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QUALIFICATION WORK

**on the topic «PHARMACOGNOSTIC STUDY OF THE
VIOLA CANINA L.»**

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ANNOTATION

The qualification work is devoted to the pharmacognostic study of herbs of *Viola canina*. The qualitative composition and quantitative content of amino acids, anthocyanins, hydroxycinnamic and phenolcarboxylic acids, flavonoids, and minerals were determined in the raw material. The diagnostic macro- and microscopic signs of grass, quality indicators of raw materials and the content of extractive substances were determined.

The main volume of the work is 43 pages. The work is illustrated with 8 figures, 12 tables.

Key words: *Viola canina*, pharmacognostic analysis, herb.

АННОТАЦИЯ

Квалификационная работа посвящена фармакогностическому изучению травы *Viola canina*. В сырье определен качественный состав и количественное содержание аминокислот, антоцианов, гидроксикоричных и фенолкарбоновых кислот, флавоноидов, минералов. Определены диагностические макро- и микроскопические признаки травы, показатели качества сырья и содержание экстрактивных веществ.

Объем основного текста работы – 43 страниц. Работа иллюстрирована 8 рисунками, 12 таблицами.

Ключевые слова: фиалка собачья, фармакогностический анализ, трава.

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LIST OF CONDITIONAL ABBREVIATIONS

BAS - biologically active substances

GLC-method - gas-liquid chromatography

PCH - paper chromatography

WHO - World Health Organization

SPHU - State Pharmacopoeia of Ukraine

MPRL - medicinal plant raw materials

TLC - thin layer chromatography

SS - standard sample

INTRODUCTION

Relevance of the topic. The search and development of new drugs based on medicinal plant materials is relevant and tends to develop. This is due to the higher level of safety of herbal medicines compared to synthetic medicines. In addition, rapid technological progress makes it possible to deepen knowledge about insufficiently studied medicinal plants, their chemical composition and use in medicine. One of the promising plants is *Viola*. Since ancient times, species of the genus *Viola* have been widely used as expectorant, mucolytic, anti-inflammatory, diuretic and choleretic agents. The herb of two species - *Viola tricolor* and *Viola arvensis* is included in the State Pharmacopoeia of Ukraine and EurPh. *Viola canina* is a promising species for pharmacognostic study of the violet genus. This species has a wide distribution area and, with a close chemical composition with the official types of violets, can be used as an additional type of raw material for the development of phytopreparations.

Therefore, the pharmacognostic study of the herb *Viola canina* is an urgent task of pharmacy.

The aim of the work was a pharmacognostic study of the herb *Viola canina*.

Research objectives:

- to analyze and summarize the data of literary primary sources on the relative distribution of violets, the chemical composition of raw materials, and their use in medicine;
- conduct a study of the qualitative composition of biologically active substances of *Viola canina* raw materials;
- establish the quantitative content of the main groups of biologically active substances;
- study the diagnostic morphological and anatomical features of the herb *Viola canina*;
- determine the main numerical indicators of *Viola canina* raw materials.

The object of the study is a comprehensive pharmacognostic study of the

herb, leaves and flowers of *Viola canina*.

The subject of the study is the detection, identification and quantitative content of substances in the herb *Viola canina*, the establishment of morphological and anatomical diagnostic features and the main numerical indicators of the herb.

Research methods – physical – determination of mass loss during drying, total ash, insoluble ash in hydrochloric acid; physical and chemical – paper chromatography, thin-layer chromatography, spectrophotometry in the UV and visible regions of the spectrum, atomic emission spectrometry, gas-liquid chromatography; chemical – reactions of identification of groups of substances; microtechnical - macro- and microscopic methods for establishing morphological and anatomical diagnostic features of raw materials; technological – selection of the extractant, the establishment of numerical indicators of raw materials; statistical – processing the results of the experiment in accordance with the requirements of the pharmacopoeia.

Practical significance of the obtained results. Experimentally proved the prospects of using the herb *Viola canina* as a promising source of biologically active substances.

Scientific novelty of the obtained results. For the first time, a comprehensive pharmacognostic study of the herb *Viola canina*, which grows in Ukraine, was carried out.

Viola canina 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 was determined in the raw materials of *Viola canina*. The mineral composition of the herb *Viola canina* was determined.

In the herb *Viola canina*, the qualitative composition and quantitative content of essential and non-essential amino acids were determined.

For the first time, *Viola canina* raw materials were determined by loss in mass on drying, total ash, and ash insoluble in hydrochloric acid. According to the

yield of extractive substances, the best extractant for the herb *Viola canina* was selected.

Approbation of the results and publication. Research results are presented at the V International Scientific and Practical Internet-Conference «Current approaches of pharmaceutical science in development and standardization of medicines and dietary supplements that contain components of natural origin», 14 april, 2023, Kharkiv.

Gontova T.M., Ibnghazala A. Prospects for pharmacognostic study of representatives of the genus Violet for the treatment of skin diseases. Current approaches of pharmaceutical science in development and standardization of medicines and dietary supplements that contain components of natural origin: The Proceedings of the V International Scientific and Practical Internet-Conference, 14 april, 2023, Kharkiv. 2023. P. 14-15.

Structure and volume of qualifying work. The qualification work is laid out on 52 pages of typewritten text, 43 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 8 figures, 12 tables. The list of references includes 66 sources.

CHAPTER 1

PHARMACOGNOSTIC STUDY OF VIOLE SPECIES (Literature Review)

1.1 Botanical characteristics of the Violet genus

Genus *Viola* L. belongs to the family *Violaceae* and includes about 700 species. The term *Viola* comes from the Greek «ῥιον ἰον viola», from an ancient ῥιον ἰον with the same meaning. The specific «canine» epithet comes from *cánis* cane: frequent, common as dogs are or loved by dogs or used to treat dogs.

To date, 664 known species of *Viola* are known, 43 of which have not yet been described. The authors divide the genus into two subgenera, 31 divisions and 20 subdivisions. The subgenus *Neoandinium* includes 139 species in 11 sections, and the subgenus *Neoandinium* includes 139 species in 11 sections and subsp. *Violet*, 525 species in 20 sections and 20 subsections [20]. Violets are well-known plants and have a long history in European folklore. The first records describing violets in Europe come from Ancient Greece [39]. Fragrant violets were sold and used in medicine since ancient times, they played a role in the description in myths, for example, about the abduction of Persephone.

This is a taxonomic group with significant intraspecific morphological variability, which is associated with easy crossing between species and their hybridization. This causes difficulties in the systematics of the genus [22, 29]. Observations of species in natural phytocenoses showed significant variability. Interpopulation differences of species according to some traits were obvious (plant height, stem branching, number of flowers per plant, pubescence the plant and leaf, the shape and color of the leaves and petals, the shape of the spur, the structure of the gynoecium, androecium, and nectaries, spur length, seeds length) and anatomical features of plants [13, 14, 16, 30, 37, 56].

Wild violets are able to settle over long distances. They grow in Hawaii, New Zealand and the Azores, tropical Africa and the Cape Province of South Africa, in southern South America. Several extant species are found on more than one

continent (eg. *V. biflora*, *V. palustris*, *V. rostrata*, *V. selkirkii*, *V. suecica*). *Viola elatior*, *Viola tanaitica* are also common in the Chernivtsi region, and in the Kharkiv region, in addition to the named species, *Viola ambigua*, *Viola Lavrenkoana*, *Viola rupestris*, *Viola suavis*, *Viola accrescens*, *Viola mirabilis* grow [5, 20]. About 40 species of violets are found on the territory of Ukraine [11].

Species of the genus *Viola* L. are highly ornamental early flowering perennials. There is an artificially created and evolutionarily developed group of hybrid violets, which taxonomists call *V. wittrockiana*, or *V. hybrida hort.* (*V. wittrokiana* Gams.) [20, 51]. This species is cultivated as an ornamental. Under the name violet *V. wittrockiana*, numerous varieties and varietal groups obtained during the selection of such wild species as *V. tricolor*, *V. altaica* and *V. lutea* are combined (Fig. 1.1.).



1



2



3



4

Fig. 1.1 Types of violets used in breeding and hybrids 1 - *V. tricolor*, 2 - *V. altaica*, 3 - *V. lutea*, 4 - *V. wittrockiana* [62, 63, 64]

Violas in cultivation prefer sunny places of growth. they are best grown in humus, moist, slightly acidic soil rich in organic material, but tolerate some drought. Modern phylogenetic studies of the *Viola* genus classified it as monophyletic [19].

Viola is a herb that usually grows in temperate climates and is characterized by several apomorphies, including unsegmented peduncles, single flowers, calyx appendages, lower petals distinctly acuminate (rarely almost sac-like), and the blade shorter than not much longer than the lateral and upper petals [21]. Cleistogamy is characteristic for species of the violet genus.

Viola canina L. (Fig 1.2) is a perennial plant 7-15 cm height. Rhizome is very short; the whole plant is bare. Stems are often numerous. Leaves are drawnly ovate, with a short blunt mucro. Stipules are narrow, subulate, and much shorter than half the length of the blade of leaf. The flowers are blue, with white faux, odorless. It has both male and female organs (hermaphrodite) and is pollinated by insects. Fruit is capsule. It is in blossom in April-May; it fruits in May-June. It is grown on the sandy banks of rivers, in glades in the forests and among the shrubs, on the slopes of the mountains until the mid-mountain belt. *V. canina* is distributed in the Albania, Altay, Austria, Baltic States, Belarus, Belgium, Bulgaria, Buryatiya, Central European Rus, Chita, Corse, Czechoslovakia, Denmark, East European Russia, Finland, France, Germany, Great Britain, Greece, Hungary, Iceland, Ireland, Italy, Kazakhstan, Krym, Netherlands, North Caucasus, North and South European Russia, Poland, Portugal, Romania, Sardegna, Sweden, Switzerland, Transcaucasus, Turkey-in-Europe, Ukraine, West Siberia, Yugoslavia in Central Asia with a temperate climate to Japan [33].

Accepted intraspecific features of *Viola canina* subsp. *canina*, *Viola canina* subsp. *ruppii* (All.) Schübl. & G. Martens, *Viola canina* subsp. *schultzii* (Billot) Kirschl [46].

The *Viola canina* (Fig. 1.3) is a species with Eurasian distribution and present from Norway to Portugal, east to the Russian Far East, Turkey and the Caucasus; North Africa and Morocco; North America and Greenland. Its habitat is

that of the moors, arid prairies, dunes and marshes where it grows in lean pastures, clearings and edges of woods, grasslands and shrubs, generally on subacid soils, from sea level to the mountain belt.



Fig. 1.3 *Viola canina* (general view) [66].

1.2 The chemical composition of species of the Violet genus

Information about the chemical composition of plants of the Violet genus is given in the table. 1.1.

Table 1.1

The chemical composition of the Violet genus plants

Biologically active substances	Name of the plant	Literary source
1	2	3
Water-soluble polysaccharides	<i>V. tricolor</i> (13%), <i>V. arvensis</i> (9,0%), <i>V. odorata</i> (11%), <i>V. canina</i> (11%)	23, 48, 49, 53
Lipids	<i>V. betonicifolia</i>	49
Vitamins	<i>V. tricolor</i> , <i>V. arvensis</i>	41, 53
Tannins	<i>V. canina</i>	23, 41, 53

Continuation of Table 1.1

1	2	3
Phenolic compounds - Arbutin - Violotoside - Phenolcarboxylic acids (p-hydroxybenzoic, vanillinic, gentisinic, protocatechuic, p-kumarova, salicylic acid, ellagic acid, gallic acid) - Hydroxycinnamic acids (caffeic, chlorogenic, neochlorogenic acids) - Anthocyanidins - Flavonoids (kaempferol, quercetin, routine, hyperoside) - Coumarins (coumarin, scopoletin, umbelliferon)	<i>V. tricolor, V. arvensis, V. canina, V. odorata, V. mirabilis, V. biora, V. elatior, V. mirabilis, V. stagnina, Viola canescens, Viola betonicifolia</i>	1, 2, 3, 9, 15, 23, 41, 48, 54, 57, 59
Essential oil	<i>V. tricolor, V. arvensis, V. canina, V. odorata, V. etrusca, V. tianshanica, V. serpens, V. hamiltoniana, V. serpens, V. etrusca, V. tianshanica,</i>	15, 23, 25, 26, 27, 42, 53
Saponins	<i>V. tricolor, V. arvensis, V. canina, V. odorata</i>	41, 53
Mineral substances	<i>V. betonicifolia, Viola odorata</i>	48, 49

Carbohydrates, phenolic compounds, saponins, proteins, fatty oil, nitrogen-containing compounds, vitamins, macro- and microelements, etc. accumulate in grass. The most studied compounds of phenolic nature: simple phenols, coumarins, phenolcarbon and hydroxycinnamic. acids, flavonoids, alkaloids, lignans, sesquiterpenes, cyclotides, etc [53].

V. canina contains anthocyanins, phenolcarboxylic acids (ferulic, salicylic), flavonoids (apigenin, vitexin, hyperoside, hesperidin, rutin), polysaccharides (water-soluble polysaccharides, pectin complex, hemicellulose A and B), essential oil. Pectin substances of *V. canina* are characterized by a low degree of esterification. The high content of free carboxyl groups in pectin substances of *V. canina* indicates their complexing ability and the possibility of recommending them as detoxicants [23].

Alkaloids, flavonoids, terpenoids, phenols, glycosides, saponins, methyl salicylate, mucus and vitamin C were found in the fragrant violet herb [1, 10]. The methanolic extract of the leaves of *V. odorata* was found to contain 15 total phenolic (35.4 mg/g) and total flavonoid (22.8 mg/g) contents [42]. Various phytochemical constituents (alkaloids, steroids, tannins, flavonoids, and saponins) has been reported in aerial parts of *V. odorata* n-hexane, butanolic, methanolic and aqueous extracts [49]. The methanolic extract of the whole plant of *V. betonicifolia* has been reported as rich source of alkaloids, flavonoids, tannins, proteins, phenolic compounds, saponins, sterols and triterpenoids [48, 49]. The dichloromethane, ethyl acetate and methanolic extract of *V. tricolor* whole plant has been reported as rich source of terpenoids, phenolic compounds, flavonoids and saponins. The methanolic extract of *V. tricolor* has been investigated for their flavonoid contents through LC-MS, HPLC and NMR and five minor flavonoids were identified [54, 59]. Various species of *Viola* has been tested for their cyclotides contents and all were proved a rich source of cyclotides. The leaves of *V. canescens* reported to have alkaloids, phenolic compounds, tannins, saponins, phytosterols and flavonoids as phytochemical constituents [23]. According to the results of high performance liquid chromatography (HPLC) and thin layer

chromatography, the leaves of *V. odorata* accumulate stigmasterol. Also flavonoids revealed rutin, vitexin, isovitexin and kaempferol-6-glucoside, anthocyanins [3, 4, 9, 18? 57].

Viola tricolor is well-known to contain polysaccharides, phenylcarbonic acids, salicylic acid derivatives, and catechins [28]. Grass of *V. canina* can be a source of water-soluble polysaccharide complexes and pectin substances. Salicylic acid was found in the herb *Viola palustris*, *V. mirabilis*, *Viola reichenbachiana*, *V. odorata*, *V. canina*, *V. tricolor*, *V. mirabilis*, *V. rupestris* [10, 41].

Anthocyanin glycosides - delphinidin, peonidin were found in the flowers of *V. tricolor*, *V. mirabilis*; violanin and the flowers of *V. bitor*, *V. elatior*, *V. mirabilis*, *V. stagnina* contain leucoanthocyanidins - leukodelphinidin and leukocyanidin [13, 15].

The leaves of *V. odorata* contain essential oil, which contains butyl 2-ethylhexyl phthalate, and 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone, etc. [42]. The linear cyclotide violacin A was isolated from fragrant violet [43].

The essential oil was isolated by hydrodistillation from the fresh aerial parts (leaves, stems, flowers) of *V. canina* and was analyzed by GC-MS. The components identified from the essential oil of fresh aerial parts of *V. canina* were twenty-five forming 98.3% of the total oil composition. Essential oil consisted mainly of acyclic diterpenoids (63.0%), acyclic alkanes (17.3%) and sesquiterpenoids (12.7%). The major component of the essential oil was phytol (55.2%). The more important sesquiterpenoids found in the essential oil of *V. canina* were α -farnesene (3.0%), germacrene D (1.9%), trans-caryophyllene (1.3%) and beta-pinene (1.2%). Phytol is also found in the herb *V. arvensis* (11.4%), *V. tricolor* (7.3%), *V. tianshanica*, *V. odorata* and *V. hamiltoniana* in small amounts [15, 22, 25, 6, 27, 32, 47, 61].

The essential oils of the leaves of *V. odorata* contain 23 volatile components, most of which were derivatives of aliphatic or shikimic acids, while the main components of the essential oil of *V. etrusca* was methyl salicylate [15,

36]. Essential oils from the aerial parts of *V. tricolor* and *V. arvensis* consisted of sesquiterpenes, aliphatic compounds, monoterpenes.

The study of the elemental composition of the stems, leaves, flowers of *V. odorata* and *V. serpens* proved that the species is a rich source of various macro- and microelements (lead, cadmium, copper, chromium, zinc, nickel, magnesium, calcium, sodium and potassium). *V. betonicifolia* is a source of various nutrients, fats, proteins, lipids and carbohydrates [49].

Thus, the pharmacognostic study of raw materials *V. canina*, which is widespread in the world, is promising [34].

1.3 Medical use of violets

The herb *V. arvensis* and *V. tricolor* are included in the European Pharmacopoeia as *Violae tricoloris herba cum flore* [31, 45].

The active chemical components of medicinal plants of the genus *Viola* have antibacterial, antiviral, antioxidant, anti-inflammatory, antitumor, neuroprotective, hepatoprotective activity, etc. [18, 53].

V. tricolor herb has been used in traditional Chinese medicine for thousands of years. Due to its anti-inflammatory and antimicrobial properties, it is considered a traditional remedy for skin diseases, and is also used in the treatment of inflammation of the lungs and chest, such as bronchitis or asthma [2, 17, 40, 49].

Recent studies have shown that violet tricolor extract can improve the appearance of the skin (calm down inflammation, reduce redness, and improve skin texture). Traditionally, *V. tricolor* was used to treat epilepsy and asthma. *V. tricolor* extract is rich in flavonoids, which are powerful antioxidants. These compounds scavenge harmful free radicals that can damage the cell and lead to inflammation. Anthocyanins exhibit antioxidant properties and inactivate free radicals, modulate intracellular mitochondrial systems, which can be effective in the treatment of metabolic disorders in the body [35, 44].

V. tricolor extract also contains triterpenoids, which have anti-inflammatory activity. The researchers concluded that *V. tricolor* extract may be useful in the treatment of inflammatory skin conditions such as eczema and psoriasis [24]. Violet extract is increasingly used in modern skin care products for inflamed skin. In addition to its anti-inflammatory properties, *V. tricolor* extract also reduces redness and hyperpigmentation caused by sun exposure, inflammation, or hormonal imbalances [58, 59]. *V. tricolor* extract helps to lighten dark spots and even out skin tone. *V. tricolor* extract has been shown to have anti-aging properties in a number of studies it could help to increase collagen production. The components of *Viola tricolor*, *Viola odorata* have an immunosuppressive effect [35].

Phytol, which is the main component of the essential oil of violet species, has antimicrobial, antitumor, cytotoxic, antimutagenic, antiteratogenic, antispasmodic, lipid-lowering, anticonvulsant, anti-inflammatory, antioxidant, anxiolytic, immunoadjuvant, antidepressant, hair growth stimulant, hair growth stimulant. Fall protection and anti-dandruff measures [15, 27, 47, 52, 61].

V. canina is used as a mild laxative, diuretic, and emollient. An aqueous infusion and a decoction of the aerial part are used for bronchitis and laryngotracheitis. An infusion of the aerial part of *V. canina* suppresses the exudative and proliferative stages of inflammation, and has a pronounced capillary-strengthening effect. The leaves are brewed as a tea and drunk for heart diseases [23]. The plant has pronounced bactericidal properties [60]. Experimental studies have shown the effectiveness of violet extract in the treatment of acute staphylococcal pneumonia. Ethanol extract showed high inhibition against *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [55, 60].

Viola flowers are edible flowers and are used in garnishes and salads. They can also be candied for a frosted effect and used to decorate cakes. Violets are good honey plants.

According to the literature, species of the genus violet have a wide distribution area in the world, the species exhibit a variety of pharmacological

effects, which is associated with the accumulation of various groups of biologically active substances, and especially phenolic substances and polysaccharides. Pharmacopoeial species of violets (*Viola tricolor*, *Viola arvensis*) have been studied to a greater extent. Given the close chemical composition of the species, pharmacognostic study of new species of the violet genus, including *V. canina*, which is widespread in different countries, is promising.

CHAPTER 2

OBJECTS, RESEARCH METHODS. IDENTIFICATION OF BAS IN VIOLA CANINA HERB

2.1 Objects of study, reagents and devices

1. The objects of the study were the grass *V. canina* and its components (leaves, flowers), harvested in May-June 2021-2022 in the vicinity of the city of Kharkov, dried by the air-shadow method [12]. For the experiments, three series of each type of raw material were used. For a preliminary chemical study, water, water-alcohol extracts were obtained, and each fractionation was carried out using chloroform and ethyl acetate. Air-dry raw materials were crushed to 3-5 mm using dismembrator grinder CO 124A.

2. For tube reactions and analysis by paper chromatography and thin layer chromatography, 5% solutions of ethyl acetate fractions and aqueous residues were prepared. Chromatographic studies were carried out on paper brand "Filtrak" (FN - 3.12), TLC on plates "Silufol" and "Sorbfil" [50, 52].

3. Solvent systems for chromatography:

- 5% acetic acid;
- 15% acetic acid;
- ethyl acetate - formic acid - water (3:1:1);
- ethyl acetate - glacial acetic acid (8:2);
- chloroform-toluene-diethyl ether-anhydrous formic acid (60:60:15:5);
- butanol - acetic acid - water (BAW) (4:1:2) and (4:1:5)
- ethyl acetate - acetic acid (95:5).

Chromatography was carried out in an ascending flow of solvents with one-time and two-time distillation on paper and in a thin layer of sorbent (TLS) at a temperature of 23°C [7, 50, 52].

4. On the chromatograms, the substances were detected by the color of the spots under daylight and filtered UV light (at wavelengths of 254 nm and 366 nm) before and after treatment with the appropriate reagents:

- a. amino acids - 0.2% alcohol ninhydrin solution;
- b. hydroxycinnamic acids, flavonoids - ammonia vapor, 2% alcohol solution of KOH;
- c. organic acids - 0.03% solution of bromocresol green methanol; ascorbic acid - 0.4% sodium solution of 2,6-dichlorophenolindophenolate aqueous;
- d. phenolcarboxylic acids, flavonoids - 3% solution of ferrum (III) chloride, 10% alcohol solution of sodium hydroxide;
- e. salicylic acid - 3% solution of ferrum (III) chloride;
- f. gallic acid - 1% potassium cyanide solution, 1% ferrum (III) chloride solution [3, 50, 52].

5. UV absorption spectra were recorded on an SF-46 spectrophotometer in cuvettes with a layer thickness of 10 mm at a concentration of substances of 1-2 10^{-3} mol in 96% ethanol in the range from 200 to 700 nm [6-8].

6. The amino acid composition of raw materials was determined by gas-liquid chromatography (GLC) on an AAA-339 amino acid analyzer (Czech Republic) after hydrolysis with hydrochloric acid.

7. Spectrophotometric determination of anthocyanins was carried out according to the method of State Pharmacopoeia of Ukraine 2.0 monograph "Dried blueberries fruits" in terms of cyanidin-3-O-glucoside chloride [6-8].

8. The mineral composition was determined on a DFS-8 atomic emission spectrophotometer. Standard solutions of metal salts in the range of measured concentrations were used to construct calibration graphs. The content of heavy metals was determined by the methods of the State Pharmacopoeia [5-8].

9. Morphological characteristics of raw materials were studied on fresh, dried raw materials using a magnifying glass and a binocular microscope MBS-9 according to the monograph of the State Pharmacopoeia. To study the anatomical structure of the epidermis of leaves, stems, and flowers, preparations

were prepared from the surface; stems were studied in transverse and longitudinal-radial sections. For work, freshly harvested and fixed raw materials were used (mixture for fixation: 96% ethanol, glycerol, purified water (1:1:1)). Biolam LOMO microscope was used to analyze the microscopic features of raw materials. Diagnostic signs were recorded with an OLYMPUS FE-140 digital camera, with partial processing of photographs using Adobe Photoshop CS2 9.0. [6].

10. To determine the weight loss during drying, an AXIS electronic laboratory balance AN 100, drying cabinet 2Sh-01-01 was used.

11. To determine the total ash and ash, not dissolved in hydrochloric acid content in raw material samples, a muffle furnace brand SNOL-1.625.1/9-115 was used. The determination was carried out according to the monograph of the State Pharmacopoeia [6-8].

12. Pharmacognostic studies were carried out according to the methods of analysis of State Pharmacopoeia 2.8 "Methods of pharmacognosy" [8].

13. Sampling and sample preparation of raw materials were performed in accordance with the requirements of State Pharmacopoeia monograph 2.8.20 [8].

14. Determination of the content of foreign impurities was carried out in accordance with the requirements of State Pharmacopoeia 2.8.2 [7].

16. Processing of the results was carried out by the method of mathematical statistics in accordance with the State Pharmacopoeia [6].

2.2 Identification of BAS in *Viola canina* herb

According to the literature, plants of the genus *Viola* contain carbohydrates, amino acids, vitamins, carotenoids, phenolcarboxylic acids, flavonoids, tannins, triterpene compounds and steroids (Chapter 1).

2.2.1 Amino acid

Amino acids are part of plant proteins and have an individual composition, which affects the pharmacological action of plant materials [52].

When adding 0.2% solution of ninhydrin alcohol to aqueous extracts of violet herb, followed by heating in a boiling water bath for 5 minutes. a violet color of the solutions was formed, which indicated the presence of free amino acids in the raw material.

Amino acids were identified by paper chromatography in the solvent system n-butanol – glacial acetic acid – purified water (BAW) (4:1:2) [50, 52]. Chromatograms were treated with 0.2% alcohol ninhydrin solution and kept in a thermostat at T=105 °C. Amino acids were detected when red-violet and yellow (proline) spots appeared. The results are shown in Table 2.1.

Table 2.1

Identification of amino acids in *V. canina* grass

Type of raw material	Amino acids										
	valine	leucine	methionine	phenylalanine	lysine	alanine *	aspartic acid*	glutamine acid*	arginine	tyrosine*	proline*
Grass	+	+	+	+	+	+	+	+	+	+	+

Note * - non-essential amino acids

11 amino acids were identified in comparison with the Rf of standard samples and their color.

2.2.2 Organic acids

The presence of organic acids was determined in aqueous extracts of the herb *V. canina*. The chromatograms were treated with 0.03% bromocresol green methanol solution. On the chromatograms, organic acids appeared as yellow spots on a green background.

Malic and citric acids were detected in the *V. canina* grass compared to Rf standard samples and stain color.

The presence of ascorbic acid was determined in aqueous extracts and proved in the solvent system ethyl acetate - acetic glacial acid (8:2) with a reliable sample of this compound. The chromatograms were treated with 0.04% sodium 2,6-dichlorophenolindophenolate aqueous solution. As a result, pink spots on a blue background were found on the chromatograms, the Rf values of which coincided with the Rf value of a reliable sample of ascorbic acid.

2.2.3 Phenolic substances

To a 1 ml aqueous extract was added 5 ml of an ammonia solution and 2 ml of a 10% solution of sodium phosphomolybdate in hydrochloric acid. Dark blue color indicated the presence of phenolic compounds in the studied samples. Iron(II) sulfate crystals were added to 2 ml of an aqueous extract of *V. canina*. A brownish-purple precipitate was formed, which confirmed the presence of phenolic compounds, including hydroxycinnamic acids, in the extracts of the herb, leaves, and flowers of *V. canina*.

Hydroxycinnamic acid derivatives were determined in aqueous, hydroalcoholic and ethyl acetate fractions by paper chromatography with standard acid samples in diluent systems: 2% acetic acid and BAW (4:1:5).

Chromatograms in UV light revealed 2 substances with blue and 1 with violet fluorescence, the color of which became bright blue after the chromatograms were treated with ammonia vapor. Subsequent processing of the chromatograms with a 0.03% solution of bromocresol green methanol led to the appearance of yellow spots on a green background, which indicated the presence of hydroxycinnamic acids.

Chlorogenic, neochlorogenic and caffeic acids were identified in the grass, leaves and flowers of *V. canina* (Table 2.2).

Phenolcarboxylic acids and their derivatives were determined by general qualitative reactions. When a 3% solution of ferrum (III) chloride was added to water-alcohol extracts, a blue color was observed, when a 10% solution of sodium hydroxide alcohol was added, a red-violet color was observed. Paper chromatography was also used in solvent systems: 5% acetic acid and BAW (4:1:2), TLC in solvent systems: ethyl acetate:acetic acid (95:5) followed by processing of chromatograms with 3% ferrum (III) chloride solution, 10% sodium hydroxide solution in alcohol. As a result, gallic acid was found (Fig. 2.1, B, Table 2.2).

Salicylic acid has a pronounced anti-inflammatory effect. It is used in cosmetology as an exfoliating, keratolytic, antimicrobial agent [10, 17, 24].

Salicylic acid in the herb was identified by TLC in the solvent system chloroform-toluene-diethyl ether-anhydrous formic acid (60:60:15:5) in the presence of a standard sample. The chromatogram was treated with a 3% solution of ferrum (III) chloride salicylic acid appeared as a red-violet spot on a brown background.

Salicylic acid was identified by TLC in the herb *V. canina* (Fig. 2.1, B, Table 2.2).

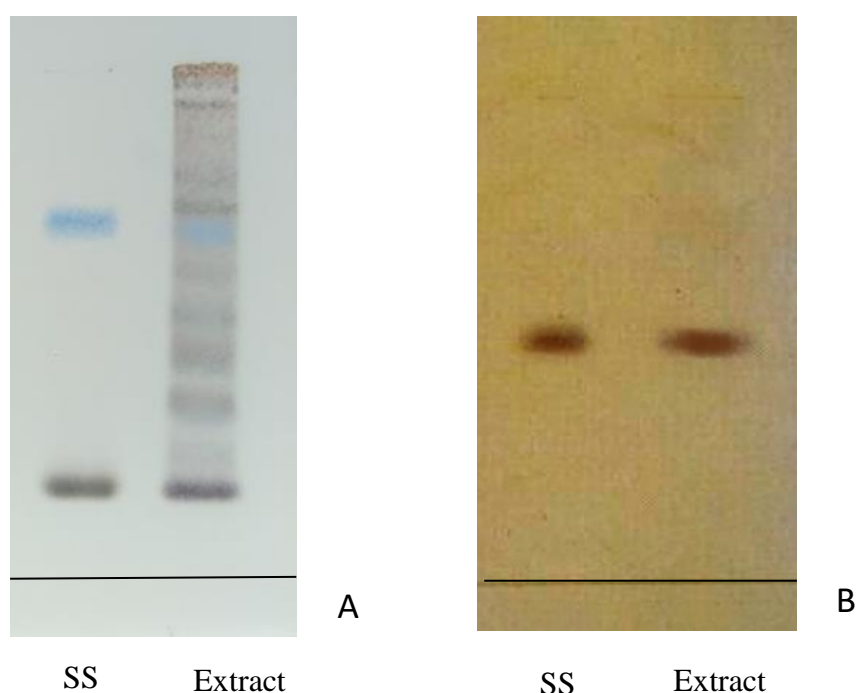


Fig. 2.1 TLC-chromatograms of gallic acid (A) and salicylic acid (B) of *V. canina* grass.

Anthocyanins. The blue color of *V. canina* flowers is due to anthocyanins. This group of substances exhibits antioxidant and cardioprotective activity, lowers blood sugar levels. [11, 35, 44].

Anthocyanins were determined in water-alcohol fractions acidified with hydrochloric acid by paper chromatography in the BAW system (4:1:5) [7, 50, 52].

In the extract of the grass and flowers of *V. canina*, we found 3 compounds of anthocyanin nature purple-lilac in daylight and lilac in UV light with $R_f=41$, $R_f=44$ and $R_f=46$, so we can assume that these are delphinidin glycosides (Table. 2.2).

This group of substances was found in alcohol and ethyl acetate extracts of the herb *V. canina* using high-quality test-tube reactions and chromatography methods [14, 38].

Cyanidin reaction according to Briant. 4-5 drops of concentrated hydrochloric acid and 1-2 pinch of metallic magnesium were added to 5 ml of water-alcohol extracts of fragrant violet. Extracts turned red. n-octanol (1/3 of the total volume) was added to the solutions and mixed. The water-alcohol layer turned dark brown (glycosides), while the octanol layer turned pink (flavonoid aglycones).

Reaction with aluminum (III) chloride. To 5 ml of aqueous-alcoholic extracts were added 10 drops of a 2% solution of aluminum (III) alcohol chloride. The solutions turned yellow.

Substances of flavonoid nature were detected using paper chromatography in solvent systems: 5% acetic acid, BAW (4:1:2). Chromatograms were treated with 10% sodium hydroxide solution in alcohol. Compounds of flavonoid nature appeared as yellow spots.

According to the results of the analysis, rutin, quercetin, and kaempferol were found in the grass and leaves of *V. canina* (Table 2.2), and rutin, quercetin, and kaempferol were found in the flowers.

Table 2.2

The results of the identification of phenolic substances in *V. canina* raw materials

Substance	Type of raw material	
	grass	flowers
Hydroxycinnamic acids		
Chlorogenic acids (Rf=0,62)	+	+
Neochlorogenic acids (Rf=0,58)	+	+
Caffeic acids (Rf=0,82)	+	+
Phenolcarboxylic acids		
Salicylic acid (Rf=0,49)	+	+
Gallic acids (Rf=0,72)	+	+
Flavonoids		
Rutin (Rf=0,46)	+	+
Quercetin (Rf=0,72)	+	+
Kaempferol (Rf=0,8)	+	+
Anthocyanins		
Substance 1 (Rf=0,41)	+	+
Substance 2 (Rf=0,44)	+	+
Substance 3 (Rf=0,46)	+	+

Note. "-" - the substance was not detected

Tannins. The detection of the presence of tannins was carried out in aqueous fractions using qualitative reactions.

Precipitation with gelatin solution. Equal amounts of freshly prepared 0.5% gelatin solution and 0.04 ml of 10% hydrochloric acid solution were added to 5 ml of water raw material lifters. Precipitation, insignificant in volume, formed in both

test tubes. This may indicate the possible presence of tannins in the studied raw materials.

Conclusions to the chapter 2

1. As a result of studying the qualitative analysis of *V. canina*, at least 11 free amino acids were identified in the grass.
2. Malic, citric and ascorbic organic acids were found in the herb *V. canina*.
3. Phenolic substances were represented by hydroxycinnamic, phenolcarboxylic acids, anthocyanins flavanoids and tannins. Neochlorogenic, chlorogenic, and caffeic acids were identified in the grass and flowers of *V. canina*; gallic and salicylic acids from phenolcarboxylic acids; and kaempferol, quercetin, and rutin from flavonoids; anthocyanins are represented by 3 substances of the delphinidin group.

CHAPTER 3

STUDY OF THE QUANTITATIVE CONTENT OF THE MAIN GROUPS OF BAS IN THE GRASS *VIOLA CANINA*

A preliminary study of the qualitative composition of *V. canina* raw materials revealed amino acids, organic acids, and phenolic compounds (Chapter 2). The next stage of the work was to determine the quantitative content of these groups of compounds and also minerals in the *V. canina* raw materials.

3.1 Study of amino acids by gas-liquid chromatography

According to preliminary analysis, nonessential and irreplaceable amino acids were found in *V. canina* raw materials. The identification of amino acids and their content of amino acids in the *V. canina* grass was determined by the GLC method using standard amino acid samples. The results of the experiment are shown in Table 3.1.

Table 3.1

Qualitative composition and quantitative content of amino acids in the herb *V. canina*

№	Amino acid name	Amino acid content*, mg/100g
1	2	3
1	Alanine	690,00
2	Valin ¹	405,00
3	Serene	410,00
4	Glycine	330,00
5	Proline	340,00
6	Leucine ¹	421,00
7	Isoleucine ¹	457,00

Continuation of Table 3.1

1	2	3
8	Threonine ¹	685,00
9	Methionine ¹	960,00
10	Arginine ¹	1375,00
11	Lysine ¹	620,00
12	Aspartic acid	1200,00
13	Glutamic acid	1060,00
14	Phenylalanine ¹	980,00
15	The amount of essential amino acids	5235,00
16	The amount of non-essential amino acids	4698,00
17	Total amount of amino acids	9933,00

Notes:

* - in terms of absolutely dry raw materials,

1 - essential amino acids

The amino acid composition of the herb *V. canina* is represented by 14 amino acids, of which 8 are essential. The total amount of amino acids was 9933 mg/100 g. Essential amino acids accumulated 5235.00 mg/100g, and nonessential amino acids 4698.00 mg/100g. Of the essential amino acids, the grass contained arginine (1375.00 mg/100g) and methionine (960.00 mg/100g) in large quantities, and of the essential amino acids, aspartic acid and glutamic acid (1200.00 and 1060.00 mg / 100 g, respectively).

3.2 Quantification of sum of organic acids

Ascorbic acid, due to its antioxidant activity, takes an important part in the metabolic processes of the body, and organic acids in the Krebs cycle. Quantitative

determination of ascorbic acid and the amount of organic acids was carried out according to the methods given in the pharmacopoeia [5, 6-8].

The results of the experiment are given in table. 3.2.

Table 3.2

The results of determining the quantitative content of organic acids in the grass *V. canina* (% , m = 5, P > 0,95)

Group of BAS	W, %	$\bar{x} \pm \Delta \bar{x}$	$S \cdot 10^{-3}$	$S_{\bar{x}} \cdot 10^{-3}$	$\bar{\epsilon}$
Ascorbic acid ¹	7,68	0,063±0,003	8,74	1,25	5,92
Organic acids ¹	7,68	1,074±0,051	4,21	1,88	5,46

Note. ¹ – в перерахунку на абсолютно суху сировину

According to the data in Table. 3.2, the content of the total organic acids in the grass was 17 times higher than ascorbic acid. The results obtained can be used in the selection of possible indicators of standardization of raw materials.

3.3 Quantification of sum of anthocyanins

According to the literature, anthocyanins, a group of substances of a phenolic nature, accumulate in parts of plants with a blue, pink color. Given the blue color of *V. canina* flowers, we studied this group of substances in the grass, which was harvested during the flowering phase and separately in flowers.

Spectrophotometric determination of anthocyanins was carried out according to the method described in the monograph “Dried blueberries fruits” of Pharmacopoeia 2.0 [6]. The amount of anthocyanins was converted to cyanidin-3-O-glucoside chloride. Used on a Specord-200 spectrophotometer, measurements were carried out at a wavelength of 528±2 nm.

The results are presented in table 3.3.

Table 3.3

The amount of anthocyanins in the herb *V. canina*

Raw material	Content, %
Grass	1,35±0,06
Flowers	2,63±0,05

Note. n=5, in terms of absolutely dry raw materials

The content of total anthocyanins was 1.9 times higher in *V. canina* flowers than in grass.

3.4 Quantification of sum of hydroxycinnamic acids

Based on the results of a preliminary study of the qualitative composition of the herb *V. canina*, substances of a phenolic nature were identified. Hydroxycinnamic acids have a wide spectrum of pharmacological activity (Chapter 1). One of the stages of the work was the determination of the quantitative content of substances of a phenolic nature, including the amount of hydroxycinnamic acids. The quantitative content of the sum of hydroxycinnamic acids was determined by the spectrophotometric method at a wavelength of 525 ± 2 nm, described in the monograph of the pharmacopeia [8]. The content of the sum of hydroxycinnamic acids was recalculated for chlorogenic acid. The results are presented in table 3.4.

Table 3.4

The content of the sum of substances of phenolic nature in the herb *V. canina*

(B %, T = 5, P > 0,95)

Group of BAS	$\bar{x} \pm \Delta \bar{x}$	$S \cdot 10^{-2}$	$S_x \cdot 10^{-2}$	$\bar{\varepsilon}$
Sum of hydroxycinnamic acids *	2,62±0,06	4,01	2,34	3,36
Sum of flavonoids *	2,05±0,08	5,71	2,64	4,34

Note. * – in terms of absolutely dry raw materials

The content of the total hydroxycinnamic acids in the herb *V. canina* was 2,62%.

3.4. Quantification of sum of flavonoids

Violet species accumulate different groups of phenolic substances, including various flavonoids, which cause a wide range of pharmacological activity of violet preparations (Chapter 1). The kaempferol, quercetin and rutin were identified from the results of chromatography of the herb *V. canina*.

The quantitative content of the sum of flavonoids in the raw material was carried out by the spectrophotometric method at a wavelength of 415 ± 2 nm in terms of rutin [8]. The results are presented in table 3.4.

The content of total flavonoids in the herb *V. canina* was 2,05%.

The data obtained will be used in the development of documentation regulating raw materials.

3.5. Mineral composition of grass

Plants in the process of life accumulate minerals. Given the place of growth and the technogenic situation, both useful minerals and heavy metals, which are dangerous to humans, can accumulate in plant tissues. Therefore, the mineral composition was studied in samples of violet raw materials.

The study of the elemental composition was carried out according to the method described in Chapter 2. The determination of heavy metals was carried out according to the requirements of the monograph 2.4.8 "Heavy metals" of the pharmacopeia [6-8]. Experimental data are shown in Table 3.5.

The mineral composition of the herb *V. canina* is represented by 19 components. Among the identified mineral substances, Na, K and Ca (1720, 1600 and 1050 $\mu\text{g}/100$ g, respectively) accumulate in the grass in large quantities. Si and Mg accumulate in significant amounts (803 and 749 $\mu\text{g}/100$ g, respectively). In the studied sample of raw materials, the content of heavy metals did not exceed the

permissible norms, except for Mo and Pb, which may be related to the place of procurement of raw materials.

Table 3.5

Mineral composition of *V. canina* grass

№	Mineral element	Content, mcg/100 g
1	K	1600,00
2	Na	1720,00
3	Ca	1050,00
4	Mg	740,00
5	Si	803,00
6	P	470,00
7	Al	250,00
8	Fe	43,00
9	Mn	180,00
10	Cu	8,00
11	Zn	6,00
12	Mo	0,04
13	Pb	0,05

Note. Hg, As, Cd, Co, Ni, Sr <0,03

Conclusions to the chapter 3

1. The amino acid composition of the herb *V. canina* is represented by 14 amino acids, of which 8 are essential. The total amount of amino acids was 9933,00 mg/100 g. Essential amino acids accumulated 5235,00 mg/100g, and nonessential amino acids 4698,00 mg/100g.

2. In *V. canina* grass, the content of ascorbic acid was 0,063%, and the amount of organic acids was 1.074%.

3. The content of total anthocyanins in the herb and flowers was 1,35 % and 2,73 % respectively. The content of total flavonoids and hydroxycinnamic acids in the herb *V. canina* was 2.05% and 2,62% respectively.

4. In the herb of *V. canina*, 19 elements were identified, of which Na, K and Ca accumulated in larger quantities. The content of heavy metals did not exceed the permissible limits, except for Mo and Pb.

CHAPTER 4

STUDY OF MORPHOLOGICAL AND ANATOMICAL FEATURES AND PARAMETERS OF THE GRASS *VIOLA CANINA*

4.1 Morphological features of grass

The grass *Viola canina* is represented by a short grassy shoot (Fig. 4.1.1). The leaves are simple, long-petiolate, with oval stipules adnate to the petiole at the base. The leaf blade is rounded or broadly kidney-shaped (Fig. 4.1.2), with a rounded apex and a notched base, a shortly crenate margin, and pinnately arcuate venation.

Flowers (Fig. 4.1.1, 4.1.3) on elongated pedicels, blue corolla, white in throat with brown veins, zygomorphic, with five oval petals.



1



2



3



4

Fig. 4.1 Morphological characteristics of the herb *V. canina*.

1 - general view of the plant, 2 - leaf blade, 3 - flower (top view), 4 - fruit box with seeds.

Stamens five, filaments short and wide, yellow anthers small elongated conical. Calyx five-membered, sepals small, lanceolate, fused at the base. The gynoecium is coenocarpous, formed by 3 carpels, the ovary is spherical, the style is short, the stigma is hooked. The fruit is a capsule (Fig. 4.1.4), opens after ripening, the seeds are brown, shiny, numerous.

Dried raw material *V. canina* consists of a mixture of parts stems, leaves and flowers, rarely come across seed capsules. It can be whole, cut (particle size 0.5-2 cm) (Fig. 4.2.1, 2) and powdered raw materials (particle size up to 3 mm).



1



2

Рис. 4.2 Dry herb of the *V. canina*.

1 – whole grass 2 – cut grass.

4.2. Anatomical signs of grass

The analysis of microscopic signs of raw materials was carried out in accordance with the pharmacopeia method [7].

The stem of *V. canina* in cross section is round, hollow, slightly ribbed (Fig. 4.3.1). The stem is covered with a single-row epidermis, covered with simple unicellular conical trichomes with a warty cuticle (Fig. 4.3.1, a). Under the epidermis in the ribs there are small 2-3-row sections of the angular collenchyma, and under the epidermis between the ribs - 2-3 rows of chlorenchyma. Below is the cortical parenchyma of rounded thin-walled cells. Endoderm pronounced. The stem is of a transitional type of structure. Vascular open collateral bundles (Fig.

4.3.1, 2) are broadly oval. The phloem is less developed than the xylem. Xylem vessels (Fig. 4.3.a, b) are porous and spiral, wide-lumen, arranged in rows.

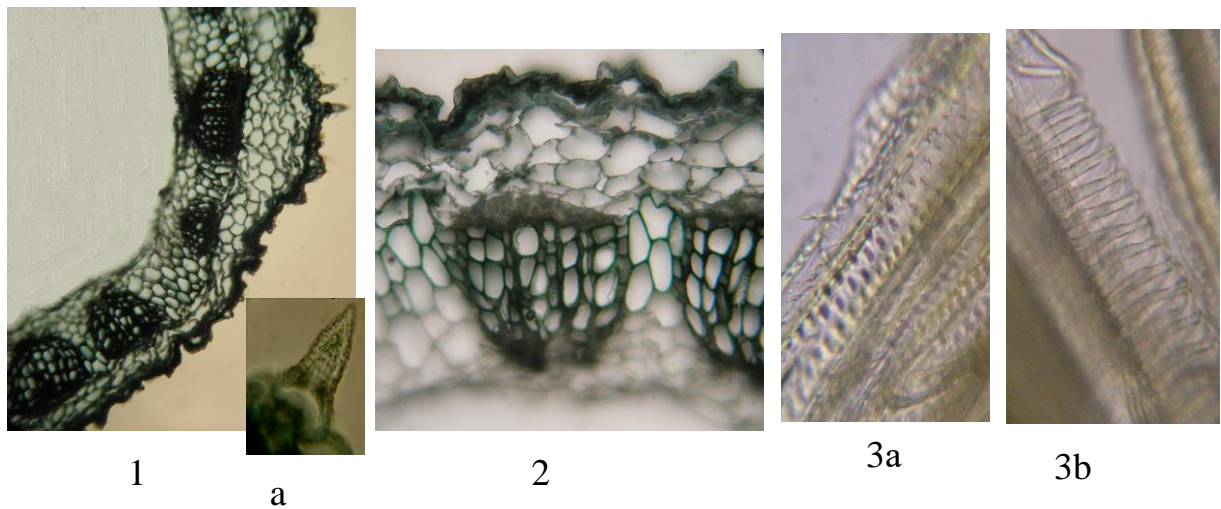


Fig. 4.3 Anatomical features of the stem of *V. canina*

1 – stem fragment on l/m, a – 1-cellular hair, 2 – open collateral bundles on the h/m, 3 – vessels: a- pitted, b- spiral.

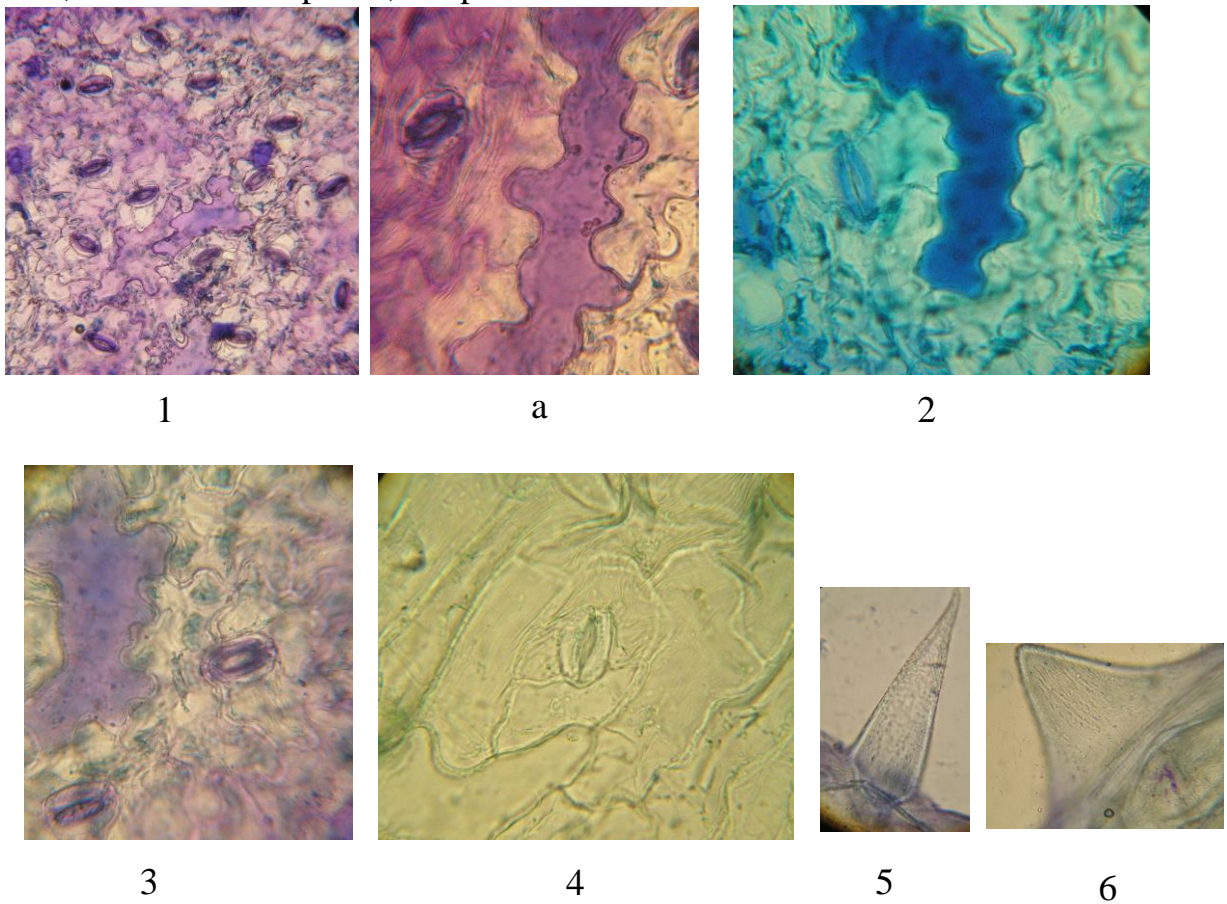


Fig. 4.4 Anatomical features of the leaf and stipule of *V. canina*.

Leaf: 1 – upper epidermis, a – view on h/m, 2 – lower epidermis, *Stipule*: 3 – upper epidermis, 4 – lower epidermis, 5 – conical 1-cellular hair with thin base 6 – conical 1-cellular hair with wide base.

The upper epidermis of the leaf is similar in structure (Fig. 4.4.1, 2) and is formed by parenchymal and prosenchymal cells, with thin, strongly sinuous membranes. The epidermis is covered with a well-defined folded cuticle. Stomata frequent, oval. The type of stomatal apparatus is anomocytic type.

The epidermis of the stipules is represented by medium-sized elongated cells; large elongated cells are less common. Stomata frequent, oval. The type of stomatal apparatus is anomocytic type.

Throughout the epidermis of the leaf and stipules, more often along the veins, conical, simple, 1-cell hairs with a narrow or wide base are rarely located (Fig. 4.4.1, 5.6). The hairs are covered with a warty cuticle. Large druses of calcium carbonate are located in the mesophyll of the leaf. Among the epidermal cells of the leaves and stipules are large oblong cells with mucus (qualitative reaction with methylene blue - blue staining) (Fig. 4.4.1, 2, 3).

The epidermis of the petals with pronounced conical papillary outgrowths (Fig. 4.5.1), the shape of the cells is oval with thin membranes and loop-like outgrowths (Fig. 4.5.2).

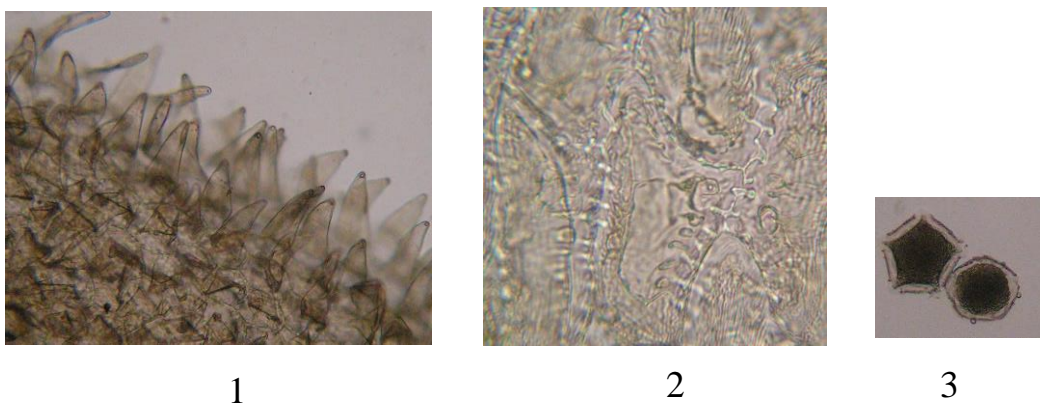


Fig. 4.5 Anatomical features of the flower of *V. canina*

1 - papillary epidermis of the petal, 2 - epidermal cells of the corolla, 3 – pollen.

Pollen grains with 5 pronounced apertures and rough exine (Fig. 4.5.3).

As a result of macroscopic analysis of the herb *V. canina*, the following diagnostic features were identified:

- the simple, long-petiolate leaves with oval stipules adnate to the petiole; the leaf blade is rounded or broadly kidney-shaped with a rounded apex and a notched base, a shortly crenate margin, and pinnately arcuate venation;
- the zygomorphic flowers with elongated pedicels, five blue oval petals, white in throat with brown veins; the sepals of the calyx are lanceolate, fused at the base;
- the stamens with short and wide filaments and yellow conical anthers; the gynoecium is coenocarpous, formed by 3 carpels, the ovary is spherical, the style is short, the stigma is hooked.
- the fruit is a bol, opens after ripening, the seeds are brown, shiny, numerous.

The diagnostic microscopic herbs *V. canina* include:

- the transitional type of stem structure with cavity and ribs; angular collenchyma 2-3-row, pronounced endoderm; open collateral vascular bundles, vessels are wide-lumen, porous and spiral;
- the leaf epidermis cells are parenchymal and prosenchymal, thin-walled and sinuous wald; the type of stomatal apparatus is anomocytic type;
- the stipules epidermis cells are medium-sized elongated; large elongated cells are less common with mucus;
- papillary epidermis of the petals;
- hairs are simple 1-cellular, conical with a warty cuticle;
- pollen grains with 5 pronounced apertures and rough exine; druses.

4.3. Determination of the main numerical indicators of raw materials

4.3.1 Loss in mass during drying

The weight loss on drying of the herb was determined according to the pharmacopeia method [8]. To determine the weight loss during drying of raw materials, the calculation formula was used:

$$X = \frac{(m - m_1) \cdot 100}{m}, X_{cp} = \frac{1}{n} \cdot \sum_{i=1}^n X_i$$

where m_n - the mass of raw materials before drying, g

m_1 - the mass of raw materials after drying, g.

The results of the experiment are shown in Table 4.1.

Table 4.1

The result of determining the loss in mass during drying of the herb *V. canina*

Sample	X_{cp}	S^2	S_{cp}	Confidence interval	ε , %
Трава	9,26	0,0384	0,2201	0,128	1,23
Цветки	9,08	0,1243	0,3380	0,196	1,92

According to the results of the experiment, the moisture content of *V. canina* grass was 9.26%, and that of flowers was 9.08%.

4.3.2 Total ash and ash, insoluble in hydrochloric acid

Determination of total ash and ash, insoluble in hydrochloric acid in violet herb was carried out according to the method described in the pharmacopoeia [8]. ash, insoluble in hydrochloric acid was determined after determining the total ash.

The content of total ash was calculated by formula 3.2:

$$X = \frac{(m_2 - m_1) * 100 * 100}{m * (100 - W)},$$

where

m_2 - mass of the crucible with ash

m_1 is the mass of the empty crucible

m - weight of raw materials

W - weight loss on drying.

The results are shown in Table 4.2.

Table 4.2

**Content of total ash and ash, insoluble in hydrochloric acid in *V. canina* grass
(n=3)**

Indicators	X_{cp}	S^2	S_{cp}	Confidence interval	ϵ , %
Total ash	3,52	0,00130	0,0361	0,02	0,59
Ash, insoluble in hydrochloric acid	1,41	0,0003	0,0173	0,010	0,71

The amount of total ash in *V. canina* was 3.52%, and ash, insoluble in hydrochloric acid was 1.41%.

4.3.2 Impurities

Determination of the content of foreign impurities was carried out in accordance with the requirements of the State Pharmacopoeia of Ukraine 2.8.2 [8, 9]. The results are shown in Table 4.3.

Table 4.3

The results of the study of the herb *V. canina* for the presence of impurities

Indicators	Rationing	Analysis results, in %	
Organic impurities	no more than 3%	1,2	corresponds
Mineral impurities	no more than 1%	0,5	corresponds
Foreign impurities	no more than 3%	2,	corresponds

The sample of the herb *V. canina* meets the requirements of the Pharmacopoeia according to the content of impurities.

When determining the presence of impurities, it was found that mineral impurities were represented by sand and small stones. The parts of the raw material that have lost the color characteristic of this type of raw material and parts of the plant (roots) acted as organic impurities.

4.4 Determination of the content of extractive substances

Determination of extractive substances in the herb *V. canina*, depending on the type of extractant, was carried out according to the method of the pharmacopeia monograph [7]. Water R, 40% ethanol, and 70% ethanol were used as the extractant. The content of extractive substances in percent (X) was calculated by formula 3.5:

$$X = \frac{m_1 * 100 * 100}{m * (100 - W)},$$

where, m - weight of raw materials in grams;

m₁ is the mass of dry residue in grams.

The results of the experiment are shown in Table 4.4.

Table 4.4

The content of extractive substances in the herb *V. canina* (n=5), B %

Extractant		
Water	40% ethanol	70% ethanol
X _{cp} = 28,65	X _{cp} = 32,46	X _{cp} = 30,54
S ² = 0,1737	S ² = 0,2045	S ² = 0,1147
S _{cp} = 0,4158	S _{cp} = 0,4526	S _{cp} = 0,3383
ε, %=1,40	ε, %= 1,40	ε, %= 1,12

Based on the research results, it can be concluded that the use of 40% and 70% ethanol as an extractant is the most appropriate in comparison with water.

Conclusions to the chapter 4

1. Morphological and anatomical analysis of the herb *V. canina* was carried out. The diagnostic macroscopic and microscopic features of the herb *V. canina* were determined.
2. According to the results of the extraction of extractives from the herb, it was found that the best extractant is 40% and 70% ethanol.

3. The indicators of the quality of Violet grass were determined, such as the loss in mass during drying, the content of total ash, the content of ash insoluble in hydrochloric acid, the content of impurities.

GENERAL CONCLUSIONS

1. A pharmacognostic study of the herb *V. canina* was carried out and the prospects for further study of this species and the development of herbal preparations based on it were shown.
2. Qualitative reactions established that the herb *V. canina* contains amino acids, organic acids, hydroxycinnamic and phenolcarboxylic acids, flavonoids, tannins.
3. At least 14 amino acids were identified by gas-liquid chromatography in the herb *V. canina*, their content was determined. The total amount of amino acids was 9933 mg/100 g. Essential amino acids accumulated 5235.00 mg/100g, and nonessential amino acids 4698.00 mg/100g.
4. Using paper and thin-layer chromatography in the herb *V. canina* was identified from organic acids malic, citric and ascorbic acids, from hydroxycinnamic acids - neochlorogenic, chlorogenic, caffeic acids, from phenolcarboxylic acids - salicylic and galic acids, from flavonoids - kaempferol, quercetin, rutin and hyperoside; from anthocyanins, 3 substances of the delphinidin group were identified. The spectrometric method in the herb *V. canina* determined the content of the total hydroxycinnamic acids (2.62%), the total flavonoids (2.05%), the total anthocyanins (1.47%). The content of total anthocyanins in the herb and flowers was 1,35 % and 2,73 % respectively.
5. The mineral composition of *V. canina* grass was studied by atomic emission spectrometry, the content of 19 elements was determined. The content of heavy metals did not exceed the permissible limits, except for lead and molybdenum.
6. Grass indicators were studied: weight loss during drying, total ash content, ash undissolved in hydrochloric acid, impurity content.
7. The best extractants for extracting extractive substances from the herb were experimentally selected - 40% and 70% ethanol.

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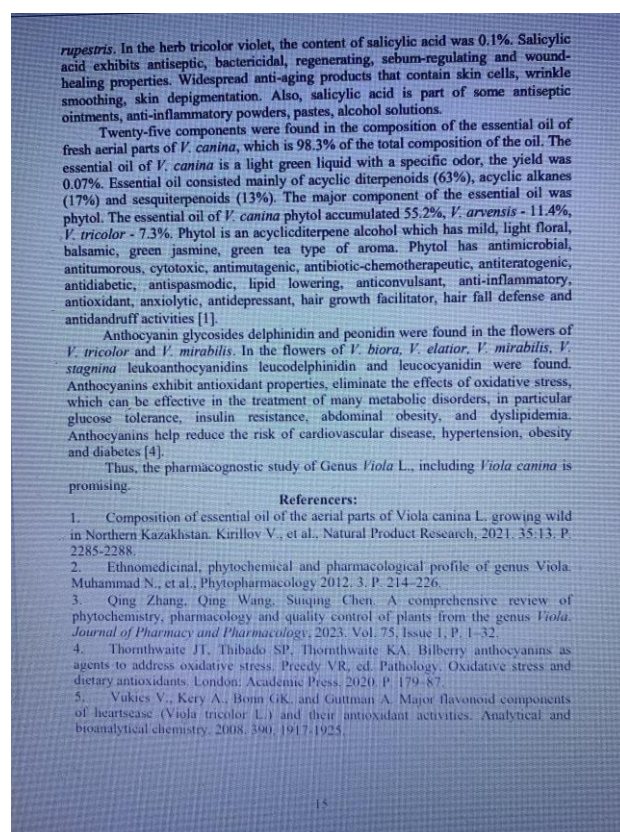
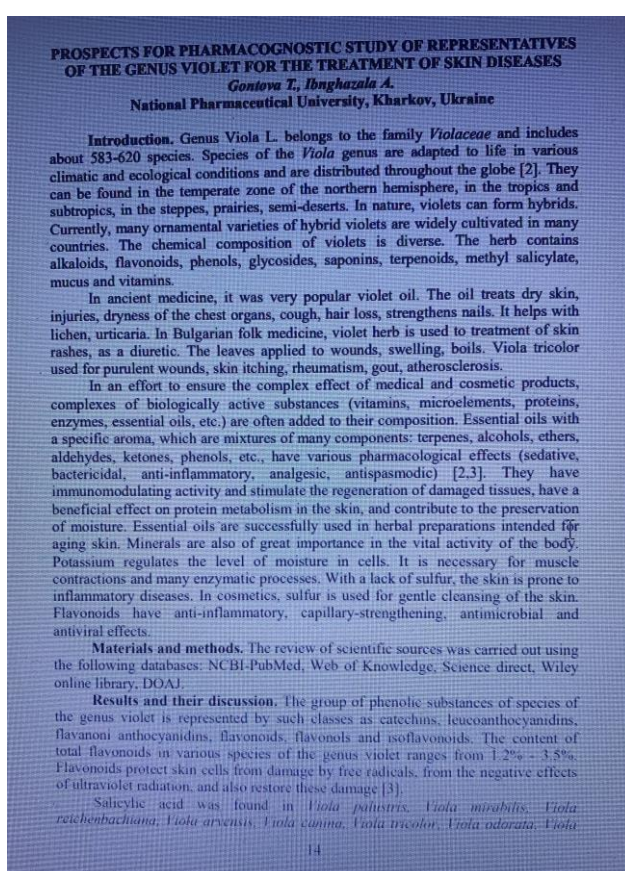
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APPENDICES

Publication on qualifying work

Gontova T.M., Ibinghazala A. Prospects for pharmacognostic study of representatives of the genus Violet for the treatment of skin diseases. Current approaches of pharmaceutical science in development and standardization of medicines and dietary supplements that contain components of natural origin: The Proceedings of the V International Scientific and Practical Internet-Conference, 14 april, 2023, Kharkiv. 2023. P. 14-15.





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**

Asmaa IBNGHAZALA

1. Topic of qualification work: «Pharmacognostic study of the Viola canina L.», 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 Viola canina 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 Viola canina; 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 – 12, pictures – 8.

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 Viola canina (Literature Review). Formatting section 1.	September 2022	done
2	Qualitative analysis of some groups of biologically active substances in Viola canina raw materials. Formation of section 2.	October 2022	done
3	Quantitative determination of some groups of biologically active substances in the herb Viola canina. Formation of section 3.	November 2022	done
4	Morphological and anatomical study of the herb Viola canina.	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

_____ Asmaa IBNGHAZALA
 _____ 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				
Ібнґхазала Асмаа	Фармакогностичне вивчення <i>Viola canina</i> L.	Pharmacognostic study of the <i>Viola canina</i> L.	професор Гонтова Т.М.	проф. Перехода Л.О.

Підстава: подання іскана, згода ректора

Ректор

Вірно. Секретар



ВИСНОВОК

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

№ 112907 від « 2 » травня 2023 р.

Проаналізувавши випускну кваліфікаційну роботу за магістерським рівнем здобувача вищої освіти денної форми навчання Ібнґхазала Асмаа, 5 курсу, _____ групи, спеціальності 226 Фармація, промислова фармація, на тему: «Фармакогностичне вивчення *Viola canina* L / Pharmacognostic study of the *Viola canina* L», Комісія з академічної доброчесності дійшла висновку, що робота, представлена до Екзаменаційної комісії для захисту, виконана самостійно і не містить елементів академічного плагіату (копіювання).

**Голова комісії,
професор**



Інна ВЛАДИМИРОВА

3%

26%

REVIEW

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

Asmaa IBNGHAZALA

on the topic: «Pharmacognostic study of the Viola canina L.»

Relevance of the topic. The study of species belonging to the same genus is promising for pharmacy. Species of the same genus have a similar chemical composition and can be used as the main or additional raw material in the production of phytopreparations. Species of the genus Viola are widely used in diseases of the upper respiratory tract, gastrointestinal tract, in dermatology. The herb Viola tricolor and Viola arvensis is included in the State Pharmacopoeia of Ukraine and EurPh. Viola canina is widespread in Europe, Ukraine. The species has a wide distribution area.

Therefore, the pharmacognostic study of Viola canina herb is an urgent task of pharmacy.

Practical value of conclusions, recommendations and their validity. For the first time, a comprehensive pharmacognostic study of the herb Viola canina, which grows in Ukraine, was carried out.

Viola canina 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 was determined in the raw materials of Viola canina. The mineral composition of the herb Viola canina was determined.

In the herb Viola canina, the qualitative composition and quantitative content of essential and non-essential amino acids were determined.

For the first time, Viola canina raw materials were determined by loss in mass on drying, total ash, and ash insoluble in hydrochloric acid. According to the yield of extractive substances, the best extractant for the herb Viola canina was selected.

Assessment of work. The qualification work was carried out at the Department of Pharmacognosy of the NUPh. Asmaa IBNGHAZALA 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 Asmaa IBNGHAZALA 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

Asmaa IBNGHAZALA

on the topic: «Pharmacognostic study of the *Viola canina* L.»

Relevance of the topic. Replenishment of the range of medicines with new herbal preparations has been and remains one of the most important problems of pharmacy. This is due to the fact that phytopreparations containing natural substances do not cause a number of negative phenomena characteristic of synthetic agents. The search for promising plants among representatives of the domestic flora, which have a sufficient raw material base and are used in traditional medicine, is an urgent task of modern pharmacy.

In this aspect, the attention of Asmaa IBNGHAZALA was attracted by *V. canina*, widely distributed in Europe and used as an expectorant, mucolytic, anti-inflammatory, diuretic and choleretic agent.

Theoretical level of work. Asmaa IBNGHAZALA 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 applicant conducted a pharmacognostic study of the herb *V. canina*. Amino acids, organic acids, hydroxycinnamic and phenolcarboxylic acids, flavonoids, tannins were found in the herb *V. canina*. The content of at least 14 amino acids was identified by gas-liquid chromatography. Malic, citric and ascorbic acids were identified from organic acids in the herb *V. canina*; from hydroxycinnamic acids - neochlorogenic, chlorogenic, caffeic acids; from phenolcarboxylic acids - salicylic and halic acids; from flavonoids - kaempferol, quercetin, rutin and hyperoside; from anthocyanins, 3 substances of the delphinidin group have been identified. The spectrometric method in the herb *V. canina* determined the content of the total hydroxycinnamic acids (2.62%), the total flavonoids (2.05%), the total anthocyanins (1.47%). The content of total anthocyanins in grass and flowers was 1.35% and 2.73%, respectively. The mineral composition of the herb *V. canina* was studied by atomic emission spectrometry, and the content of 19 elements was determined. The content of heavy metals did not exceed the permissible limits, except for lead and molybdenum. Grass indicators were studied: weight loss during drying, total ash content, ash undissolved in hydrochloric acid, impurity content. Experimentally, the author selected the best extractants for extracting extractive substances from grass - 40% and 70% 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 *Viola canina* 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 Asmaa IBNGHAZALA on the topic "Pharmacognostic study of the Viola canina L." 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)-03)англ Асмаа ІБНГХАЗАЛИ на тему «Фармакогностичне вивчення *Viola canina* L.».

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)-03)англ Асмаа ІБНГХАЗАЛИ на тему «Фармакогностичне вивчення *Viola canina* L.» до захисту в Екзаменаційній комісії.

Голова
Завідувачка кафедри
Секретар

_____ **Ольга МАЛА**
_____ **Микола КОМІСАРЕНКО**

НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

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

Направляється здобувач вищої освіти Асмаа ІБНГХАЗАЛА до захисту кваліфікаційної роботи
за галуззю знань 22 Охорона здоров'я
спеціальністю 226 Фармація, промислова фармація
освітньою програмою Фармація
на тему: «Фармакогностичне вивчення *Viola canina* L.»
Кваліфікаційна робота і рецензія додаються.

Декан факультету _____ / Світлана КАЛАЙЧЕВА /

Висновок керівника кваліфікаційної роботи

Здобувач вищої освіти Асмаа ІБНГХАЗАЛА в процесі виконання кваліфікаційної роботи освоїла і використала 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. Асмаа ІБНГХАЗАЛА 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 Асмаа ІБНГХАЗАЛА 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 /