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DEVELOPMENT OF TECHNOLOGY FOR THE PRODUCTION OF A DRY EXTRACT BASED ON PLANT RAW MATERIALS OF CALENDULA OFFICINALIS L. FLOWERS AND MELISSA OFFICINALIS L. HERB

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Abstract
In recent years, the proportion of medicinal products derived from plant raw materials has been steadily increasing in modern medicine. One of the main reasons for the growing demand for plant-based drugs is their mild effect on the human body, the relatively low incidence of adverse reactions, as well as their affordability. Therefore, expanding the range of medicinal products obtained from plant raw materials is of great importance for the field of medicine [1].
In the Republic of Uzbekistan, comprehensive and systematic reforms have been implemented in recent years, aimed at the conservation of medicinal plant resources, sustainable and rational use of natural resources, establishment of specialized plantations, and development of processing technologies for medicinal plants [2].
Furthermore, proposals have been introduced by the Ministry of Agriculture of the Republic of Uzbekistan, the Ministry of Innovative Development, the State Forestry Committee, and the Agency for the Development of the Pharmaceutical Industry under the Ministry of Health to establish, starting from May 1, 2020, clusters for the cultivation, storage, primary and secondary processing of medicinal plants (hereinafter referred to as “medicinal plant clusters”), as well as to specialize regions in medicinal plant cultivation [3].

Keywords: Sedative medicinal products; state pharmacopoeia; european pharmacopoeia; World Health Organization; biologically active substances; central nervous system; plant raw materials; regulatory documents.

Introduction

The selection of sedative medicinal products for the treatment of nervous system disorders remains a relevant issue in modern medicine. Despite the availability of synthetic sedatives, anxiolytics, and antidepressants, plant-based remedies are widely used in clinical practice for conditions such as increased nervous excitability, neuroses, sleep disorders, and neurasthenia. According to the World Health Organization, up to 80% of the world’s population prefers medicinal products derived from plant sources [4].
It is well established that plant-based medicinal products exert a milder effect compared to synthetic drugs, while their efficacy is often more comprehensive and stable due to the complex of natural bioactive constituents. In addition, phytopreparations are characterized by

lower toxicity, which allows their long-term use with minimal adverse effects on the body. For this reason, plant-based medicinal products are considered among the most effective and safe sedative agents in modern medical practice. Medicinal plants with sedative properties are widely used in the treatment of nervous system disorders. Against the background of enhanced inhibitory processes, certain plants contribute to hypotensive and antioxidant effects, as well as to the improvement of cerebral circulation. Taking into account the initial state of the central nervous system, increased excitability, and neurocirculatory disorders, the differential selection of medicinal plant preparations enables the achievement of a targeted neuroprotective effect [5].

Sedative medicinal plants used in the territory of Uzbekistan are widely applied in both pharmaceutical practice and traditional medicine, exerting a mild calming effect on the human body. They contain biologically active compounds that contribute to the regulation of the nervous system, reduction of stress, and improvement of sleep quality. Among the most commonly used sedative medicinal plants in the Republic are **Valeriana officinalis**, **Melissa officinalis**, **Mentha piperita**, and **Calendula officinalis**, as well as many other plant species possessing sedative properties. These plants are produced in various pharmaceutical dosage forms, including infusions, extracts, tablets, and syrups [6, 7].

Currently, the importance of sedative preparations derived from medicinal plants in the pharmaceutical industry is steadily increasing. The development of new, effective, and safe medicinal products based on plant raw materials, the in-depth study of their composition, and their production using modern technologies represent one of the promising scientific directions in pharmaceutical and medical research. Therefore, the development of next-generation sedative preparations based on biologically active compounds derived from medicinal plants, as well as the investigation of their pharmacological properties, is of significant scientific and practical importance [8].

Sedative medicinal plants are of particular relevance due to their mild yet effective action on the nervous system, and their pharmaceutical and therapeutic applications require further development. For this purpose, **Herba Melissae officinalis L.** and **Flores Calendulae officinalis L.** were selected as the objects of the present study [9,10].

Aim of the study. To develop a technology for obtaining a dry extract based on plant raw materials of **Herba Melissae officinalis L.** and **Flores Calendulae officinalis L.**

Material and Methods

One of the most promising forms of processing herbal mixtures and medicinal plant raw materials is the production of dry extracts. During the preparation of laboratory samples of the dry extract, ethanol of various concentrations was used as the extraction solvent. In order to ensure the maximum yield of biologically active compounds, the extraction conditions were selected on a scientific basis. In this context, factors such as the degree of comminution of the raw material, temperature conditions, type of extractant, and extraction methods were systematically studied. The quantitative evaluation of the obtained extract was carried out by determining the content of water-soluble extractive substances, as well as the total flavonoid content calculated in terms of rutin, quercetin, and β-carotene equivalents [11].

The extraction of biologically active compounds from medicinal plant raw materials represents one of the most critical stages in pharmaceutical technology. Flavonoids, alkaloids, saponins, glycosides, tannins, and other bioactive compounds present in plants constitute the key components determining the therapeutic efficacy of medicinal products. Ensuring the complete extraction of these compounds, preserving their native state, and maintaining the quality characteristics of the final extract require the appropriate selection of extraction methods and conditions [11].

In order to determine the optimal degree of grinding of the plant raw material during the extraction process, samples of the raw material were taken. The raw material was ground to a particle size of 1–10 mm and passed through sieves. Table 1 presents the results of determining the optimal degree of grinding of the studied herbal mixture. The experiments were carried out three times (n=3).

Table 1 Amount of extractive substances according to the particle size

Degree of comminution, mm	**Total extractives content, %**
1-2	26,21 ±0,32
3-5	**23,52 ±0,27**
5-7	22,84 ±0,25
7-10	19,75 ±0,21

It is well known that extracts obtained from plant raw materials with a particle size of 1–2 mm were highly turbid and contained a significant amount of ballast substances.

According to the experimental data (Table 1), the optimal particle size for the studied mixture was determined to be 3–5 mm, as this range ensured the maximum yield of extractive substances and total flavonoids. Under these conditions, the content of extractive substances reached 23,52 ±0,27%.

In the course of our experiments, in order to select the most appropriate extractant, solvents ranging from hot water to ethanol of various concentrations were employed (Table 2). The experiments were performed in triplicate (n=3).

Accordingly, hot water at 60 °C, as well as ethanol at concentrations of 70% and 96%, were used as extraction media.

Table 2 **Effect of extractant type on the content of bioactive compounds**

Extractant type	**Total extractives content, %**	**Total flavonoid content (in rutin equivalents) %**
Water (60^0 C)	22,72±0,06	2,78±0,04
70% Ethanol	21,65±0,73	4,31±0,06
96% Ethanol	42,26±0,42	5,15±0,07

As shown in Table 2, the content of extractive substances obtained using water at 60 °C was higher compared to that obtained with 70% ethanol. However, significant difficulties were encountered during the drying of the extract obtained with this solvent, as the resulting dry mass exhibited a resinous and sticky consistency.

Extraction with 96% ethanol, particularly under heating conditions, resulted in comparatively better yields of extractive substances and total flavonoids (expressed as rutin equivalents). Therefore, 96% ethanol was selected as the optimal extractant.

In pharmaceutical production, several extraction methods are employed, among which maceration, percolation, and supercritical fluid (CO_2) extraction are the most widely used. Each method has its own advantages and limitations and is selected depending on the chemical nature of the active compounds present in the plant material and the intended type of final product.

Before selecting the optimal composition, the yield of dry extract obtained from each medicinal plant material was determined. Accordingly, equal amounts of plant raw materials were taken. From Melissa officinalis, 4 g of dry extract was obtained from 50 g of raw material at a ratio of 1:10, while from Calendula officinalis, 6 g of dry extract was obtained at the same 1:10 ratio.

Based on the obtained experimental results, the plants were combined into a herbal mixture, and the subsequent experiments focused on selecting an alternative extraction method for obtaining the dry extract.

In order to achieve the maximum extraction of biologically active compounds from the proposed compositions, various extraction methods were investigated. The content of extractive substances and total flavonoids obtained using 96% ethanol, depending on the extraction method, is presented in Table 3. The experiments were conducted three times.

Table 3. **Effect of extraction methods on the content of bioactive compounds**

Extraction methods	Extractive substances content, %	The amount of flavonoids in terms of rutin %	The amount of flavonoids in terms of quercetin %.
Percolation	28,13±0,22	3,28±0,07	1,08±0,03
Reflux maceration	20,72±0,16	2,37±0,03	0,98±0,06
Continuous hot extraction (Soxhlet extraction)	46,72±0,04	5,13±0,06	1,24±0,05

Initially, three different extraction methods were employed to obtain extracts from the plant raw materials. In one approach, the plant material was soaked in the solvent for several days. However, the extracts obtained using this method demonstrated relatively low values of dry residue content, as well as reduced total flavonoid content expressed in rutin and quercetin equivalents.

In addition, a significant limitation of soaking (maceration) and percolation methods was not only the low yield of dry extract but also the high consumption of ethanol. Therefore, during the course of the study, continuous hot extraction (Soxhlet extraction) was selected as the optimal method, as it ensures reduced solvent consumption, enhanced extraction of biologically active compounds under the influence of temperature, and improved time efficiency.

At the initial stage of the study, dried parts of the investigated plants were used. Each type of plant raw material was ground separately using a milling device to a particle size of 3–5 mm and accurately weighed using an analytical balance.

For Melissa officinalis, phenolic compounds, flavonoids, and water-soluble constituents are most effectively extracted using 80–90% ethanol. This concentration range allows for a balanced extraction of both polar and lipophilic components.

Calendula officinalis contains flavonoids, carotenoids, and triterpenoids with predominantly lipophilic properties; therefore, 96% ethanol was selected as an effective extractant.

A mixture of 500 g of the ground plant raw materials was extracted with 5 L of 96% ethanol. To ensure the maximum recovery of biologically active compounds, the extraction process was carried out under closed conditions. For this purpose, continuous hot extraction (Soxhlet extraction) was employed. The ethanol was heated above its boiling point (above 70 °C) and subsequently condensed using a reflux condenser, allowing continuous extraction for 30 minutes.

After completion of the extraction process, the obtained extract was cooled and prepared for the subsequent filtration stage.

Filtration is one of the key technological processes used for the purification of extracts obtained from plant raw materials. It is aimed at removing mechanical impurities and insoluble particles present in the extract. This process is based on passing the liquid through a porous filter medium, which retains solid particles while allowing the filtrate to pass through. As a result, the clarity of the extract is improved and its quality parameters are enhanced.

During the extraction process, along with biologically active compounds, various ballast substances are also released from plant raw materials. These include fine residues of plant tissues, fragments of cell walls, starch granules, pectin complexes, and other mechanical impurities. The presence of these particles leads to turbidity of the extract, which may negatively affect its storage stability and the efficiency of subsequent technological processes. Therefore, filtration represents an essential stage in extract purification.

In our experiments, the cooled extract was initially filtered through paper filters. Subsequently, the filtrate was transferred into a centrifuge. The filtrate was centrifuged at 5000 rpm for 2 min 27 s at 25 °C**.** During centrifugation, the extract was separated into two phases: a liquid phase containing biologically active compounds and a sediment consisting of fine solid particles of plant origin. The obtained liquid phase was then subjected to further purification by re-filtration through a membrane filter with a pore size of 0.22 μm.

In order to remove ethanol from the extract, it was placed into a rotary evaporator, and the evaporation process was carried out at a temperature not exceeding 40 °C. As a result, the major portion of the solvent was removed, yielding a concentrated extract rich in biologically active compounds, which was subsequently placed in a freezer. The frozen concentrated extract was then subjected to drying using a lyophilization (freeze-drying) apparatus.

This method is one of the most effective drying techniques widely used in modern pharmaceutical technology, as it allows maximum preservation of the natural properties of biologically active compounds present in the extract. The subsequent stage involved the drying process, for which a lyophilization method was selected. Lyophilization is based on

the preliminary freezing of the liquid or concentrated extract, during which the water contained in the system forms ice crystals. The frozen extract is subsequently transferred into the vacuum chamber of the lyophilizer, where sublimation occurs under reduced pressure, enabling the removal of water without passing through the liquid phase. This method ensures the preservation of thermolabile biologically active compounds and improves the stability of the final dry extract.

As a result of the study, the dry extract obtained by lyophilization exhibited a light and porous structure, low moisture content, and good solubility. Furthermore, lyophilized extracts demonstrated high physicochemical stability and retained their quality characteristics over an extended storage period.

Subsequent experiments were focused on evaluating the antioxidant properties of the dry extracts obtained from each plant individually, as well as from their combined mixture.

The determination of antioxidant activity enables the assessment of the extract's anti-inflammatory potential, cytoprotective properties, and overall health benefits. Antioxidant compounds neutralize free radicals, thereby protecting cells from oxidative damage. Moreover, antioxidant activity is closely associated with the high content of bioactive compounds in the extract, particularly polyphenols and flavonoids, reflecting their pharmacological and biological efficacy. Therefore, antioxidant activity serves as an important scientific indicator for evaluating the quality, biological activity, and pharmaceutical applicability of the obtained dry extracts [12].

The antioxidant activity of SEDDS (Self-Emulsifying Drug Delivery Systems) was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which allows the determination of the free radical scavenging capacity of substances. This method is considered a simple and reliable colorimetric technique.

The principle of the method is as follows: the DPPH free radical exhibits a purple color, which, upon reaction with antioxidant compounds, is reduced to a yellow-colored product. This color change is measured spectrophotometrically at a wavelength of 517 nm.

A decrease in absorbance indicates the antioxidant activity of the sample.

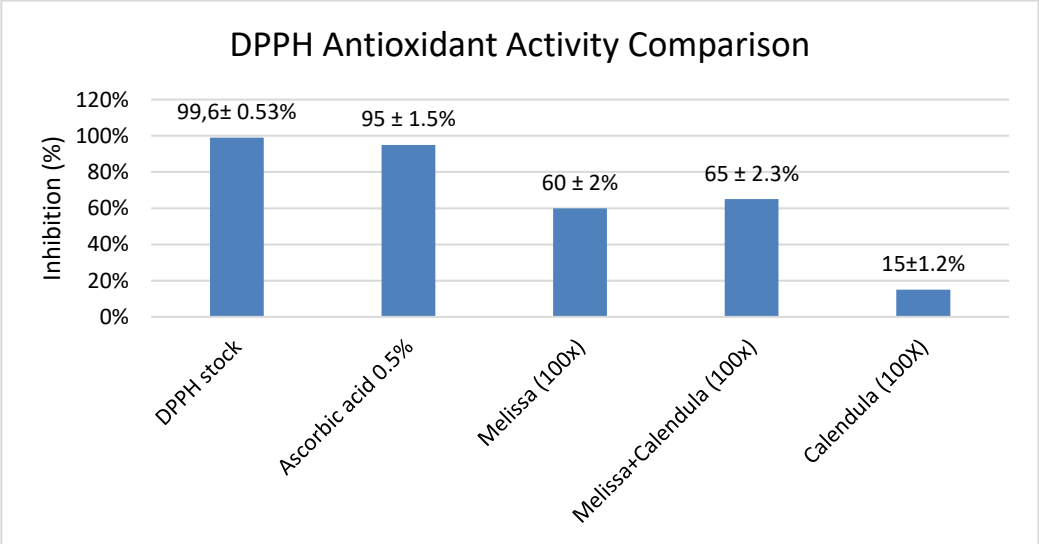

Figure1. Antioxidant activity of dry extracts obtained from the studied plant raw materials (n = 3).

The antioxidant activity of different samples was evaluated using the DPPH assay, and the results were expressed as “Inhibited ROS (%).

According to the obtained results:

The antioxidant activity assay demonstrated that the combined Melissa officinalis and Calendula officinalis extract exhibited higher radical scavenging activity (65 ± 2.3%) compared with Melissa officinalis extract alone (60 ± 2.0%), whereas Calendula officinalis extract showed relatively low activity (15 ± 1.2%). Ascorbic acid (0.5%) used as the reference standard demonstrated strong antioxidant activity (95 ± 1.5%).

Conclusion

According to the experimental data, the optimal particle size of the herbal mixture was determined to be 3–5 mm. Ethanol (96%) was selected as the optimal extractant, and the extraction process was carried out using a Soxhlet apparatus under continuous hot extraction conditions.

The yield and composition of dry extracts obtained from **Melissa officinalis** and **Calendula officinalis** were analyzed using HPLC (high-performance liquid chromatography).

The antioxidant activity of the dry extracts was evaluated using the DPPH assay, confirming their ability to effectively neutralize free radicals. The study established the optimal extraction conditions for obtaining dry extracts from Melissa officinalis and Calendula officinalis. HPLC analysis confirmed the presence of biologically active compounds, including flavonoids, phenolic compounds, and carotenoids. The obtained extracts demonstrated antioxidant activity in the DPPH assay, with the combined extract showing enhanced activity compared to the individual extracts.

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