МІНІСТЕРСТВО ОСВІТИ І НАУКИ УКРАЇНИ
НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ
ФАКУЛЬТЕТ ЕКОЛОГІЧНОЇ БЕЗПЕКИ, ІНЖЕНЕРІЇ ТА ТЕХНОЛОГІЙ
КАФЕДРА БІОТЕХНОЛОГІЇ

ДОПУСТИТИ ДО ЗАХИСТУ
Завідувач випускової кафедри
_____ М.М. Барановський
«___» _____2021

ДИПЛОМНА РОБОТА

(ПОЯСНЮВАЛЬНА ЗАПИСКА)

ЗДОБУВАЧА ВИЩОЇ ОСВІТИ ОСВІТНЬОГО СТУПЕНЯ «БАКАЛАВР»
СПЕЦІАЛЬНІСТЬ 162 «БІОТЕХНОЛОГІЇ ТА БІОІНЖЕНЕРІЯ»
ОСВІТНЬО-ПРОФЕСІЙНА ПРОГРАМА «ФАРМАЦЕВТИЧНА БІОТЕХНОЛОГІЯ»

Тема: «Технологія отримання сухого екстракту біологічно активних речовин з коренів Вовчого тіла болотяного»

Виконавець: студентка 404 гр. ФЕБІТ Скрипець Д.Ю.

Керівник дипломної роботи: к.т.н, доцент Кузнєцова О.О.

Нормоконтролер: Дражнікова А.В.

КИЇВ 2021

MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
NATIONAL AVIATION UNIVERSITY
FACULTY OF ENVIRONMENTAL SAFETY, ENGINEERING AND TECHNOLOGIES
DEPARTMENT OF BIOTECHNOLOGY

ALLOWED TO DEFENCE
Head of Department
_____M.M. Baranovskyy
«___» _____2021

BACHELOR THESIS

(EXPLANATORY NOTE)

GRADUATE OF HIGHER EDUCATION OF THE EDUCATIONAL DEGREE "BACHELOR"
SPECIALTY 162 «BIOTECHNOLOGY AND BIOENGINEERING»
EDUCATIONAL PROFESSIONAL PROGRAM «PHARMACEUTICAL BIOTECHNOLOGY»

Theme: «Technology of obtaining dry extract of biologically active substances from the roots of Marsh cinquefoil»

Executor: student of group 404Б FESET Skripets' D.Y.

Supervisor: PhD, Associate Professor Kuznietsova O. O.

Standards inspector: Drazhnikova A.V.

KYIV 2021

NATIONAL AVIATION UNIVERSITY

Faculty of Environmental Safety, Engineering and Technologies

Department of Biotechnology

Specialty: 162 «Biotechnology and Bioengineering»

EPP: «Pharmaceutical Biotechnology»

APPROVED

Head of the Department

_____ M.M. Baranovskyy

«___» _____2021

TASK

to perform a thesis

Skripets Daria Yuriivna

1. Theme of degree work: «Technology of obtaining dry extract of biologically active substances from the roots of Marsh cinquefoil» approved by the Rector «11» of May 2021 № 715/ст

2. Term of work execution: from «10» of May 2021 to «14» of June 2021.

3. Content of explanatory note: Introduction. Characteristics of Marsh cinquefoil. Study of the content of biologically active substances of marsh cinquefoil. Technology of dry extract from the roots of marsh cinquefoil. Conclusions. References.

5. List of necessary graphic (illustrative) material: 7 fig., 5 tables.

НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ

Факультет екологічної безпеки, інженерії та технологій

Кафедра біотехнології

Спеціальність: 162 «Біотехнології та біоінженерія»

Освітньо-професійна програма «Фармацевтична біотехнологія»

ЗАТВЕРДЖУЮ

Завідувач кафедри

_____М.М. Барановський

«___»_____2021 р.

ЗАВДАННЯ

на виконання дипломної роботи

Скрипець Дар'я Юріївна

1. Тема дипломної роботи: «Технологія отримання сухого екстракту біологічно-активних речовин з коренів Вовчого тіла болотяного» затверджена ректором від «11» травня 2021 р. № 715/ст.
2. Термін виконання роботи: з «10» травня 2021 р. по «14» червня 2021 р.
3. Зміст пояснювальної записки: Вступ. Характеристика вовчого тіла болотяного. Дослідження вмісту біологічно-активних речовин вовчого тіла болотяного. Технологія отримання сухого екстракту з коренів вовчого тіла болотяногою. Висновки. Список використаних джерел.
4. Перелік обов'язкового графічного (ілюстративного) матеріалу: 7 рис., 5 таблиць.

№	Task	Executio	Signature of the
1	Coordination of the content of the thesis with the thesis supervisor	10.05.21 – 12.05.2021	
2	Selection of information soutces	12.05.2021– 17.05.2021	
3	Future work concept development	17.05.2021– 20.05.2021	
4	Processing information sources	20.05.2021– 25.05.2021	
5	Registration of the thesis	25.05.2021– 30.05.2021	
6	Formulation of conclusions	30.05.2021– 05.06.2021	
7	Thesis defense	14.06.21	

5. Календарний план-графік

6. Date of issue of the task: «10» of May 2021

Supervisor of degree thesis _____ Kuznietsova O. O.

Task for execution was taken over by _____ Skripets' D.Y.

№ з/п	Завдання	Термін виконання	Підпис керівника
1	Узгодження змісту дипломної роботи з дипломним керівником	10.05.21 – 12.05.2021	
2	Підбір джерел інформації	12.05.2021 – 17.05.2021	
3	Розробка концепції майбутньої роботи	17.05.2021– 20.05.2021	
4	Обробка джерел інформації	20.05.2021– 25.05.2021	
5	Оформлення дипломної роботи	25.05.2021– 30.05.2021	
6	Формулювання висновків	30.05.2021– 05.06.2021	
7	Захист дипломної роботи	14.06.2021	

5. Дата видачі завдання «10» травня 2021 р.

Керівник дипломної роботи _____ Кузнєцова О.О.

Завдання прийняла до виконання _____ Скрипець Д.Ю.

ABSTRACT

Explanatory note to the diploma thesis « Technology of obtaining dry extract of biologically active substances from the roots of Marsh cinquefoil», 49 p., 5 tables, 7 figures, 59 references.

Object of investigation - Marsh cinquefoil

Subject of investigation - dry extract of biologically active substances from the roots of Marsh cinquefoil

The purpose of the work - to improve the technology of obtaining dry extract of biologically active substances from the roots of Marsh cinquefoil

In the nomenclature of medicines a significant place is occupied by drugs produced from medicinal plants, as they have a number of advantages over synthetic drugs. Phytopreparations have become especially popular today. The analysis of the content of biologically active substances in such a medicinal plant as Marsh cinquefoil is carried out. The pharmacological activity of biologically active substances of Marsh cinquefoil was analyzed. Possibilities of application of medicines and biologically active additives on the basis of this medicinal plant are considered. As a result of the literature analysis, it was found that a promising technology is to obtain a dry extract from the roots of Marsh cinquefoil with the subsequent use of the obtained extract for the production of dosage forms of medicines (tablets and capsules). To optimize the extraction stage, optimal regime parameters such as temperature and extraction duration were determined. The optimal extractant and the degree of grinding of the plant raw material are selected as well.

The results of the work can be used for the development of drugs containing biologically active substances from the roots of Marsh cinquefoil.

MARSH CINQUEFOIL, BIOLOGICALLY ACTIVE SUBSTANCES, EXTRACT, REGIME PARAMETERS

РЕФЕРАТ

Пояснювальна записка до дипломної роботи «Технологія отримання сухого екстракту біологічно активних речовин з коренів Вовчого тіла болотяного», 49 с., 5 таблиць, 7 рисунків, 59 літературних джерел.

Об'єкт дослідження – Вовче тіло болотяне

Предмет досліджень – сухий екстракт біологічно-активних речовин з коренів вовчого тіла болотяного

Мета роботи – вдосконалення технології отримання сухого екстракту біологічно-активних речовин з коренів Вовчого тіла болотяного.

Методи дослідження – мікробіологічні, фізико-хімічні, аналітичні.

У номенклатурі лікарських засобів значне місце займають препарати з лікарської рослинної сировини, так як вони мають цілий ряд переваг в порівнянні з синтетичними препаратами. Особливу популярність на сьогодні набули фітопрепарати. В роботі проведений аналіз вмісту біологічно активних речовин в такій лікарській рослині як Вовче тіло болотяне. Проаналізовано фармакологічну активність біологічно активних речовин Вовчого тіла болотяного. Розглянуто можливості застосування лікарських засобів і біологічно активних добавок на основі цієї лікарської рослини. В результаті проведеного літературного аналізу з'ясовано, що перспективною технологією є отримання сухого екстракту з коренів Вовчого тіла болотяного з подальшим використанням отриманого екстракту для виробництва дозованих лікарських препаратів (таблеток і капсул). Для оптимізації стадії екстрагування були визначені оптимальні режимні параметри, такі як температура та тривалість екстрагування. Вибраний оптимальний для цих цілей екстрагент та ступінь подрібнення рослинної сировини.

Результати роботи можуть бути використані для розробки препаратів, що містять біологічно активні речовини з коренів Вовчого тіла болотяного.

ВОВЧЕ ТІЛО БОЛОТЯНЕ, БІОЛОГІЧНО-АКТИВНІ РЕЧОВИНИ, ЕКСТРАКТ, РЕЖИМНІ ПАРАМЕТРИ

CONTENT

LIST OF SYMBOLS AND ABBREVIATIONS

BAS – biologically active substances

DE – dty extract

MP – medicinal plants

INTRODUCTION

Topicality. Based on the analysis of literature data on the pharmacological properties of Marsh cinquefoil, as well as chemical components that are part of it, we can conclude that, despite the active use in medicine, Marsh cinquefoil has significant potential for its expansion further use.

Currently, Marsh cinquefoil is used mainly in non-dosage forms - decoction, tincture, cream, while the variety of chemical composition and spectrum of pharmacological properties of the medicinal plant indicates the need to develop dosage forms, more accurate and convenient for oral administration. Such dosage forms include tablets and capsules based on an extract from the roots of Marsh cinquefoil. That is, the development and optimization of technology for obtaining an extract of biologically active substances from the roots of Marsh cinquefoil is an urgent task.

Object of investigation – Marsh cinquefoil.

Subject of investigation – dry extract of biologically active substances from the roots of Marsh cinquefoil.

Purpose of the work – Improvement of technology for obtaining dry extract of biologically active substances from the roots of Marsh cinquefoil.

Tasks for execution of the bachelor thesis:

1. To investigate the content of biologically active substances in the roots of Marsh cinquefoil.
2. To investigate the pharmacological action of drugs from the roots of Marsh cinquefoil.
3. To determine the optimal regime parameters of the process of extraction of biologically active substances from the roots of Marsh cinquefoil.
4. To improve the technology of obtaining a dry extract from the roots of Marsh cinquefoil.

Methods of investigation – microbiological, physico-chemical, analytical.

ВСТУП

Актуальність роботи. На основі аналізу літературних даних про фармакологічні властивості вовчого тіла болотяного, а також хімічних компонентів, що входять до його складу, можна зробити висновок про те, що, незважаючи на активне застосування в народній та науковій медицині, вовче тіло болотяне має значний потенціал для розширення його подальшого використання.

В даний час вовче тіло болотяне застосовується в основному в недозованих лікарських формах - відвар, настоянка, крем, в той час як різноманітність хімічного складу і спектр фармакологічних властивостей лікарської рослини свідчить про необхідність розробки саме дозованих лікарських форм, більш точних і зручних для перорального введення. До таких лікарських форм відносяться таблетки і капсули на основі екстракту з коренів вовчого тіла болотяного. Тобто розробка та оптимізація технології отримання екстракту біологічно-активних речовин з коренів вовчого тіла болотяного є актуальним завданням.

Об'єкт дослідження – Вовче тіло болотяне.

Предмет досліджень – сухий екстракт біологічно-активних речовин з коренів вовчого тіла болотяного.

Мета роботи – вдосконалення технології отримання сухого екстракту біологічно-активних речовин з коренів вовчого тіла болотяного.

Завдання до виконання дипломної роботи:

1. Дослідити вміст біологічно-активних речовин в коренях вовчого тіла болотяного.
2. Дослідити фармакологічну дію препаратів з коренів вовчого тіла болотяного.
3. Визначити оптимальні режимні параметри процесу екстрагування біологічно-активних речовин з коренів вовчого тіла болотяного.
4. Вдосконалити технологію отримання сухого екстракту з коренів вовчого тіла болотяного.

Методи досліджень – мікробіологічні, фізико-хімічні, аналітичні.

CHAPTER 1
CHARACTERISTICS OF MARSH CINQUEFOIL

1.1. Botanical characteristics of Marsh cinquefoil

Marsh cinquefoil (Fig. 1.1) was described in 1753 by Carl Linnaeus. The genus *Comarum* comes from the Greek word χομαρου, meaning "fruit of the strawberry tree" (*Arbutus unedo*). This is the name of Marsh cinquefoil in the 18th century to show its relationship to this tree. The species name -*palustris* translated from Latin means "swamp" and indicates the habitat of the plant [1]. In Germany it popular names are Fiinffingerkraut, Sie-benfiigerkraut, Sumpf-fiinf-oder, Blutauge, Gdnsekraut. In England - Red March Cinquefoil, Marsh Potentilla, in France - Comaret des malais, in Poland - Siedmopalecznik blotny, and in America - Purple marshlocks [2 – 4].

Fig. 1.1. Marsh cinquefoil – *Comarum palustre* L.

Marsh cinquefoil is a perennial semi-shrub with a creeping rhizome and rising reddish woody stems. The leaves are dark green, gray-felt below, with stipules. The lower

leaves are imparipinnate, with five or seven leaves, the upper - trifoliate. Leaves are oblong, serrate. The flowers are dark red with a podchashem, collected in a brush. Petals of the corolla, sepals and petals of the calyx are five. Corolla petals are shorter than calyx, dark red. The fruit is a multi-seeded, disintegrating into flattened achenes with filamentous columns. Height 30 - 100 cm. Flowering time is from June to July [5 – 10].

It grows in swamps and wetlands of forest and forest-steppe parts of Ukraine (Male Polissya) and western Ukrainian forests. The species is included in the lists of protected plants of Vinnytsia, Dnipropetrovsk, Donetsk, Zakarpattia, Ivano-Frankivsk, Luhansk, Kharkiv, Khmelnytsky, Chernivtsi regions.

1.2. Phytochemical composition of Marsh cinquefoil

The following biologically active substances, such as phenolic acids, essential oils, saponins, polysaccharides and pectins, small amounts of alkaloids, vitamins, flavonoids and tannins, were found in Marsh cinquefoil [11 – 17].

In the aboveground part, such groups of biologically active substances as phenolic carboxylic acids, essential oil in the amount of 0.03 - 0.06%, flavonoids 0.45-1.38%, tannins up to 13% [130], saponins 1%, vitamins C (in leaves) and polysaccharides were found. Organic acids were found in Marsh cinquefoil as well [6, 11, 19, 20]. The flavonoid glycoside gossipitrin was found in the stems [21]. Essential oil 0.68%, flavonoids 0.01-0.20% and tannins 6.0-16.0% were found in the underground part of Marsh cinquefoil [5, 6, 22. 23].

Organic acids of marsh sabelnik are represented by isovaleric (Fig. 1.2) and isobutyric acids (Fig. 1.3). Of the phenolic carboxylic acids, ellagic, ferulic, p-coumaric, and mustard acids were found in Marsh cinquefoil. In the composition of the essential oil a-pinene, terpeneol; methylheptenone and citronellal were found. The flavonoid glycoside gossipitrin was found in the stems, and quercetin (Fig. 1.5) and kaempferol (Fig. 1.6) were found in the flowers. K.F. Blinova, and then E. Ya. Lyukshenkova and co-workers, showed that tannins of Marsh cinquefoil belong to the group of condensed catechins, such

as (+) - gallocatechin and (-) - epigallocatechin, which are present in the free state, as well as flobafen - insoluble product of polymerization of condensed tannins [24].

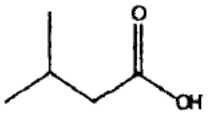

Fig. 1.2. Isovaleric acid

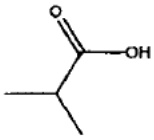

Fig. 1.3. Isobutyric acid

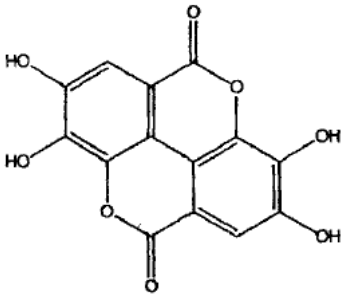

Fig. 1.4. Ellagic acid

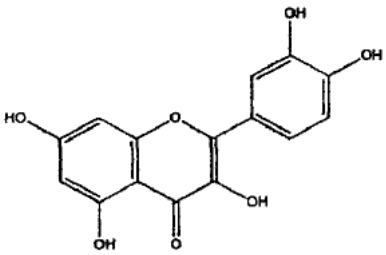

Fig. 1.5. Quercetin

The study of the UV spectrum of the solution of Marsh cinquefoil extract shows the identity with the spectrum of (+) - catechin, which confirms the majority of condensed catechins in Marsh cinquefoil [26].

In some works, the macro- and microelement composition of Marsh cinquefoil has been studied. It contains elements such as potassium, calcium, magnesium, iron, sodium, manganese, copper, zinc, cobalt, chromium, aluminum, barium, nickel, strontium, lead,

lithium, vanadium, molybdenum, tin, phosphorus, bismuth, silver, titanium, zirconium, scandium and beryllium [6, 26, 11, 27, 28].

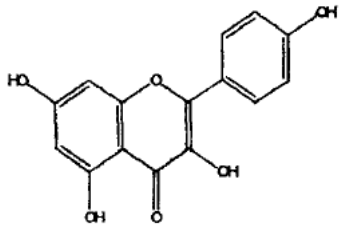

Fig. 1.6. Kaempferol

1.3. Pharmacological activity and medical use of Marsh cinquefoil and its preparations

Marsh cinquefoil has astringent, anti-inflammatory, wound-healing, analgesic and hemostatic effects, it is used for diarrhea, flu, colds, bleeding [22, 29 – 31].

In folk medicine, aqueous extracts from the rhizomes of Marsh cinquefoil are used, as tannins accumulate in larger quantities in the underground part of the plant and more easily pass into water than into an organic solvent [13, 32].

It is known to use an aqueous infusion of rhizomes or from the aboveground part of the Marsh cinquefoil as an astringent for the treatment of dysentery and diarrhea [1]. Decoctions of herbs and roots with rhizomes, exhibiting anti-inflammatory properties, are used in dental disease (acute toothache), to strengthen the gums, and in other inflammatory diseases of the oral cavity [33]. Marsh cinquefoil medications increase the body's resistance to colds and viral diseases due to the immunomodulatory ability of the plant [12]. Extracts from Marsh cinquefoil show antibacterial activity, they are used in the treatment of tuberculosis, chronic and hereditary syphilis. In folk and scientific medicine, a decoction and tincture of Marsh cinquefoil rhizomes are used in inflammatory processes in the lungs and bronchi [1, 21].

Medications of Marsh cinquefoil are used in gynecology as a hemostatic for bleeding during difficult childbirth, as well as wound healing, analgesic for gastralgia, gastric cancer, neuralgia, breast tumor [1, 34, 14, 20].

In folk medicine, drugs from the rhizomes of Marsh cinquefoil are used as an antipyretic, astringent, analgesic and hemostatic agent for rheumatism, bleeding and digestive disorders. Drugs from the grass of Marsh cinquefoil are used as wound healing and anti-inflammatory drugs. Decoction of the whole plant - for acute toothache, ulcerative bleeding gums, pulmonary tuberculosis, throat diseases (sore throats), metabolic disorders and uterine bleeding. Chopped fresh grass of Marsh cinquefoil is applied to purulent wounds, tumors on the body and hemorrhoids to resorb them.

In recent years, the interest of researchers in Marsh cinquefoil has increased, as its drugs have given good results in the complex treatment of bone and joint diseases (arthritis, osteoarthritis, gout, radiculitis, osteochondrosis) [29]. Acting on the metabolism in the body, Marsh cinquefoil tincture improves the structure of cartilage, by increasing its strength and elasticity, restores the composition of synovial fluid, relieves inflammation, which helps to restore the basic functions of the joints. A clinical study of Marsh cinquefoil medicines on patients with diseases of the joints and spine showed that patients who received Marsh cinquefoil in the acute phase showed good tolerability and no complications [35]. At increased loads on the musculoskeletal system, medines from Marsh cinquefoil also proved to be a tonic [36, 37]. According to many scientists, it is necessary to continue research of Marsh cinquefoil in this direction further [35, 37].

The pharmacological action of Marsh cinquefoil is due to the properties of the complex of biologically active substances, mainly flavonoids and tannins, essential oils, polysaccharides and other compounds in the complex [14, 20].

The pharmacological properties of flavonoids, which are part of Marsh cinquefoil, are known. Quercetin, for example, has a capillary-strengthening (vitamin P) and anti-inflammatory effect [38]. Antioxidant, antispasmodic activity has been experimentally established in quercetin [38]. When studying the antiviral activity of flavonoids, it was found that some types of Herpes are sensitive to quercetin. Quercetin and kaempferol in the experiment showed hypocholesterolemic activity, and quercetin was more active. Catechins are able to show higher vitamin P activity than flavonols. A comparative study of 29 flavonoids on cytotoxic activity on HeLa cells showed that some flavonoids, including quercetin, had inhibitory effects on cancer cells, and catechins with

hydroxylation at positions 3 ', 4' and 5 ', in particular (-) - epigallocate, proved to be highly effective [39, 40]. The kaempferol glycoside was found to have antileukemic activity in experiments on mice. Studies in recent years have shown that quercetin and a number of other flavonoids can inhibit the destruction of the myelin sheath of nerve fibers in autoimmune diseases: Alzheimer's, Parkinson's, multiple sclerosis, etc. [39]. 7-glucoside gossypetin - gossipitrin, the structure of which is established in 1939 in experiments on rats, showed hepatoprotective activity [41, 42]. Yaque and co-authors experimentally demonstrated the antioxidant activity of gossipitrin and its ability to prevent asthma [43]. The pharmacological properties of the flavonoids included in Marsh cinquefoil make it promising for study in these areas.

Tannins of Marsh cinquefoil, both in the aboveground and underground parts of the plant are dominant in the composition of biologically active substances and set a certain direction of biological effects on the body [23]. Tannins themselves have astringent, anti-inflammatory, antimicrobial properties [4].

Based on the analysis of literature data on the pharmacological properties of Marsh cinquefoil, as well as chemical components that are part of it, one can conclude that, despite the active use in folk and scientific medicine, Marsh cinquefoil has a significant potential for further use. Currently, Marsh cinquefoil is used mainly in non-dosed dosage forms - decoction, tincture, cream, while the variety of chemical composition and spectrum of pharmacological properties of Marsh cinquefoil indicates the need to develop dosage forms, more accurate and convenient for oral administration. Such dosage forms include tablets and capsules based on the extract of Marsh cinquefoil roots.

1.4. Technology of drugs from medicinal plant Marsh cinquefoil

Drugs of Marsh cinquefoil are still on the market in the form of a powder of crushed raw materials for the preparation of infusion and tincture in home conditions. Only a few enterprises produce drugs in the form of tincture and tablets of crushed raw materials, taking into account their technological features. The pharmaceutical enterprise "Evalar" produces "Sabelnik Evalar", “Artrozanet” produces aqueous-alcoholic extract from roots

and rhizomes of Marsh cinquefoil, "Sabelnik Evalar" tablets containing powder rhizomes of Marsh cinquefoil and cream "Sabelnik Evalar" containing hydroalcoholic and oil extracts of Marsh cinquefoil rhizomes.

Based on biologically active substances contained in the plant and their pharmacological activity, the technology of tincture and dry extract from rhizomes of Marsh cinquefoil was developed [12]. To prepare the tincture, it was used 40% ethanol as an extractor, based on what exactly this concentration is used by traditional medicine (vodka) and based on that alcohol of this concentration extracts the most amount of extractive substances of Marsh cinquefoil. Extraction was carried out by the method of percolation resulting in five parts by weight extraction from one part of the raw material. It was found that the content of polyphenols in the drug should be 1.5-2.0%. A dry extract was obtained by the method of fast-flowing repercolation using 40% ethanol as an extractant and the dry extract yield was at least 18.4% and the content of polyphenols is not less than 34% [12].

The technology of oil extract from the herb of Marsh cinquefoil was developed by Lazukina and other researchers to isolate lipophilic components from raw materials. For this, the method of maceration with preliminary swelling of raw materials in ethanol 70% and method of two-phase extraction (ratio - feed: polar phase: non-polar phase 1:10:10). Soybean oil was used as a non-polar phase, as polar - ethanol 70% and propylene glycol 70%. It was found that for extraction of raw materials by the method of two-phase extraction, the yield of the sum of chlorophylls accounted for 64.5%. The resulting extract was used to develop gels [44].

E.V. Malyuk developed a method for obtaining a liquid extract from a herb Marsh cinquefoil. The extract was obtained with 70% ethanol in a battery of 6 diffusers in a ratio (1: 1) by the repercolation method. In this work the content of the sum of flavonoids for standardization of extract liquid was used, and the amount was in the range from 0.393% to 0.401% [45,46].

Despite the wealth of knowledge about the medicinal plant Marsh cinquefoil; little has been done in the field of drug technology development from this plant. In folk and scientific medicine, mainly decoction and tincture are used. Thus, it represents the

theoretical and practical interest in the development of dry extract technology from Marsh cinquefoil and receiving dosed drugs based on it.

1.5. Conclusions to chapter

1. On the basis of the analysis of data of the literature it is established that in traditional and modern medicine drugs of Marsh cinquefoil are used in the form of tinctures, decoctions, ointments containing alcohol and oil extracts, and tablets from crushed raw materials.

2. The analysis of the content of various groups of biologically active substances in Marsh cinquefoil testifies to the high therapeutic potential of this plant, so it is necessary to continue its study.

3. Based on the assessment of information on the applied methods of extraction of medicinal plant raw materials, it is necessary to select the method and establish the factors influencing the process. This will reduce the duration of extraction, reduce the consumption of extractant and increase the yield of biologically active substances. Therefore, it is necessary to develop a science-based approach to the technology of the extract and dosage forms.

CHAPTER 2
STUDY OF THE CONTENT OF BIOLOGICALLY ACTIVE SUBSTANCES OF MARSH CINQUEFOIL

2.1. Methods of phytochemical analysis

To confirm the presence of the established biologically active substances in the rhizomes of the Marsh cinquefoil, characteristic color reactions to tannins, flavonoids, alkaloids, saponins, coumarins, cardiac glycosides, anthracene derivatives were performed. Quantitative content of tannins, flavonoids, alkaloids and water-soluble polysaccharides and pectin substances has been determined [47 – 52]. Since the pharmacological action of the plant depends on the presence of these biologically active substances.

2.1.1. Tanning tests

2.1.1.1. Qualitative determination of tannins

Characteristic color reactions with 1% solution of gelatin in 10% solution of sodium chloride, 5% solution of potassium dichromate and solution of ferroammonium alum are used for detection of tannins [23, 24, 51].

Obtaining extraction from raw materials. 5 g of the crushed raw material were placed in a 250 ml flask, filled with 100 ml of purified boiling water and boiled on the tile for 5 minutes, and filtered. The obtained extract was used for reactions [53]:

- Reaction with gelatin solution ©
- Reaction with iron (III) salts
- Reaction with potassium dichromate solution.

The following reactions were used to distinguish tannin groups:

- Reaction with lead acetate medium in acetic acid medium.

• Reaction with bromine water

2.1.1.2. Quantitative determination of tannins

Determination of the amount of the total tannins was carried out by three methods: permanganatometric [23, 51, 54, 55], permanganatometric with preliminary precipitation of tannins with 5% gelatin solution and spectrophotometric one.

Determination of tannins by permanganate metric method. The quantitative content of tannins in the raw material was determined by the permanganatometric method described in [55].

Methodology. About 2.0 g (accurately weighed) of crushed raw Marsh cinquefoil rhizomes were placed in a conical flask with a capacity of 500 ml, 250 ml of water heated to boiling was poured in and boiled under reflux on an electric stove with a closed spiral for 30 minutes with frequent stirring.

The liquid was cooled to room temperature and filtered about 100 ml into a 250 ml conical flask through cotton wool so that particles of raw material did not get into the flask. Then 25 ml of the obtained extract was taken with a pipette into a flask with a capacity of 750 ml, 25 ml of solution was added indigosulfonic acid and 500 ml of water, stirred and titrated with 0.02M potassium permanganate solution.

In parallel, a control experiment was carried out, titrating 25 ml of indigosulfonic acid in 500 ml water, (indigosulfonic acid was prepared from 1 g of indigo carmine, which was dissolved in 25 ml of concentrated acid, then another 25 ml of concentrated acid was added (carefully pouring the solution into water)).

2.1.2. Tests for flavonoids

2.1.2.1. Qualitative determination of flavonoids

The presence of flavonoids was determined by characteristic color chemical reactions.

2.0 g of raw material was labeled in a 100 ml flask with a section and poured into 20 ml 70% ethanol. The flask was connected to a reflux condenser and heated on a boiling water bath for 10 minutes. The extract was cooled, filtered and used to carry out qualitative reactions to the content of flavonoids [53, 56, 57].

• Cyanidine reaction or Shinoda test

• Reaction with aluminum chloride

2.1.2.2. Quantative determination of flavonoids

The method of differential spectrophotometry based on the measurement of the optical density of the complex of flavonoid compounds with a solution of aluminum chloride is used for the quantitative determination of flavonoids.

Methodology.1,0 g of crushed raw material with a particle size of up to 2 mm was placed in a flask with a thin section with a capacity of 200 ml. To these 100 ml of 70% ethanolwere added, the contents of the flask were shaken and weighed with an error of 0.01 g. The flask was connected to a reflux condenser and heated on a boiling water bath for 30 minutes with periodic shaking to wash the particles from the walls. The flask was then cooled to room temperature, weighed again and, if necessary, 70% alcohol was added to the original weight. The extract was filtered through a folded paper filter into a 100 ml volumetric flask, discarding the first 20 ml of filtrate. 1 ml of the filtrate is placed in a 25 ml volumetric flask, 5 ml of a 2% solution of aluminum chloride 1 in 95% alcohol are added, the volume of the solution is adjusted to the mark with 95% alcohol and mixed. After 30 minutes, the optical density of the solution was measured on a spectrophotometer at a wavelength of 410 nm in a cuvette with a layer thickness of 10 mm.

The reference solution was a solution consisting of 1.0 mm extraction and 0.1 ml of concentrated acetic acid, brought to the mark with 95% ethanol in a 25 ml volumetric flask. In parallel, under the same conditions, the optical density of a solution of a standard image of rutin was measured using 95% ethanol as a reference solution.

2.1.3. Research on the presence of alkaloids

Colored sedimentary reactions were used for qualitative analysis of alkaloids. The quantitative content was determined by the gravimetric method [24, 53].

2.1.3.1. Qualitative determination of alkaloids

1.0 g of crushed plant material was placed in a flask with a capacity of 100 ml, 10 ml of 1% hydrochloric acid solution was poured in and heated in a boiling water bath for 5 minutes. After cooling, the extract was filtered through a paper filter.

The extract was poured into tubes of 0.5 ml and in; each tube was carefully, dropwise, added the appropriate reagent for the determination of alkaloids;

- Wagner's reagent.
- Mayer's reagent.
- Dragendorff's reagent.
- Scheibler's reagent.
- Sonnenstein's reagent.
- Picric acid solution.

2.1.3.2. Quantative determination of alkaloids

The method is based on the separation of alkaloids from raw materials in the form of bases, their subsequent purification and gravimetric determination of the content.

10 g of air-dry crushed raw material with a particle size passing through a sieve with holes 1 mm in diameter, with an error of 0.01 g, was poured into a bottle with a stopper with a capacity of 250 ml. Poured into a bottle 150 ml of dichloroethane, 7 ml of ammonia solution, the contents were shaken for 15 minutes and left to stand until the next day.

The next day, the contents of the bottle were shaken again for 15 minutes. The extract was filtered, 10 ml of water was added, shaken, the water was separated (removal of ammonia), 15 ml of the extract corresponding to 1.0 g of plant material was measured.

Then the extract is poured into a separating funnel with a capacity of 200 ml, the cylinder was rinsed twice with 10 ml organic solvent, which was added to the measured extraction. Of extract, alkaloids were extracted with 1% hydrochloric acid solution, gently shaking the extract each time for 2 minutes sequentially with 20, 15, 10 and 5 ml of 1% hydrochloric acid solution until complete extraction of alkaloids. Each time after the separation of the liquid, we proceeded as follows: since the layer organic solvent in the separating funnel below, it was temporarily poured into a flask, and the transparent the aqueous layer was filtered through a smooth filter pre-moistened with water into a second separatory funnel with a capacity of 200 ml. The organic layer was poured into the first separatory funnel and acid was added. The completeness of the extraction of alkaloids by acid was checked with a general alkaloid reagent. For this, 5 drops were placed on a watch glass from the last extraction, and a drop of Wagner's reagent was added. No sediment or haze testified to the complete extraction of alkaloids. Then the filter was washed twice with 1% hydrochloric acid solution, 5 ml each, attaching wash water to total acid extraction. The filtrate was podslushivaet with ammonia solution to alkaline reaction with phenolphthalein and recovered alkaloids with chloroform, shaking successively with 20, 15 and 10 ml of extractant for 3 minutes. After settling and complete separation of the layers, the chloroform extracts were filtered through a smooth filter, on which 5.0 g of anhydrous sodium sulfate was previously placed, in a dry conical flask with a capacity of 100 ml. To check the completeness of the extraction, 5 drops of the last chloroform extraction were evaporated on a watch glass, the residue was dissolved in a 1% solution of hydrochloric acid, and a drop of a general alkaloid reagent was added. After checking the completeness of the extraction, the filter was washed twice with 5 ml of chloroform. Chloroform was distilled off water bath to 1-2 ml, its residue was removed by blowing air with a pear until the chloroform odor completely disappeared. The precipitate was dried at 70 ° C to constant weight and weighed. The content of alkaloids was determined in % of the loaded raw material.

2.1.4. Research on the presence of saponins

0.5 g of air-dry crushed raw material was placed in a test tube, poured in 5 ml of water and heated in a water bath for 15 min. The liquid was filtered and poured into 2 test tubes. In one test tube 5 ml of hydrochloric acid (0.1 mol l) was poured, in another - 5 ml of sodium hydroxide solution (0.1 mol/l). The liquids were shaken vigorously for 3 minutes. The appearance of the foam proved the presence of saponins. The columns of foam in the test tubes were approximately the same, which indicated the presence of a group of triterpene saponins in the rhizomes of the Marsh cinquefoil.

The presence of saponins was also determined by thin layer chromatography. On the chromatographic plate "Sorbfil" PTSH-P-A-UV microspray was applied to the studied extracts and chromatographed in the system n-butanol-methanol-water (5:3:1). Developer - 25% solution of phosphorus-molybdic acid in ethanol. The appearance of brown spots indicated the presence of saponins [53].

2.1.4.1. Research on the presence of coumarins

Lactone test. The reaction is based on the ability of coumarins when heated in an alkaline medium to form a layer of yellow, soluble in water, which when acidified turn into the desired products, insoluble in water. Pour 1 ml of the stock solution into a test tube, add 0.5 ml of 10% sodium or potassium hydroxide solution, and heat in a boiling water bath. In the presence of coumarins appears, a yellow color. The contents of the test tube are cooled, add 4 ml of distilled water, 10% hydrochloric acid solution to an acidic reaction (litmus test) [53]. The appearance of a precipitate or turbidity of the solution, indicating the possible presence of coumarins in the raw material, was not observed.

The nitrogen coupling reaction is based on the ability of coumarins to form colored products with aromatic amphibian compounds. To 1.0 ml of the stock solution was added 3.0 ml of sodium hydroxide solution (0.1 mol/l), heated in a water bath, cooled and mixed with 1.0 ml of freshly prepared diazotized sulfanilic acid solution. In the presence of

coumarins, depending on their chemical structure, there is a color from red-orange to cherry red. The reaction to the extract from the rhizomes of the saber is negative.

Preparation of diazo reagent. 5.0 ml of a solution of sulphanilic acid (4.5 g of sulphanilic acid and 45; 0 ml of concentrated hydrochloric acid, in 500 ml of water) is added to a volumetric flask with a capacity of 100 ml, placed on ice, add 2.5 ml of 10% sodium nitrite solution . The mixture is left on ice for 5 minutes, then another 10 ml of 10% sodium nitrite solution 62 is added, shaken, left on ice for 5 minutes, and the volume of the solution is adjusted to the mark with water. The reagent is stored on ice.

The presence of coumarins can also be determined by thin layer and paper chromatography using various solvent systems. The chromatograms are viewed in UV light. Coumarins, depending on the structure, fluoresce bright blue, greenish-blue, purple, green: - This reaction of the extract from the rhizomes of the saber is negative.

As a result of the conducted research it was established the absence of coumarins in this kind of raw materials.

2.1.5. Analysis of polysaccharides and pectin substances

To isolate polysaccharides and pectin substances from the rhizomes of Marsh cinquefoil 10.0 g of air-dry ground raw material with a particle size passing through a sieve with holes with a diameter of 2.0 mm was purified with chloroform and 96% ethanol. The meal was dried to remove the odor of solvents and subjected to double extraction with water while heating in a boiling water bath in a reflux condenser for 4 hours (ratio of raw material and extractant 1:20). The obtained extracts were combined, filtered and evaporated on a rotary film evaporator at a temperature of 40-50 ° C, reducing the volume of the extract to 1/20 of the original volume.

From the obtained concentrates, the number of water-soluble polysaccharides was precipitated with 96% ethanol. The precipitate was separated by centrifugation and sequentially treated with 96% ethanol, diethyl ether and dried at room temperature to air-dry state.

2.1.6. Generalized results of phytochemical study of rhizomes of Marsh cinquefoil

The dominant groups of substances of Marsh cinquefoil are tannins and flavonoids. The content of condensed catechin tannins in the raw material was 4.21 ± 0.01%, flavonoids 0.26 ± 0.01% and alkaloids 0.092 ± 0.004%. Water-soluble polysaccharides and pectin substances were contained in amounts of 8.62 ± 0.6% and 12.82 ± 0.20%, respectively.

2.2. Conclusions to chapter

1. A photochemical study of the rhizomes of Marsh cinquefoil was carried out. It has been found that they contain tannins, flavonoids, saponins, alkaloids, water-soluble polysaccharides and pectin.

2. To quantify the amount of tannins in the raw material, the spectrophotometric method of analysis was chosen as more accurate and less labor-intensive. The content of tannins in the rhizomes of the saber marsh by this method was 4.25 ± 0.10%.

3. Differential spectrophotometry method was used for quantitative determination of flavonoids in raw materials. The content of flavonoids in the rhizomes of Marsh cinquefoil in terms of rutin was 0.26 ± 0.01%.

CHAPTER 3
TECHNOLOGY OF OBTAINING DRY EXTRACT FROM THE ROOTS OF MARSH CINQUEFOIL

To develop the technology, it is necessary to determine the technological parameters of raw materials, select the extractant that maximally extracts the active biologically active substances that provide pharmacological action and as few concomitant and ballast substances as possible, optimize the extraction process and study the extraction kinetics. Therefore, it is necessary to choose the mode of obtaining a dry extract (DE) from medicinal plant raw materials and to develop a method of standardization of extracts for the main biologically active substances.

3.1. Determination of optimal extraction conditions

Dry extracts are one of the most rational ways of processing of medicinal herbs providing the maximum extraction of active substances and a possibility of creation of the standardized phytopreparation.

In this regard, in the development of an anti-inflammatory agent based on Marsh cinquefoil, it is proposed to obtain a dry extract as a substance to create finished forms.

The technological scheme of obtaining a dry extract of Marsh cinquefoil includes the stages of solid-phase extraction, evaporation of the obtained extract, purification and drying of the concentrated extract.

To determine the optimal conditions of the extraction stage, we studied the effect of the extractant, temperature regime, raw material-extractant ratio, duration and the number of extraction stages on the yield of biologically active substances (BAS).

Water and ethanol of various concentrations were used in the selection of the extractant.

The obtained data are presented in table 3.1

Table 3.1

The dependence of the yield of extractives and the sum of polyphenolic compounds on the type of extractant

Extractant	Yield of extractive substances, %	The yield of the sum of polyphenols connections in terms of (+) - catechin,%
Purified water	16,95	8,38
25% ethanol	20,11	11,01
40% ethanol	23,60	13,39
50% ethanol	23,68	13,54
70% ethanol	23,72	13.57

From the data presented in Table 3.1 it follows that the yield of the sum of extractives and polyphenolic compounds increases with increasing ethanol concentration. The use of water as an extractant makes it difficult to filter and evaporate, thereby increasing the duration of the process. The use of 70% ethanol causes the extraction of lipophilic substances that degrade the quality of the dry extract. The most optimal is 25 and 40% ethanol. In addition, pharmacological studies have shown that the dry extract obtained by the use of 40% ethanol has the most pronounced anti-inflammatory activity. Based on this, 40% ethanol was selected as the extractant.

The degree of grinding of the raw material has a great influence on the extraction process. Based on the obtained experimental data, it was determined that the optimal degree of grinding of raw materials is 3 mm (Table 3.2). Rurther reduction of the particle size leads to difficulties in the filtration process, obtaining turbid extracts. An increase in particle size leads to a decrease in the yield of BAS.

Table 3.2

Yield of the sum of extractives and the sum of polyphenolic compounds depending on the particle size

Degree of grinding, mm	Yield of extractive substances. %	The yield of the sum of polyphenols connection in terms of (+) - catechin,%
1	Not filtered	-
2	24,72	13,56
3	23/64	13,39
5	22,55	13,11
7	19,26	12,15

It was also experimentally shown that the increase in temperature has a positive effect on the efficiency of the extraction process, increasing the yield of extractives and polyphenolic compounds (Table 3.3).

Table 3.3

Yield of extractives and the amount of polyphenolic compounds depending on the extraction temperature

Temperature of extraction, ° C	Yield of extractive substances, %	The yield of the sum of polyphenols connection in terms of (+) - catechin,%
20	15,07	9,37
40	21,33	10,25
50	22,85	11,52
60	23,18	12,97
70	23,52	12,99
80	23,86	13,14
90	23,88	13,15

It is proposed to carry out the extraction of raw materials at a temperature of (60 ± 5) ° C. This temperature regime provides a high yield of extractives and biologically active substances with minimal energy costs.

Also, the optimal phase ratio corresponding to 1:10 (raw material - extractant) was selected experimentally. The increase in the volume of the extractant is unjustified, due to a slight increase in the yield of BAS, and also leads to increased production costs and, respectively, the cost of the drug substance (Table 3.4).

Table 3.4

The dependence of the yield of extractives and the sum of polyphenolic compounds on the ratio of raw materials and extractant

Ratio of raw materials and extractant	Yield of extractive substances,%	The yield of the sum of polyphenols connection in terms of (+) – catechin
1:8	21,43	12,18
1:10	21,52	12,28
1:12	21,13	12,54
1:15	21,71	12,71

In order to determine the optimal process duration and extraction stages, the time of reaching the equilibrium concentration in the raw material-extractant system was established. To do this, 40% ethanol in a ratio of 1:10 was added to 5 g of crushed raw material (3 mm) and infused for 1 h, then heated in a water bath at a temperature of (60 ± 5) ° C with reflux for 30 min , 1 h, 1.5 h, 2 h, 2.5 h. Than the mixture was cooled to room temperature, filtered and the content of the sum of extractives and polyphenolic compounds were determined. The extraction was repeated three times, pouring the raw material with a new portion of the extractant. The obtained data are given in Table 3.5.

Table 3.5

Yield of extractives and the sum of polyphenolic compounds

Duration of extraction	Yield of extractive substances,%			The yield of the sum of polyphenols compounds in terms of (+) – catechin,%		
	I	I	II	I	II	III
30 min.	19,89	5,96	2,57	9,26	5,04	1,93
1 hour	21,08	6,98	2,64	10,18	4,99	2,08
1,5 hours	21,47	7,34	2,72	11,61	5,02	2,29
2 hours	21,60	7,80	2,94	12,23	5,17	2,34
2,5 hours	21,52	7,95	3,10	12,30	5,19	2,37
3 hours	21,55	7,93	3,12	11,98	5,20	2,28

The presented results indicate that the equilibrium state at the first, second and third contact of the phases is reached after 2 hours. At the same time at the first contact 60-65% of extractive substances and polyphenolic compounds pass to extractant, at the second - 20-25% and 8-10% at the third contact. Thus, triple extraction provides depletion of raw materials by an average of 85-95%.

In order to accelerate the extraction process, the extraction of raw materials should be carried out with constant stirring, because there is a renewal of the surface of the contacting phases, which leads to an increase in the driving force of the process.

Thus, it is optimal to carry out three stages of extractions for 2 hours each (after infusion for 1 hour) of raw materials with a degree of grinding of 3 mm in a ratio of 1:10 using as an extractant 40% ethanol and heating to 60 ° C.

3.2. Technological process of production of dry extract from Marsh cinquefoil

Sanitary treatment and preparation of production

Technological process in the production of dry extract from Marsh cinquefoil roots begins, first of all, with observance of industrial and personal hygiene of service personnel, aimed at ensuring the production of a high quality finished product, on exclusion of microbial contamination of the drug during production, storage and transportation.

Preparation of the extractant

The manufacture of galenic preparation is associated with the use in significant quantities of various extractants necessary for the extraction of biologically active substances from plant materials. For these purposes, purified water is especially widely used, as well as ethanol of various concentrations, depending on the BAS contained in the raw material.

Purified water is obtained by distilling drinking water coming from the city water supply network. The general principle of obtaining is that drinking water is poured into a distillation apparatus (evaporator) and heated to a boiling temperature. All non-volatile impurities that were in the source water are remained in the distillation apparatus. Installations for obtaining purified water may have different capacities: The choice depends on the scale of the production.

Grinding of raw materials. The raw material is crushed using electric cutter according in order to obtain particles size from 0,5 – 1,0 mm. Then the entire crushed mass of raw materials in layers are loaded into the extractor, wetting with water.

Extraction of raw materials. For the extraction of raw materials, a percolator was used (height-to-diameter ratio 3: 1). In production, various types of extractors are used, depending on several factors affecting the process such as scale of extraction, the type of

extractant, the nature of extractives, etc. The main task is to obtain a high quality extract; with a high yield of active ingredients and few accompanying and ballast substances.

Evaporation. The evaporation was carried out using a rotary type evaporator on a boiling water bath. Evaporation entails an increase in the concentration of biologically active substances in the extract, since when heated some part of the extractant is converted into a vaporous state and in the form of vapour is removed from the liquid medium. Evaporation process in pharmaceutical production is widely used in obtaining liquid and thick extracts and is an intermediate stage in the production of dry extracts:

Drying. Drying of the thickened extract was carried out in an infrared dryer. Drying is carried out to a residual moisture content of no more than 5%.

Shredding. The resulting dry extract was ground in a porcelain mortar to a particle size of less than 1 mm and sieved through sieves with holes 1 mm in diameter.

Packing and packaging. The dry extract was packaged in double polyethylene bags.

The technological scheme is presented in Fig. 3.1.

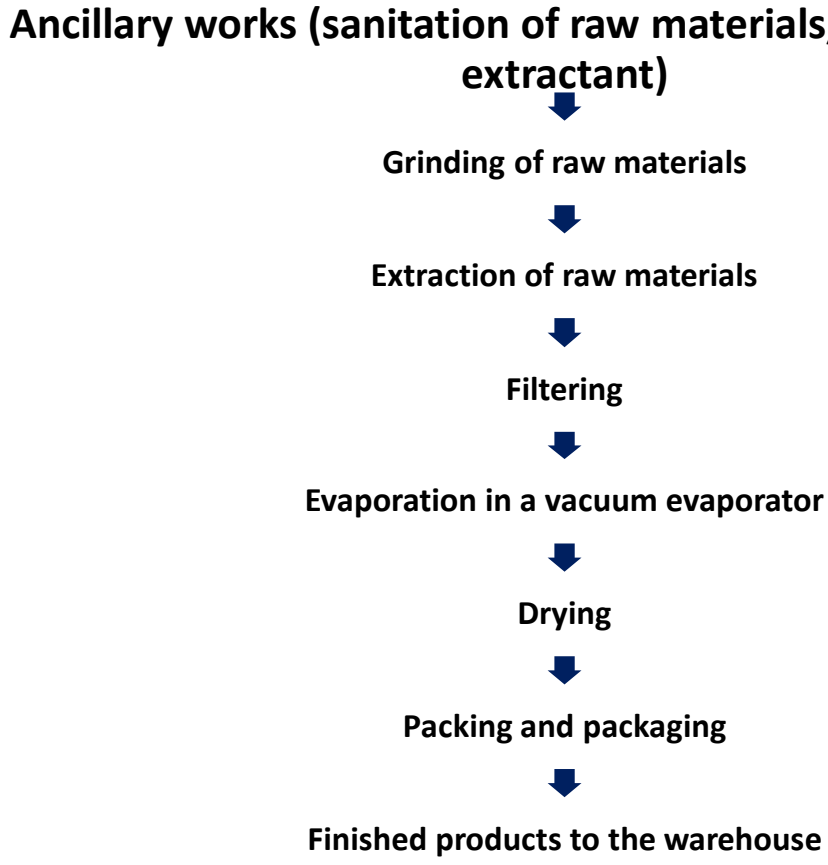

Fig. 3.1. Technological scheme of obtaining dry extract from the roots of Marsh cinquefoil

3.3. The value of indicators of quality and standardization of dry extract from the roots of Marsh cinquefoil

In order to standardize phytopreparations, certain qualitative and quantitative indicators are taken into account. In our work, we took into account the presence of a wide variety of physicochemical methods of analysis widely used in practice. The standard indicators of the quality of dry extract include appearance, color, smell, taste [58].

The dry extract from the roots of Marsh cinquefoil is a hygroscopic powder of dark brown color, bitter taste with a pleasant odor, easily soluble in water, insoluble in organic solvents. Dry extract from the roots of Marsh cinquefoil contains a complex of biologically active substances, in particular, the sum of tannins and flavonoids, responsible for its therapeutic effect. In the dry extract, the content of the total tannins was determined, since they are the main components of biologically active substances in Marsh cinquefoil.

3.3.1. The validity of the dry extract from the roots of Marsh cinquefoil

For the qualitative analysis of the dry extract, methods for determining the quality of raw materials were proposed, which include the general color reaction to tannins.

Methodology. 0.5 g of dry extract was placed in a 50 ml flask, 20 ml of distilled water was added. The flask was heated in a boiling water bath as needed. The resulting solution was filtered through a paper filter and used for qualitative reactions.

1. Reaction with gelatin solution. 2-3 drops of 1% gelatin solution in 10% sodium chloride solution were added to 2-3 ml of the solution. The formation of a white precipitate from the formed gelatin thanides, soluble in an excess of the reagent, indicated the presence of tannides.

2. Reaction with iron salts. To 3 ml of the solution was added 2 drops of a 1% alcoholic solution of iron chloride or iron-ammonium alum, shaken, after 3 minutes a dark green color was observed, upon standing a black precipitate was formed. This indicates the presence of condensed tannides.

3. Reaction with potassium dichromate. To 3-5 ml of the solution 2-3 drops of a 5% solution of potassium dichromate was added. A yellow-brown precipitate was observed.

3.3.2. The value of tannins in dry extract from the roots of Marsh cinquefoil

The dominant BAS of Marsh cinquefoil are tannins, and their presence is preferably used for standardization of dry extract.

Methodology. Dry extract analysis was performed on an SF-56 spectrophotometer. The UV spectrum of the solution of aqueous extraction of raw materials converged with the spectrum of catechin and has an absorption maximum at a wavelength of 277 ± 2. Analytical wavelength - 277 nm; purified water served as a reference solution; tannin preparation (FS 42-2217-84), which has an absorption maximum of 275 ± 2 nm in the UV86 spectrum, was used as a standard.

0.1 g of dry extract (accurately weighed) was dissolved in 200 ml of distilled water. 1 ml of the obtained extract solution was placed in a 25 ml volumetric flask and also diluted with purified water to the mark. In parallel, a standard tannin solution (standard sample) was prepared. About 0.02 g (accurately weighed) of tannin was placed in a 200 ml measuring flask, 100 ml of purified water was added, dissolved, then brought to the mark with the same solvent. 5 ml of the resulting tannin solution was placed in a 50 ml volumetric flask and diluted with purified water (standard sample solution) [55].

3.3.3. Determination of residual moisture

There was determined that the content of residual moisture in 7 series of Marsh cinquefoil extract does not exceed 5% (table), which corresponds to the requirements.

3.3.4. Determination of the content of heavy metals

There was determined that the content of heavy metals in 7 series of Marsh cinquefoil extract did not exceed 0.01%, which corresponds to the requirements.

3.3.5. The determination of the solubility of the dry extract from Marsh cinquefoil

In the pharmacopoeia, solubility means the property of a substance to dissolve in different solvents adopted by the State Pharmacopoeia. The dry extract from the rhizomes of Marsh cinquefoil contains substances of different chemical nature and different solubility, which determine the degree of its dissolution in various solvents. In this regard, the solubility of the dry extract was evaluated as a percentage of the total sample.

3.3.6. Preliminary hygroscopicity of dry extract from the roots of Marsh cinquefoil

The study of the hygroscopicity of the dry extract is necessary to select the optimal storage conditions. When moisture is absorbed, activation of some chemical processes is possible, which can lead to a change in the quality of the extract. When stored in the light, the color and shape of crystals change. Air humidity is one of the main factors that actively affect the stability of drugs. An increase in air humidity affects the physical properties of hygroscopic dosage forms. As a result, the appearance, color, concentration of the medicinal substance may change. As a result of these processes, decomposition products are formed, and the pharmacological activity of drugs decreases, up to an increase in the toxicity of the drug. Medicines with pronounced hygroscopic properties should be stored in glass containers, hermetically sealed with a cork embedded in paraffin, etc.

3.3.7. Determination of shelf life of the dry extract from the roots of Marsh cinquefoil

In order to establish the shelf life of the dry extract, there were manufactured and stored 5 series of the drug. Since the dry extract is a hygroscopic one, the packaging and storage conditions of the drug are selected in accordance with this feature. The extract was packed in double polyethylene bags; storage was carried out in a dry, dark place.

The determination of the shelf life of dry extracts was carried out by monitoring the qualitative and quantitative indicators of the dry extract during storage under natural conditions [59]. Quantification of the amount of tannins in dry extracts was carried out by the spectrophotometric method. The observation period for the stability of the dry extract was from 6 months up to 2 years. Since the dry extract has kept its properties unchanged for each of the selected qualitative and quantitative indicators, that allowed us to recommend a shelf life of at least 2 years.

3.4. Conclusions to chapter

1. Economically justified conditions for the extraction of biologically active substances from plant raw materials were selected.

2. The technology of obtaining dry extract from the roots of Marsh cinquefoil has been developed, which ensures the maximum yield of extractive substances and a complex of biologically active substances.

3. The quality indicators of the dry extract have been determined. It has been established that the content of the total tannins in the extract must be at least 26%.

4. The hygroscopicity of the extract was studied at a relative air humidity of 59.2%, 80.6% and 100%. The dry extract of Marsh cinquefoil rhizomes is hygroscopic, and as a result of the study, the need for its storage at a relative humidity of no higher than 60% was established.

5. It was found that the dry extract retains its quality for 2 years when stored in natural conditions.

CONCLUSIONS

1. Analysis of the content of different groups of biologically active substances in Marsh cinquefoil indicates a high therapeutic potential of this plant.

2. It is determined that the pharmacological effect of medicines based on biologically active substances of the plant is anti-inflammatory, antipyretic, hemostatic, antihypertensive and wound-healing one.

3. In the study of the process of extraction of biologically active substances from the roots of Marsh cinquefoil it was found that it is optimal to carry out three extractions lasting 2 hours of raw materials with a grinding degree of 3 mm in the ratio of raw materials and extractant 1:10. As an extractant, the optimal use of 40% ethanol. The optimum process temperature is 60 ° C.

4. Based on the determination of the optimal modes of the extraction process, the technology of obtaining a dry extract from the roots of Marsh cinquefoil was improved.

REFERENCES

1. Валягина Е.Т. Лекарственные растения России. Целительные травы / Е.Т. Валягина - СПб.: Агропромиздат, 1997. - 287 с.

2. Sams T. Healing Herbs. A beginner's guide to identifying, foraging, and using medicinal plants / T. Sams – Beverly: Fair Winds Press. – 2015. – 235 p.

3. Blumenthal M. Herbal medicine. Expanded commission E monographs / M. Blumenthal, A. Goldberg, J. Brinckmann // Integrative Medicine Communications, 2000. - P. 520.

4. Rhodas A. F. The vascular flora of Pennsylvania / A.F. Rhodas, W.M. Klein // Annotated checklist and atlas American Philosophical society. Philadelphia, 1993.– 636 p.

5. Гаммерман А.Ф. Лекарственные растения (Растения - целители) / А.Ф. Гаммерман, Г.Н. Кадаев, А.А. Яценко-Хмелевский // М.: Высшая школа,1990.- 544 с.

6. Гончарова Т.А. Энциклопедия лекарственных растений. Лечение травами. / Т.А. Гончарова. - Т. 2 - М.: Изд. дом "МСП", 1999. – 524 с.

8. Дикорастущие растения России / Отв. ред. А. Л. Буданцев, Е.Е. Лесиовская. — СПб.: СПХФА, 2001.- 663 с.

9. Лавренов В.К. Полная энциклопедия основных лекарственных растений / В.К. Лавренов, Г.К. Лавренова. - М.: «АСТ, Сталкер», 2007. - 796 с.

10. Лебеда А.Ф. Лекарственные растения / А.Ф. Лебеда, Н.И. Джуренко, А.П. Исайкина, В.Т.Собко. - М.: 2004. — 912 с.

11. Киров Е.И. Фотохимическое изучение солянки холмовой (*Salsola collina Pall.*), сабельника болотного (*Comarum palustre* L.) и дихоризандры букетоцветковой (*Dichorisandra thyrsiflora Mikan.)* / Е.И.Киров, Г.А., Ковальская, О.В.Чайкина // Материалы VII международного съезда «Актуальные проблемы создания новых лекарственных препаратов природного происхождения» Фитофарм 2003. Спб.: 2003. - С . 530-531.

12. Наумчик Ф.Н. Фитохимическое исследование сабельника болотного (*Comarum palustre*) и приготовление из него некоторых лекарственных препаратов:

дисс. на степень кандидата фармацевтических наук: 15.00.02 / Г.Н.Наумчик; ЛХФИ. -Л., 1964. - 185 с.

13. Одуладжа Дж. Исследование биологически активных веществ в корневищах Comarum palustre // Материалы I (IX) Международной конференции молодых ботаников в Санкт-Петербурге. Май 2006, С. 265 - 266.

14. Пастушенков Л.В. Лекарственные растения. Использование в народной медицине и быту. / Л.В. Пастушенков, А.Л. Пастушенков, В.А. Пастушенков. - Л.: Лениздат, 1990. - 384 с.

15. Скляревская Н.В. Полисахариды сабельника болотного (*Comarum palustre* L.) / Н. В. Скляревская, Л. Ф. Стрелкова // Разработка, исследование и маркетинг новой фармацевтической продукции. Сборник научных трудов. Вып. 62. Пятигорск 2007. — С 111-112.

16. Фотохимическое изучение солянки холмовой (Salsola collina Pall.), сабельника болотного (*Comarum palustre* L.) и дихоризандры букетоцветковой (*Dichonsandra thysiflora Mikan*.) / Е. И. Киров, Г. А. Ковальская, Т. А. Кукушкина, В. И. Долгов // Материал VII международного съезда «Актульные проблемы создания новых лекарственных препаратов природного происхождения». Phytopharm 2003. СПб.: Пушкин, 2003. - С. 530-533.

17. Фотохимическое исследование лекарственного растительного сырья // Методические указания для выполнения лабораторных работ. - СПб.: СПХФИ, 1994. -61с.

18. Скляревская Н.В. Сравнительное исследование сырья: Трава и корневища сабельника болотного (*Comarum palustre* L.) / Н.В. Скляревская, Н.В. Сыровежко, Е.Г. Шеховцова // Материалы международной научно-практической конференции, посвященной 85-летию академии «Выпускник фармацевтического вуза (факультета) в прошлом, настоящем и будущем» 09.09.04 -13.09.04 / Санкт-Петербургская государственная химико-фармацевтическая академия. — Спб.: СПХФА, 2004. - С. 195- 198.

19. Наумчик Ф.Н. Фотохимические изучение сабельника / Р.К. Наумчик, П.Э. Розенцвейг // Материалы научной конференции, посвященной итогам работы за 1961-1962: ЛХФИ: Л., 1963. - С. 101-102.

20. Лікарські рослини: Енциклопедичний довідник / Під ред. А.М. Гродзінського. – К.: Видавництво «Українська енциклопедія» ім. М.П. Бажана, Український виробничо-комерційний центр «Олімп», 1992. – 544 с.

21. Буданцев А.Л. Растительные ресурсы / А.Л. Буданцев. - СПб.: Мир и семья - 95, 1996. - 334 с.

22. Платонов В.В. Сабельник болотный (*Comarum palustre L.*) в медицинской практике (Обзор литературы) / В.В. Платонов, А.А. Хадарцев, А.А. Беляева // Клиническая медицина и фармакология. – 2019. – Том 5. - № 4. – С. 66-70.

23. Чемесова И. И. Определение содержания дубильных веществ в корневищах *Comarum palustre* L. и настойки из него спектрометрическим методом / И. И. Чемесова, Д. В. Чижиков // Растительные ресурсы. - 2004. -вып. 3. - С. 122-128.

24. Минина С. А. Химия и технология фитопрепаратов. / С.А. Минина, И. Е. Каухова. М.: ГЭОТАР-МЕД., 2004. – 54 с.

25. Жукова О. Л. Разработка методик качественной и количественной оценки полифенольных соединений в сабельника болотного экстракте сухом/ О.Л.Жукова // Разработка, исследование и маркетинг новой фармацевтической продукции: Сборник научных трудов. Вып. 61. Пятигорск, 2006. – С. 201-203.

26. Исследование микроэлементного состав объектов растительного происхождения / Д. А. Муравьева, О. И. Попов, Н. М. Мартинова и др // Достижения современной фармацевтической науки и образования — практическому здроохранению: Материалы юбилейной нуч-иракгич. Конф. Конф. — Пермь, 1997. - Стр. 224.

27. Малюк Е.В. Изучение элементного: состава в надземной части сабельника» болотного (*Comarum Palustre* L.) / Е. В. Малюк, О. Н. Денисенко // Разработка, исследование и маркетинг новой фармацевтической-продукции. Сборник научных трудов. Вьш: 61. Пятигорск, 2006. – С. 34-35.

28. Уделнова Т.М. Микроэлемент, экология и здоровье человека / Т.М. Удельнова, С.Г. Торчин, В.А. Ягодин // Успехи современной биохимии. -1990. - Вып 2. - С. 279.

29. Изучение противовоспалительной активности сабельника болотного экстракта жидкого / Е.В. Малюк, С.А. Кулешова, О.Н. Денисенко, М. Н Ивашев // Разработка, исследование и маркетинг новой фармацевтической продукции. Сборник научных трудов. Вып. 62. Пятигорск 2007. – С. 502 - 503.

30. Макарова С.В. Фармакогностическое изучение надземной части сабельника болотного, разработка технологии сухого экстракта на её основе / С.В. Макарова, Н.В. Скляревская // Тезисы докладов региональной научной конференции студентов и аспирантов «Молодые ученые-практическому здравоохранению». Санкт-Петербург, 18-19 апреля 2007.- СПб.: С1ТХФА, 2007. -С 35-37.

31. Малюк Е.В. Фармакогностическое изучение травы сабельника болотного: автореф. дисс. канд. фармацевтическх наук: 15.0.0.01: / Е.В. Малюк; ТШФА. - Пятигорск., 2007. 24с.

32. Одуладжа Дж.О. Выбор условий экстрагирования корневищ сабельника болотного./ Дж.О. Одуладжа, Е.В. Дрожжина // Разработка, исследование и маркетинг новой фармацевтической продукции. Сборник научных трудов. Пятигорск 2007. Вып. 62. -Стр. 194-195.

33. Пастушенков Л.В. Лекарственные растения. «Использование в народной медицине и быту». / Л.В. Пастушенков, А.Л. Пастушенков В.А. Пастушенков. - Л.: Лениздат, 1990. - 384 с.

34. Ериневич М.А. Информационный поиск перспективных лекарственных растений. / М.А. Гриневич // Л.: «Наука», 1990. - 40 с.

35. Sapozhnikova, S. K. Phytopreparation "Artrozanet" in complex treatment of the support-impellent apparatus infalation / S. K. Sapozhnikova, L. A. Prokopjeva, A.K. Vinogradov *et al* //Material of the 8th International Congress "Actual problem of creation of new medicinal preparations of natural origin". Phytopharm 2004, Mikkeli, Finland, 2004. P. 581-582.

36. Одуладжа Дж. О. Разработка капсул сухого экстракта сабельника болотного для хронических заболеваний опорно-двигательного аппарата / Дж.О. Одуладжа, Д.В. Чижиков // Вестник развития науки и образования. — 2007. — No 4. - С. 6-8.

37. Прокопьева Л.А. Об исследовании влияния БАД «сабельник-Эвалар» в комплексном лечении воспалительных обменно-дистрофических заболевании опорно-двигательного аппарата / Л.А. Прокопьева, А.К. Виноградов // Материалы VII международного сьезда: «Актуальные проблемы создания новых лекарственных препаратов природного происхождения» Фитофарм, 2003. Спб., 2003. - С. 454 - 457.

38. Hertog M.GX. Pronounced effect of nutritional flavonoid - quercertin on health / M.G.L. Hertog, P.C.H. Holman // Eur J Clin Nutr. - 1996. - No 50. - P 63-71.

39. Flavonoids inhibit myelin phagocytosis by macrophages; a structure-activity relationship study. / J.A. Hendriks, H.E. de Vries, S.M.A. van der Pol [et al] // Biochemical Pharmacology. - 2003. - Vol. 65. - P.877-885.

40. Yanagihara K. Antiproliferative effect of isoflavons on human cancer cell lines established from the gastrointestinal tract / K. Yanagihara, A. Ito, T. Toge, M. Numoto // Cancer Research. – 1993. – Vol. 53. – № 23. – P. 5815-5821.

41. Jones T.K. Pharmacology& pathophysioloy of leukotrienes / T.K Jones, G. Letts, CC. Chan et al. // Chem. Biol and Clin. Aspects, - 1989. - P. 309-403.

42. Perez-Tfueba G. Protective effect of gossypitrin on carbon tetrachlorid - induced in vivo hepatotoxicity / G. Perez-Tfueba, C. Ramos-Guanche, B. Marquez-Hernandez // Redox Report. – 2003. – Vol. 8. - № 4. – P. 215-221.

43. Yaque J. G. Gossipetrin: the antiasthmatic principle isolated from the flowers of Talipariti elatum and an approach to its possible mechanism of action. / J.G. Yaque, A.C. Cuellar, P.J.M. Abreu. 2006.

44. Лазукина М.А., Скляревская Н.В. и др. Разработка технологии масляного экстракта из травы сабельника болотного и изучение его биологической активности // Тезисы докладов региональной научной конференции студентов и аспирантов «Молодые ученые-практическому здравоохранению». Санкт-Петербург, 18-19 апреля 2007. -СПб.: СПХФА, 2007. -С 157-158.

45. Малюк Е.В. Фармакотехнологическое изучение надземной- части сабельника болотного - *Comarum palustre* L. / Е.В. Малюк, О.Н. Денисенко, Н.В. Кобильченко // Разработка, исследование и маркетинг новой фармацевтической продукции. Сборник научных трудов. Вып. 61. Пятигорск, 2006. — Стр. 36-37.

46. Малюк Е.В. Фармакотехнологическое изучение надземной части сабельника болотного – *Comarum palustre* L. / Е.В. Малюк, О.Н. Денисенко, Н.В. Кобильченко // Разработка, исследование и маркетинг новой фармацевтической продукции; Сборник научньк трудов: Вып. 59: Пятигорск, 2004. - С 36.

47. Гринкевич Н.И. Химический анализ лекарственных растений / Н.И. Гринкевич. - М .: Высшая школа, 1983. - С. 82-93.

48. Малюк Е.В. Фармакотехнологическое изучение надземной- части сабельника болотного - *Comarum palustre* L. / Е.В. Малюк, О.Н. Денисенко, Н.В. Кобильченко // Разработка, исследование и маркетинг новой фармацевтической продукции. Сборник научных трудов. Вып. 61. Пятигорск, 2006. — Стр. 36-37.

49. Малюк Е.В. Изучение элементного: состава в надземной части сабельника болотного (*Comarum Palustre* L.) / Е.В. Малюк, О.Н. Денисенко // Разработка, исследование и маркетинг новой фармацевтической-продукции. Сборник научных трудов. Вьш: 61. Пятигорск 2006.- С. 34-35.

50. Малюк Е.В. Фармакотехнологическое изучение надземной части сабельника болотного – *Comarum palustre* L. / Е.В. Малюк, О.Н. Денисенко, Н.В. Кобильченко // Разработка, исследование и маркетинг новой фармацевтической продукции; Сборник научньк трудов: Вып. 59: Пятигорск, 2004.-С 36.

51. Одуладжа Дж. О. Стандартизация корневищ сабельника болотного экстракта сухого по дубильным веществам. / Дж.О. Одуладжа, Д.В. Чижиков // Разработка, исследование и маркетинг новой фармацевтической продукции. Сборник научных трудов. Пятигорск, 2007. Вып. 62. -Стр. 340-34.

52. Одуладжа Дж.О. Сабельник болотный - Источник медицинских препаратов / Дж.О. Одуладжа, Д.В. Чижиков // Фармация. - 2007. - No 7. -С. 45-48.

53. Фотохимический анализ лекарственного растительного сырья: Методические указания к лабораторным занятиям / Под редакцией К.Ф. Блиновой. СПб: СПХФИ, 1993.-60 с.

54. Государственная фармакопея СССР X изд. М., 1966. 1079 с.

55. Государственная фармакопея СССР вып. 1 и 2 /МЗ СССР- XI изд., доп. – М.: Медицина, 1989,1990. - 826 с.

56. Лавренов В.К. Польная энциклопедия основных лекарственных растений / В.К. Лавренов, Г.К. Лавренова. - М.: «АСТ, Сталкер», 2007. - 796 с.

57. Минина С. А. Химия и технология фитопрепаратов. / С.А. Минина, И.Е. Каухова. - М.: ГЭОТАР-МЕД. 2004. – 54 с.

58. Технология и стандартизация лекарств. Под редакцией акад. В. П. Гиоргиевского и проф. Ф. А. Конева. - Харьков: ООО «Ирег», 1996. -784 с.

59. Машковский М.Д. Проблема стабильности и сроков годности лекарств / М. Д. Машковский // Фарматека. 1996. - No 1. — С. 38.