florida Plants <https://florida.plantatlas.usf.edu/Genus.aspx?id=956>.
19. Belorio M., Gomez M. Psyllium: A useful functional ingredient in food systems. *Critical Reviews in Food Science and Nutrition.* 2020. № 21. P. 1-12.
20. Brum J.M., Gibb R. D., Peters J.C. Mattes Satiety effects of psyllium in healthy volunteers. *Appetite.* 2016. № 105. P. 27-36.
21. CABI (2022) ‘*Plantago major* (broad-leaved plantain)’, CABI Compendium. CABI International. doi: 10.1079/cabicompendium.41814.
22. Cabrera-Jaime S., Martínez C., Ferro-García T., Giner-Boya P., Icart-Isern T., Estrada-Masllorens J.M., Fernández-Ortega P. Efficacy of *Plantago major*, chlorhexidine 0.12% and sodium bicarbonate 5% solution in the treatment of oral mucositis in cancer patients with solid tumour: A feasibility randomised triple-blind phase III clinical trial - *Eur. J. Oncol. Nurs.* 2018., 32. P. 40-47.
23. Chemical composition of essential oils from *plantago lanceolata* L. leaves extracted by hydrodistillation. Bajer T. et al., *J. Food Sci. Technol.* 2016, Mar., 53(3), 1576-1584.
24. Chemical constituents and medical benefits of *Plantago major*. Adom. M. B. et al. *Biomedicine and Pharmacotherapy* 2017. № 96. P. 348–360.
25. Comparative study between cold and hot water extracted polysaccharides from *Plantago ovata* seed husk by using rheological methods. Zhou. P., et al. *Food Hydrocolloids.* 2020. № 101. P. 105-125.
26. Comparison of structure and antioxidant activity of polysaccharides extracted from the leaves of *Plantago major* L., *P. media* L. and *P. lanceolata* L. Lukova. P. et al. *Bulgarian Chemical Communications.* 2017. № 49. P. 282–288.
27. Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. Gibson G.R., et al. *Nutrition Research Reviews.* 2004. №17 (2). P. 259-275.

28. Drincovich M. F., Voll L. M., Maurino V. G. Editorial: On the Diversity of Roles of Organic Acids. *Front Plant Sci.* 2016. № 016; P. 1592-1599.
29. Effects of *Plantago lanceolata* L. extract on full-thickness excisional wound healing in a mouse model. Kurt B., et. al., *Biotech. Histochem.* 2018. 93(4). P. 249- 257.
30. Farokhi F., Khaneshi F. Histopathologic changes of lung in asthmatic male rats treated with hydro-alcoholic extract of *Plantago major* and theophylline - Avicenna J. *Phytomed.* 2013. 3(2). 143- 151.
31. Fractionation and physicochemical characterization of psyllium gum. Guo, Q., et al. *Carbohydrate Polymers.* 2008. № 73 (1). P. 35–43.
32. Functional role of anthocyanins in the leaves of *Quintinia serrata*. Gould KS, Markham KR, Smith RH, Goris JJ. A. *Cunn. J Exp Bot.* 2000. 51(347). P.1107-15.
33. Immunomodulatory activities of flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds of *Plantago* species Chiang L.C., et. al., *Planta Med.* 2003. 69(7). P. 600-604.
34. Indigenous knowledge of the use of medicinal plants in the North-West of Morocco and their biological activities, Bouyahya A. et.al., *European Journal of Integrative Medicine* 2017, Vol. 13. P. 9-25.
35. Influence of abiotic stress during soybean germination followed by recovery on the phenolic compounds of radicles and their antioxidant capacity. Swigonska S, et. al., *Acta Soc Bot Pol.* 2014. 83(3). P. 209–218.
36. In vitro effects of a novel polysaccharide from the seeds of *Plantago asiatica* L. on intestinal function. Hu J.L., Nie S.P., Li C., Xie M.Y. *Int. J. Biol. Macromol.* 2013., 54. P. 264-269.
37. Lertpipopmetha K., Kongkamol C., Sripongpun P. Effect of Psyllium Fiber Supplementation on Diarrhea Incidence in Enteral Tube-Fed Patients: A Prospective, Randomized, and Controlled Trial. *Journal of Parenteral and Enteral Nutrition.* 2019. № 43 (6). P.759-767.

38. Medicinal plants resource science : handbook for students of higher schools / V.S. Kyslychenko, L.V. Upyr, I.G. Zinchenko, O.A. Kyslychenko, S.I. Stepanova; ed. by V.S. Kyslychenko. – Kharkiv : NUPh : Golden Pages, 2012. – 168 p.
39. Melese E., Asres K., Asad M., Engidawork E. Evaluation of the antipeptic ulcer activity of the leaf extract of *Plantago lanceolata* L. in rodents - *Phytother. Res.* 2011. 25(8). P. 1174-1180.
40. Meletis CD, Barker JE. Therapeutic uses of amino acids. *Alternative and Complementary Therapies.* 2005. 11. P. 24–28.
41. Molecular properties and immunomodulatory activities of a water-soluble heteropolysaccharide isolated from *Plantago asiatica* L. leaves. Yin. J., et al. *Natural Product Research.* 2019. № 33 (11). P. 1678–1681.
42. Moroccan medicinal plants traditionally used for the treatment of skin diseases: From ethnobotany to clinical trials. Ajjoun M., Kharchoufa L., Merrouni I. A., Elachouri M. *Journal of Ethnopharmacology* 2022, Vol. 297. 115532.
43. n-Hexane Insoluble Fraction of *Plantago lanceolata* Exerts Anti-Inflammatory Activity in Mice by Inhibiting Cyclooxygenase-2 and Reducing Chemokines Levels. Fakhrudin N., et. al., *Sci. Pharm.* 2017. 85 (1).
44. Oskolski A., Vuza N., Shipunov A. Stem and Leaf Anatomy of *Aragoa* (Plantaginaceae): In Search of Lost Rays Plants 2021. №10. 1773-1788.
45. Osmolovskaya N., Vu D.V., Kuchaeva L. The role of organic acids in heavy metal tolerance in plants // *Biological Communications.* 2018. Vol. 63, 1. P. 9–16.
46. Ou S, Lu S, Yang S. Effects of enhanced UV-B radiation on the content of flavonoids in mesophyll cells of wheat. *Imaging Radiat Res.* 2018. 1(1). P. 1–9.
47. Pharmacognosy: textbook for students of higher / V.S. Kyslychenko, L.V. Lenchyk, I.G. Gurieva et al.; ed. by V.S. Kyslychenko. – Kharkiv : NUPh : Golden Pages, 2019. - 584 p.
48. Pharmacological Evaluation of *Mentha spicata* L. and *Plantago major* L., Medicinal Plants Used to Treat Anxiety and Insomnia in Colombian Caribbean

Coast. Caro D.C., et. al., Evid. Based Complement. Alternat. Med. 2018. P. 5921514.

49. Phenylalanine ammonia-lyase activity and content of flavonoid compounds in wheat seedlings at the action of hypothermia and hydrogen sulfide donor. Kolupaev YuE, et. al., Ukr Biochem J. 2018. 90(6). P. 12-20.

50. Physicochemical characterization, antioxidant and anti-proliferative activities of a polysaccharide extracted from psyllium (*P. ovata*) leaves. Kumar. M., et al. *International Journal of Biological Macromolecules*. 2018. № 118. P. 976–987.

51. *Plantago asiatica* L. Ameliorates Puromycin Aminonucleoside-Induced Nephrotic Syndrome by Suppressing Inflammation and Apoptosis Kho M.C., et. al., *Nutrients*. 2017. 9(4).

52. *Plantago major* in Traditional Persian Medicine and modern phytotherapy: a narrative review. Najafian Y., Hamed S.S., Farshchi M.K., Feyzabadi Z. *Electron. Physician*. 2018. 10(2). P. 6390-6399.

53. Prebiotic activity of poly- and oligosaccharides obtained from *Plantago major* L. leaves. Lukova. P., et al. *Applied Sciences (Switzerland)*. 2020. № 10 (8). P. 2648.

54. Promotion of wound healing by *Plantago major* L. leaf extracts-ex vivo experiments confirm experiences from traditional medicine. Zubair M., et.al., *Nat. Prod. Res.* 2016. 30(5). P. 622-624.

55. Renoprotective Effect of *Plantago Major* Against Nephrotoxicity and Oxidative Stress Induced by Cisplatin. Parhizgar S., et. al., *Iran. J. Kidney Dis.* 2016. 10(4). P. 182-188.

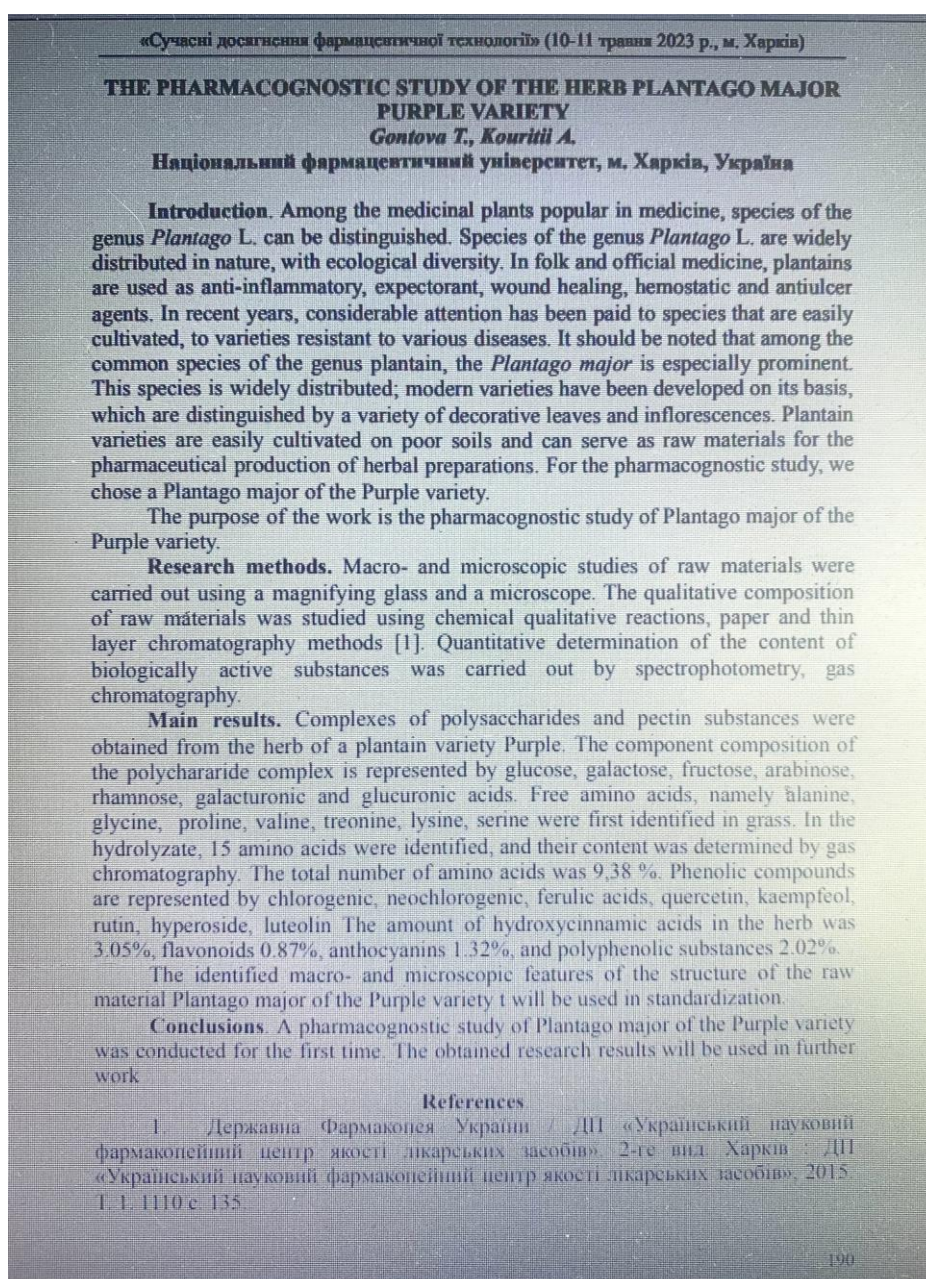
56. Rimi M., Dr. Arvind Negi, Dr. Manish Mishra. *Plantago major* L. a cynosure of modern medicine: A review. *World journal of pharmaceutical research*. 2021. Vol. 10 (12). P. 650-675.

57. Stability Study and a 14-Day Oral Dose Toxicity in Rats of Plantain Leaf Extract (*Plantago lanceolata* L.) Syrup. Mansoor K., et. al., *Sci. Pharm.* 2017. 85(1).
58. Tan H., Nie S. Deciphering diet-gut microbiota-host interplay: Investigations of pectin. *Trends in Food Science & Technology*. 2020. № 106. P. 171-181.
59. The Effects of *Plantago major* on the Activation of the Neutrophil Respiratory Burst. Reina E., et. al., *J. Tradit. Complement. Med.* 2013. 3(4). P. 268-272.
60. The Plant List. A working list of all plant species. URL :http://www.theplantlist.org/tpl1.1/search?q=Plantago&_csv=on (Date of access: 22.02.2023).
61. Tinkov A.A., Nemereshina O.N., Suliburska J., Gatiatulina E.R., Regula J., Nikonorov A.A., Skalny A.V. Comparative Analysis of the Trace Element Content of the Leaves and Roots of Three *Plantago* Species - *Biol. Trace Elem. Res.* 2016, Sep., 173(1), 225-230. doi: 10.1007/s12011-016-0626-2.
62. Van Tienderen. P.H. Morphological variation in *Plantago lanceolata*: limits of plasticity. *Evol. Trends Plants* 4. 1990. P. 35-40.
63. World Checklist of Selected Plant Families. Kew Science. URL :<https://powo.science.kew.org/results?q=plantago> (Date of access: 22.02.2023).

APPENDICES

Publication on qualifying work

Gontova T., Kouritii A. The pharmacognostic study of the herb *Plantago major* Purple variety. X International Scientific-Practical Conference «Modern achievements of pharmaceutical technology» dedicated to the 60th anniversary of the birth of Doctor of Pharmaceutical Sciences, Professor Gladukh Ievgenii Volodymyrovych, which will take place on May 10-11, 2023, Kharkiv. 2023 – P. 190.



Continuation of Appendices A






National University of Pharmacy
 Національний фармацевтичний університет
 Department of Technologies of Pharmaceutical preparations
 Кафедра технологій фармацевтичних препаратів

CERTIFICATE № 035
 СЕРТИФІКАТ

This is to certify that
 Цим засвідчується, що

Kouritii Adam

has participated in the X International Scientific-Practical Conference
 брав(ла) участь у X Міжнародній науково-практичній конференції
"MODERN ACHIEVEMENTS OF PHARMACEUTICAL TECHNOLOGY"
«СУЧАСНІ ДОСЯГНЕННЯ ФАРМАЦЕВТИЧНОЇ ТЕХНОЛОГІЇ»
 dedicated to the 60th anniversary of the birth of Doctor of Pharmaceutical Sciences,
 присвяченої 60-річчю з дня народження доктора фармацевтичних наук
 професора Гладух Євгенія Володимировича

May 10-11, 2023, Kharkiv, Ukraine
 10-11 травня 2023 року, Харків, Україна

Rector of the NUPh, prof.
 Ректор НФаУ, проф.

Head of the Department of Technologies of Pharmaceutical preparations, prof.
 Голова кафедри технологій фармацевтичних препаратів, проф.



Alla KOTVITSKA
 Алла КОТВИЦЬКА

Oleksandr KUKHTENKO

National University of Pharmacy

Faculty for foreign citizens' education
Department pharmacognosy

Level of higher education master

Specialty 226 Pharmacy, industrial pharmacy
Educational program Pharmacy

APPROVED
The Head of Department
pharmacognosy
Olga MALA_____

“28” of September 2022

ASSIGNMENT
FOR QUALIFICATION WORK
OF AN APPLICANT FOR HIGHER EDUCATION

Adam KOURITII

1. Topic of qualification work: «Pharmacognostic study of the Plantago major Purple variety», supervisor of qualification work: Name SURNAME, PhD, prof., Tetiana GONTOVA, approved by order of NUPh from “6” of February 2023 № 35
2. Deadline for submission of qualification work by the applicant for higher education: April 2023.
3. Outgoing data for qualification work: Pharmacognostic study of the herb Plantago major Purple variety to establish the prospects for use in pharmaceutical practice was carried out according to the plan of research work of the Department of Pharmacognosy.
4. Contents of the settlement and explanatory note (list of questions that need to be developed): to analyze literature data on the topic of work, to conduct a qualitative analysis of raw materials, to quantify some groups of biologically active substances in the grass, leaves and flowers of Plantago major Purple variety; identify diagnostic morphological and anatomical features of raw materials, determine the main numerical indicators of raw materials, the content of extractives.
5. List of graphic material (with exact indication of the required drawings):
Tables – 11, pictures – 10.

6. Consultants of chapters of qualification work

Chapters	Name, SURNAME, position of consultant	Signature, date	
		assignment was issued	assignment was received
1	Tetiana GONTOVA professor of higher education institution of department Pharmacognosy	09.2022	09.2022
2	Tetiana GONTOVA professor of higher education institution of department Pharmacognosy	10.2022	10.2022
3	Tetiana GONTOVA professor of higher education institution of department Pharmacognosy	11.2022-12.2022	11.2022-12.2022
4	Tetiana GONTOVA professor of higher education institution of department Pharmacognosy	01.2023	01.2023

7. Date of issue of the assignment: «28» _ September __ 2022

CALENDAR PLAN

№	Name of stages of qualification work	Deadline for the stages of qualification work	Notes
1	Summary of Literature Data on Botanical Characterization, Chemical Composition and Application of the Plantago major Purple variety (Literature Review). Formatting section 1.	September 2022	done
2	Qualitative analysis of some groups of biologically active substances in Plantago major Purple variety raw materials. Formation of section 2.	October 2022	done
3	Quantitative determination of some groups of biologically active substances in the herb Plantago major Purple variety. Formation of section 3.	November 2022	done
4	Morphological and anatomical study of the herb Plantago major Purple variety.	January 2023	done
5	Determination of indicators of quality of raw materials. Formation of section 4.	February 2023	done
6	Registration of work and preparation for defense.	March-April 2023	done

An applicant of higher education
Supervisor of qualification work

_____ Adam KOURITII
 _____ Tetiana GONTOVA

ВИТЯГ З НАКАЗУ № 35
По Національному фармацевтичному університету
від 06 лютого 2023 року

нижченаведеним студентам 5-го курсу 2022-2023 навчального року, навчання за освітнім ступенем «магістр», галузь знань 22 охорона здоров'я, спеціальності 226 – фармація, промислова фармація, освітня програма – фармація, денна форма здобуття освіти (термін навчання 4 роки 10 місяців та 3 роки 10 місяців), які навчаються за контрактом, затвердити теми кваліфікаційних робіт:

Прізвище студента	Тема кваліфікаційної роботи	Посада, прізвище та ініціали керівника	Рецензент кваліфікаційної роботи
• по кафедрі фармакогнозії			
Курітії Адам	Фармакогностичне вивчення подорожника великого сорту Пурпурний.	Pharmacognostic study of the Plantago major Purple variety.	професор Гонтова Т.М. проф. Перехода Л.О.

Підстава: подання декана факультета ректору

Ректор

Вірно. Секретар



ВИСНОВОК

**Комісії з академічної доброчесності про проведену експертизу
щодо академічного плагіату у кваліфікаційній роботі
здобувача вищої освіти**

№ 112906 від « 2 » травня 2023 р.

Проаналізувавши випускну кваліфікаційну роботу за магістерським рівнем здобувача вищої освіти денної форми навчання Курітії Адам, 5 курсу, _____ групи, спеціальності 226 Фармація, промислова фармація, на тему: «Фармакогностичне вивчення подорожника великого сорту Пурпурний / Pharmacognostic study of the Plantago major Purple variety», Комісія з академічної доброчесності дійшла висновку, що робота, представлена до Екзаменаційної комісії для захисту, виконана самостійно і не містить елементів академічного плагіату (компіляції).

**Голова комісії,
професор**



Інна ВЛАДИМИРОВА

4%

33%

REVIEW

**of scientific supervisor for the qualification work of the master's level of higher education
of the specialty 226 Pharmacy, industrial pharmacy**

Adam KOURITII

on the topic: «Pharmacognostic study of the *Plantago major* Purple variety»

Relevance of the topic. It is worth noting that promising raw materials for the creation of pharmaceutical preparations are medicinal plants. In the pharmaceutical industry, the expansion of the range of modern effective and safe medicines based on plant extracts can be achieved by introducing cultivated raw materials into new original medicines. The potential possibilities of phytotherapy are significant, as plants have inherent therapeutic properties, namely anti-inflammatory, analgesic, diaphoretic, diuretic, choleric, reparative, soothing, immunostimulating, hemostatic, antiviral, bactericidal and others. The use of plantain for the treatment of various diseases is widely known. Preparations based on the leaves of wild species of the genus *Plantago* are used in medicine as anti-inflammatory, hypnotic, analgesic, anti-allergic agents. Plantain is an integral part of various breast fees. The plant is very popular in modern folk medicine. Traditional healers recommend an infusion of plantain leaves for diarrhea, hay fever, inflammatory processes in the bladder, hemorrhoids, fever. Ointment with the addition of dried plantain powder is effective for pustular lesions of the skin.

The author set a goal to conduct a pharmacognostic study of a cultivated variety of plantain major, namely Plantain Purple. This is an urgent task and in the future will make it possible to expand the raw material base for the production of phytopreparations based on psyllium.

Practical value of conclusions, recommendations and their validity. For the first time, a comprehensive pharmacognostic study of the Plantain major Purple variety, which is cultivated in Ukraine, was carried out. *Plantago major* Purple variety contains polysaccharides, phenolcarboxylic and hydroxycinnamic acids, flavonoids, anthocyanins, amino acids, organic acids, tannins.

For the first time, the content of the sum of hydroxycinnamic acids, the sum of flavonoids and the sum of anthocyanins in the raw materials of the variety Plantain major Purple was determined. The mineral composition of the herb *Plantago major* Purple variety was determined.

In the *Plantago major* Purple variety, the qualitative composition and quantitative content of essential and non-essential amino acids were determined.

Complexes of polysaccharides and pectins were obtained from the herb of a Plantain major Purple variety in the flowering phase, their content, component composition and quantitative indicators of quality were determined. For both complexes, the weight loss during drying, of total ash were determined.

For the first time, an analysis of the macro- and microscopic features of the leaves of *Plantago major* Purple variety herb was carried out, the indicators of the quality of raw materials and the content of extractive substances were determined when using water and 40% ethanol.

For the first time, *Plantago major* Purple variety raw materials were determined by loss in mass on drying, and total ash. According to the yield of extractive substances, the best extractant

for the Plantago major Purple variety was selected.

Assessment of work. The qualification work was carried out at the Department of Pharmacognosy of the NUPh. Adam KOURITII learned and put into practice the basic methods of pharmacognostic analysis of medicinal raw materials, proved to be a competent, responsible and hardworking researcher, gained experience in independent work with scientific literature, in a scientific laboratory.

General conclusion and recommendations on admission to defend. The obtained results of the research in terms of relevance, scientific and practical significance meet the requirements for qualifying works, therefore the presented qualifying work of Adam KOURITII can be recommended for public defense in the examination committee of NUPh.

Scientific supervisor _____

Tetiana GONTOVA

«7th» of April 2023

REVIEW

for qualification work of the master's level of higher education, specialty 226 Pharmacy, industrial pharmacy

Adam KOURITII

on the topic: «Pharmacognostic study of the Plantago major Purple variety»

Relevance of the topic. Along with synthetic drugs, a significant place in therapy is occupied by herbal medicines, which have a wide range of pharmacological actions, have low toxicity, mild action, are able to quickly eliminate exacerbation symptoms, and do not have negative side effects with prolonged use. Most often in the complex therapy of diseases of the respiratory system, phytopreparations are used as expectorant, anti-inflammatory, immunostimulating, antimicrobial agents. One of the ways to increase phytopreparations is to expand the spectrum of action of pharmacopoeial medicinal plants and study the possibility of using closely related species in scientific medicine. From this point of view, plants of the plantain genus are of great interest. To solve the problem of the quality of raw materials, the conservation of wild species in nature, it is relevant to study varieties and cultivated species.

In this aspect, the attention of Adam KOURITII was attracted by Plantago major Purple variety, widely distributed in Europe and used as an expectorant, mucolytic, and anti-inflammatory, agent.

Theoretical level of work. Adam KOURITII conducted a literature search on the topic at a sufficient theoretical level. The content of the work is fully consistent with the goals and objectives set by the applicant for higher education.

Author's suggestions on the research topic. The author conducted a pharmacognostic study of the herb Plantago major Purple variety. The literature on the study of the systematics of the genus Plantago, the issues of breeding varieties based on plantain, the chemical composition of raw plantains, their use in folk and official medicine was analyzed. Complexes of polysaccharides and pectins were obtained from the herb of a large variety of plantain purple in the flowering phase, their content, component composition and quantitative indicators of quality were determined. The content of polysaccharides and pectins in the grass of the purple plantain variety was 10.8% and 6.76%, respectively. For both complexes, the weight loss during drying, the content of total protein and the content of total ash were determined. For the first time, the qualitative composition and quantitative content of 15 amino acids in the herb Plantago major Purple variety herb was studied by gas chromatography. Among organic acids, malic, citric, tartaric and ascorbic acids were identified. The amount of organic acids and ascorbic acid in the grass was determined (0,34 % i 0,56 % in accordance). Hydroxycinnamic acids, flavonoids were identified for the first time in the raw materials of Plantago major Purple variety herb. Quantitative content of the amount of flavonoids in the herb was (0,87%), the amount of hydroxycinnamic acids (3,05%), the amount of anthocyanins (1,32%), the amount of polyphenols (2,02%). The mineral composition of raw materials and the content of heavy metals were studied for the first time. For the first time, an analysis of the macro- and microscopic features of the leaves of Plantago major Purple variety herb was carried out, the indicators of the

quality of raw materials and the content of extractive substances were determined when using water and 40% ethanol.

Practical value of conclusions, recommendations and their validity. The work is completed in full and includes tables with statistical data of the experiment, drawings and photos. The research results can be used in the identification and standardization of the Plantago major Purple variety raw materials. The scientific provisions and conclusions given in the work have an evidence-based experimental base.

Disadvantages of work. In the work there are unsuccessful expressions and grammatical mistake.

General conclusion and assessment of the work. Qualification work Adam KOURITII on the topic «Pharmacognostic study of the Plantago major Purple variety» according to the results of research and the volume of the experiment performed, it can be presented for defense at the Examination Commission of the National University of Pharmacy.

Reviewer _____

prof. Lina PEREKHODA

«10th» of April 2023

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ВИТЯГ З ПРОТОКОЛУ № 13
засідання кафедри фармакогнозії

«19» квітня 2023 року

м. Харків

засідання кафедри

фармакогнозії

Голова: завідувач кафедри, канд. фарм. наук, доцент Мала О. С.

Секретар: канд. фарм. наук, ас. Комісаренко М. А.

Присутні: доц. Мала О. С., проф. Кошовий О. М., проф. Гонтова Т. М., проф. Криворучко О. В., проф. Ковальова А.М., доц. Бородіна Н. В., доц. Демешко О. В., доц. Машталер В. В., доц. Очкур О. В., ас. Гончаров О. В., ас. Комісаренко М. А.

ПОРЯДОК ДЕННИЙ:

1. Представлення кваліфікаційних робіт до захисту в Екзаменаційній комісії НФаУ.

1. СЛУХАЛИ: Про представлення до захисту в Екзаменаційній комісії НФаУ кваліфікаційної роботи здобувачки вищої освіти випускного курсу (Фм18(5,10д)- англ 03 гр.) Адама КУРІТІІ на тему «Фармакогностичне вивчення *Plantago major* сорту Пурпурний».

Науковий керівник: проф. Татьяна ГОНТОВА.

Рецензент: завідувачка кафедри медичної хімії, д.фарм.н., професор Ліна ПЕРЕХОДА.

В обговоренні кваліфікаційної роботи брали участь: зав. каф. доц. Мала О.С., проф. Кошовий О.М., доц. Бородіна Н.В., доц. Демешко О.В., доц. Очкур О.В., ас. Гончаров О.В.

1. УХВАЛИЛИ: Рекомендувати кваліфікаційну роботу здобувачки вищої освіти факультету з підготовки іноземних громадян групи (Фм18(5,10д)- англ 03 гр.) Адама КУРІТІІ на тему «Фармакогностичне вивчення *Plantago major* сорту Пурпурний» до захисту в Екзаменаційній комісії.

Голова
Завідувачка кафедри

_____ **Ольга МАЛА**

Секретар

_____ **Микола КОМІСАРЕНКО**

НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

**ПОДАННЯ
ГОЛОВІ ЕКЗАМЕНАЦІЙНОЇ КОМІСІЇ
ЩОДО ЗАХИСТУ КВАЛІФІКАЦІЙНОЇ РОБОТИ**

Направляється здобувач вищої освіти Адам КУРІТІІ до захисту кваліфікаційної роботи за галуззю знань 22 Охорона здоров'я спеціальністю 226 Фармація, промислова фармація освітньою програмою Фармація на тему: «Фармакогностичне вивчення *Plantago major* сорту Пурпурний». Кваліфікаційна робота і рецензія додаються.

Декан факультету _____ / Світлана КАЛАЙЧЕВА /

Висновок керівника кваліфікаційної роботи

Здобувач вищої освіти Адам КУРІТІІ в процесі виконання кваліфікаційної роботи освоїв і використав на практиці різні методи фармакогностичного аналізу досліджуваної сировини.

Отримані результати досліджень за актуальністю, науковим та практичним значенням відповідають вимогам, які висуваються до кваліфікаційних робіт, тому представлена робота може бути рекомендована до публічного захисту у Екзаменаційну комісію Національного фармацевтичного університету. Адам КУРІТІІ допускається до захисту даної кваліфікаційної роботи у Екзаменаційній комісії Національного фармацевтичного університету.

Керівник кваліфікаційної роботи _____ Тетяна ГОНТОВА

«7» квітня 2023 року

Висновок кафедри про кваліфікаційну роботу

Кваліфікаційну роботу розглянуто. Здобувач вищої освіти Адам КУРІТІІ допускається до захисту даної кваліфікаційної роботи в Екзаменаційній комісії.

Завідувачка кафедри фармакогнозії

_____ Ольга МАЛА

«19» квітня 2023 року

Qualification work was defended
of Examination commission on

« ___ » of June 2023

With the grade _____

Head of the State Examination commission,

DPharmSc, Professor

_____ / Oleh SHPYCHAK /