STUDY OF THE CONTENT OF BIOLOGICALLY ACTIVE SUBSTANCES AND DEVELOPMENT OF SPECIFICATIONS FOR A DENSE EXTRACT FOR THE TREATMENT OF CHEILITIS

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Introduction

Cheilitis is an inflammatory disease of the lip tissues, which may be accompanied by erythema, peeling and cracks. Its causative agents are streptococcal or yeast-like fungi of the genus Candida (candidamycotic) angulitis. Cheilitis significantly reduces the quality of all areas of the patient's life and can be caused by various reasons that must be determined for proper pharmacotherapy. Treatment of this disease depends on the etiology and should be comprehensive: sanitation of the focus of infection in the oral cavity, local therapy with the use of analgesic, antiseptic, anti-inflammatory, antipruritic, epithelializing and vitamin drugs [1].

One of the most important problems in the treatment of cheilitis is the selection of drugs that have pronounced pharmacological activity, minimal side effects and are suitable for long-term use. This is the advantage of herbal drugs. Medicinal plant raw materials (MPRM) are a source of trace elements, vitamins, amino acids, proteins, polyphenols, unsaturated fatty acids, etc. During the pharmacotherapy of cheilitis, it is recommended to use multicomponent drugs with a complex effect, however, the range of drugs on the pharmaceutical market based on MPRM for the treatment of cheilitis is limited [2, 3].

MPRM of burdock root reduces the development of inflammatory processes, inhibits lipid peroxidation processes, normalizes the antioxidant-prooxidant balance in the body, eliminating the manifestations of the inflammatory process [4].

MPRM of common oak bark exhibits anti-inflammatory, antimicrobial, astringent effects, and thanks to the latter, it eliminates contamination with pathogenic bacteria in the affected area, preventing the occurrence of secondary infection [5].

MPRM of marigold flowers has anti-inflammatory, wound healing, bactericidal effects, accelerates the processes of tissue regeneration and epithelialization [6].

The feasibility of using drugs based on MPRM is determined by a number of their advantages over synthetic drugs: substances of natural origin have high biological activity and are well absorbed by skin cells; complications during their use occur much less frequently, and the total therapeutic effect is effective; each natural compound contains many different biologically active substances (BAS), which determines their broad therapeutic effect in contrast to the narrowly targeted action of synthetic drugs [7, 8].

Thus, it is urgent to develop the composition and technology, as well as methods for standardizing a new remedy for the treatment of cheilitis using MPRM, which is not inferior in effectiveness to synthetic drugs, is suitable for long-term use, and has significantly fewer side effects.

The aim of our work was standardization of the content of biologically active substances and the development of a draft specification for the dense aqueous extract for the pharmacotherapy of cheilitis.

Materials and methods

Dense aqueous extract was obtained at the Department of Pharmaceutical Technology of Drugs of the National University of Pharmacy under the supervision of prof. L. I. Vyshnevska. The ratio of components in the composition of the dense extract is burdock root: oak bark: marigold flowers 5: 1: 1.5, respectively. The phytocomplex is a viscous mass of dark green color with a specific smell of components. The chemical composition of the dense extract is represented by a wide range of BAS (polysaccharides, tannins, flavonoids, phenolcarboxylic acids, saponins), which exhibit a complex effect of ingredients: antibacterial, antifungal, anti-inflammatory and wound healing [9, 10].

The reagents used during the physical and chemical studies met the requirements of the SPhU [11].

Description. Determination and control of the appearance and organoleptic properties of the dense extract (color and odor) were carried out using visual and organoleptic methods according to SPhU [11].

Identification of the main BAS in the obtained dense extract was carried out by thin-layer chromatography (TLC) using plates with a silica gel layer F_{254}P.

Polysaccharides were determined after preliminary acid hydrolysis on Silicagel 60 plates from the company "Merk" using a mobile phase of a mixture of solvents water - chloroform - glacial acetic acid (10 : 60 : 70) compared with a solution of a mixture of standard samples of fructose (PAS SPhU, cat. number M0260, series 1) and glucose (CAS 50-99-7). Detection was carried out with a methanol solution of aniline with diphenylamine in an acidic medium.

To identify tannins, the mobile phase was used: water R – anhydrous formic acid R – ethyl acetate R (5 : 10 : 85), compared with a standard sample of catechin with detection of dried plates with anisaldehyde [11].

The determination of flavonoids was carried out using the mobile phase anhydrous formic acid R – water R – ethyl acetate R (10 : 10 : 80) compared with standard samples of caffeic acid (PAS SPhU, PAS EP or RSS), chlorogenic acid and rutin (PAS SPhU, PAS EP or RSS). Detection was performed with methanolic solutions of diphenylboronic acid aminoethyl ester and macrogol in UV light at a wavelength of 365 nm.

The determination of the moisture content of the dense aqueous extract was carried out in accordance with the requirements of SPhU [11].

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The quantitative content of polysaccharides was determined by the gravimetric method described in SPhU for the quantitative assessment of polysaccharides in the article "Althaea roots (Althaeae radix)", "Althaea grass (Althaeae herba)" [11].

The polysaccharide content in the thick extract, in grams, was calculated using the formula:

$$X = \frac{(m_2 - m_1) \cdot 250 \cdot 100}{m \cdot 25} = \frac{(m_2 - m_1) \cdot 1000}{m},$$

where m – mass of a portion of dense extract, g;

m_1 – filter mass, g;

m_2 – mass of filter with sediment, g.

The content of polysaccharides (inulin and other water-soluble), in grams, must be at least 0.75 g per 100.0 g of dense extract.

Quantitative determination of the amount of flavonoids in terms of hyperoside was carried out by spectrophotometric method according to the method of the monograph of SPhU "Calendula tincture" [11].

The content of the sum of flavonoids, in grams, in terms of hyperoside, was calculated by the formula:

$$X = \frac{A \cdot 0{,}625}{m},$$

where A – optical density of the test solution at a wavelength of 425 nm,

m – mass of a portion of dense extract, g.

The specific absorption index of hyperoside was used, which is equal to 500. The content of flavonoids in terms of hyperoside should be at least 0.006 g per 100.0 g of dense extract.

The quantitative content of tannins was determined by the spectrophotometric method, as specified in the SPhU article "Determination of tannins in medicinal plant raw materials" (2.8.14) and the monograph "Oak bark" [11].

The tannin content in the dense extract, expressed as pyrogallol, in grams, was calculated using the formula:

$$X = \frac{0{,}625 \cdot (A_1 - A_2) \cdot m_2}{A_3 \cdot m_1},$$

where A_1 – optical density of the test solution at a wavelength of 425 nm;

m_1 – mass of the test sample, g;

m_2 – mass of pyrogallol, g.

The content of tannins in terms of pyrogallol must be at least 0.030 g per 100.0 g of dense extract.

Results and Discussion

Extracts from plant raw materials are widely used in various industries: pharmaceutical, medical, perfumery and cosmetic, food industry. In the production of medicines, the use of extracts based on MPRM is becoming increasingly relevant [12].

Extracts are summary substances obtained by extracting MPRM and are concentrated extracts containing the sum of BAS. The BAS content depends on factors such as the raw materials included in the extract, the extractant, the extraction time, etc. [13].

Previous studies have substantiated the composition of the phytocomposition (burdock root: common oak bark: marigold flowers (5:1:1.5)) and developed a technological process for obtaining dense aqueous extract based on it [9, 14].

Accordingly, the next stage of our work was the identification and determination of the quantitative content of BAS in the composition of the proposed dense aqueous extract, as well as the development of specifications for the dense aqueous extract.

According to literature data, the aqueous fraction of the developed extract based on MPRM: burdock roots, common oak bark, marigold flowers may contain inulin-type fructan with a degree of polymerization of 20-24 with promising functional properties, tannins, carotenoids, resins, mucus, bitterness (calendula), flavonoids, triterpene glycosides, saponins, phytoncides, etc. [4, 5, 6].

Identification of the qualitative and quantitative content of the main groups of BAS (polysaccharides, flavonoids, tannins) in the developed dense extract was carried out by the TLC method. As reference solutions for the identification of polysaccharides, the WRS of fructose and glucose were used, the WRS of flavonoids - rutin, chlorogenic and caffeic acids, and the WRS of tannins - catechin [11]. The results of the determination of the main BAS are shown in Fig. 1.

As can be seen from Fig. 1, the following zones appeared on the chromatograms of the test solution of the dense extract: (A) light gray fluorescence zone – glucose; dark brown fluorescence zone – fructose; (B) yellow-brown fluorescence zone – rutin, blue fluorescence zone – chlorogenic acid, light blue fluorescence zone – caffeic acid; (C) brown fluorescence zone – catechin.

The quantitative content of the main groups of BAS in the obtained dense extract was determined by the gravimetric method (polysaccharides) and spectrophotometric method (flavonoids in terms of hyperoside, tannins in terms of pyrogallol) [11].

The content of polysaccharides (inulin and other water-soluble), in grams, must be at least 0.75 g per 100.0 g of dense extract.

The content of flavonoids in terms of hyperoside must be at least 0.006 g per 100.0 g of dense extract.

The content of tannins in terms of pyrogallol should be at least 0.030 g per 100.0 g of dense extract.

The results of the quantitative determination of the main groups of BAS and the metrological characteristics of the average result of 6 samples of dense extract are given in Table 1.

For the developed dense extract, a draft specification for the intermediate product – summary aqueous dense extract was drawn up based on the results of research on quality indicators in accordance with the requirements of SPhU and EP (description, identification, quantification, moisture content) [11, 15]. The results are given in Table 2.

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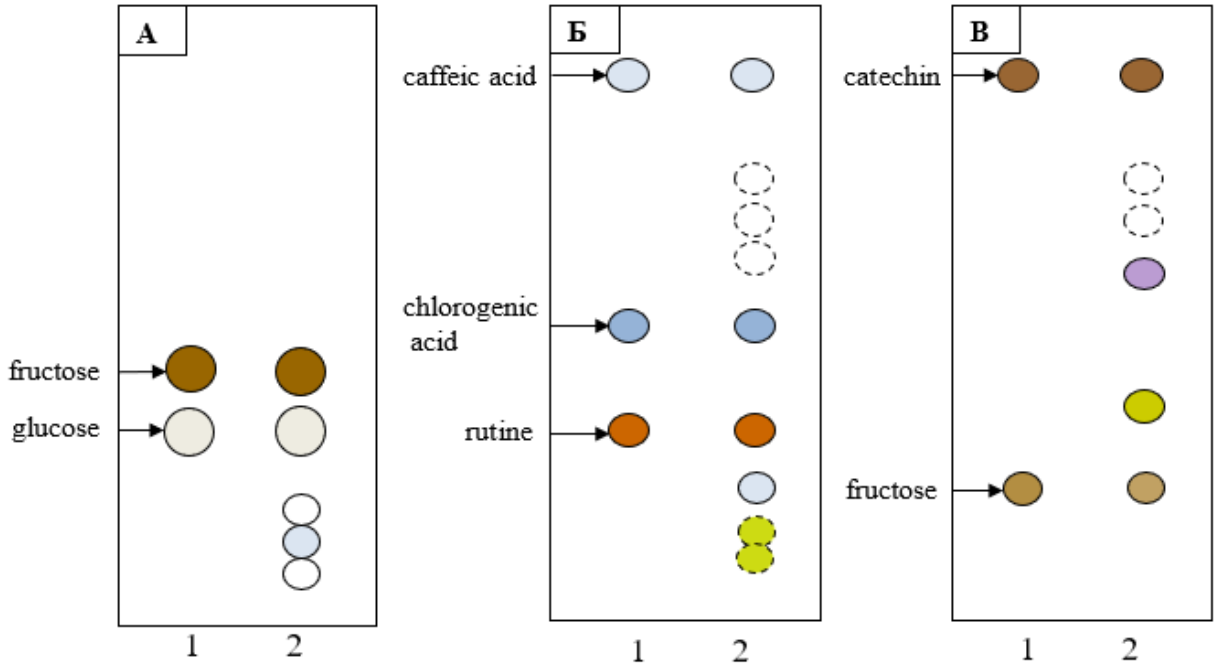

Fig. 1. Scheme of the chromatogram of the identification of BAS in the dense extract: (A) polysaccharides, (B) flavonoids, (C) tannins: 1 – reference solution; 2 – test solution of the dense extract

Table 1. Metrological characteristics of the average result of the quantitative determination of BAS in dense extract

No. series	BAS content, g	S^2	S	$S_{\bar{x}}$	Δx	$\Delta\bar{x}$	$\bar{\varepsilon}$, %	ε, %
Polysaccharides								
1	0,834	0,0007	0,0273	0,0111	0,0701	0,0286	3,47	8,51
2	0,827							
3	0,871							
4	0,813							
5	0,808							
6	0,792							
Flavonoids, calculated as hyperoside								
1	0,0065	$5{,}0\cdot10^{-8}$	0,0002	0,0001	0,0006	0,0002	3,73	9,14
2	0,0066							
3	0,0062							
4	0,0067							
5	0,0068							
6	0,0063							
Tannins, in terms of pyrogallol								
1	0,0302	$3{,}0\cdot10^{-8}$	0,0002	0,0001	0,0005	0,0002	0,64	1,58
2	0,0303							
3	0,0307							
4	0,0305							
5	0,0306							
6	0,0304							

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Table 2 – Specification for the developed dense aqueous extract

Indicator	Acceptable limits	Control methods
Description	Thick mass of greenish-brown color with a specific odor	SPhU: organoleptically
Identification: – polysaccharides – flavonoids – tannins	Spots corresponding to the Rf value and color of the spots should appear on the chromatogram of the test solution: WRS fructose and glucose. WRS chlorogenic and caffeic acids, rutin. WRS catechin, fructose. Other, less noticeable spots may be present	SPhU: thin layer chromatography method
Moisture, %	Not higher than 25	SPhU
Quantitative determination: – polysaccharides – flavonoids in terms of hyperoside – tannins in terms of pyrogallol	 not less than 0.75 g in 100.0 g of extract not less than 0.006 g in 100.0 g of extract not less than 0.03 g in 100.0 g of extract	SPhU: gravimetric method spectrophotometric method spectrophotometric method

So, as a result of the research, the standardization of the aqueous dense extract for the treatment of cheilitis was carried out according to the main groups of BAS (polysaccharides, flavonoids, tannins) and a draft specification was drawn up for the developed dense extract.

Conclusions

1. A study was conducted to identify and quantify the main groups of BAS (polysaccharides, flavonoids, tannins) of the dense aqueous extract, which may indicate its quality.
2. The studied indicators are included in the specification: description, identification (polysaccharides (fructose, glucose), substances of flavonoid nature (caffeic, chlorogenic acids, rutin), tannins (catechin)), moisture (no more than 25%), quantitative determination (polysaccharides, flavonoids in terms of hyperoside, tannins in terms of pyrogallol).
3. A draft specification has been drawn up for the developed dense aqueous extract for the pharmacotherapy of cheilitis.

Prospects for further scientific research

Thus, the team of authors considers the dense aqueous extract, containing burdock root, oak bark, and marigold flowers, promising for further research and development of modern, effective, and safe drugs for the pharmacotherapy of cheilitis.

Conflict of interest: absent**.**

Study of the content of biologically active substances and development of specifications for a dense extract for the treatment of cheilitis

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Introduction. Cheilitis is an inflammatory disease of the lips, which significantly affects the patient's quality of life. Treatment of this disease should be comprehensive and depend on its origin. The drugs of choice in the treatment of cheilitis are herbal remedies that contain a wide range of biologically active substances and have a pronounced pharmacological activity, minimal side effects and are suitable for long-term use. Promising medicinal plant raw materials for the pharmacotherapy of cheilitis are burdock roots, common oak bark and marigold flowers. Burdock roots have the property of reducing the development of inflammatory processes, oak bark has anti-inflammatory, antimicrobial and astringent activity, marigold flowers have anti-inflammatory, wound-healing and bactericidal effects. Previous studies have obtained a dense aqueous extract for the treatment of cheilitis based on the mentioned above medicinal plant raw materials. The wide spectrum of the chemical composition of the dense extract includes various components, such as polysaccharides, tannins, flavonoids, which exhibit antibacterial, antifungal, anti-inflammatory and wound-healing activity. Therefore, it is relevant to develop methods for identifying and quantifying the main biologically active substances of a new phytocomplex for the treatment of cheilitis. **Aim of the study.** Identification and study of the content of the main biologically active substances of the dense aqueous extract for the treatment of cheilitis and development of the specification of the phytocomplex. **Materials and methods.** The object of the study was a previously developed dense aqueous extract based on medicinal plant raw materials: burdock root: oak bark: marigold flowers (5:1:1.5). The standardization of the developed phytocomplex for the treatment of cheilitis was carried out according to the methods and recommendations given in the State Pharmacopoeia of Ukraine 2.0. **Results.** The analysis of the quantitative and qualitative composition of the main groups of biologically active

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substances (polysaccharides, flavonoids, tannins) in the developed dense aqueous extract was carried out using the thin-layer chromatography method. According to the results of the research, the presence of fructose and glucose, rutin, chlorogenic and caffeic acids, as well as catechin in the dense aqueous extract was identified. The quantitative determination of the main groups of biologically active substances in the dense aqueous extract was carried out using the gravimetric method (polysaccharides) and the spectrophotometric method (flavonoids in terms of hyperoside and tannins in terms of pyrogallol). The content of polysaccharides per 100.0 g of thick extract must be at least 0.75 g. The content of flavonoids per 100.0 g of dense extract in terms of hyperoside must be at least 0.006 g. The content of tannins per 100.0 g of dense extract in terms of pyrogallol must be at least 0.030 g. The results of the conducted experimental studies were used as the basis for the draft specification for the developed dense aqueous extract according to the following indicators: description, identification, quantification, moisture content. **Conclusion.** It is proposed to identify the main groups of biologically active substances of the dense extract (polysaccharides, tannins, flavonoids) by the method of thin-layer chromatography. Quantitative methods have been developed: polysaccharide - by the gravimetric method, tannins and flavonoids - by the spectrophotometric method. A draft specification has been drawn up for the developed thick aqueous extract.
Key words: dense aqueous extract, biologically active substances, identification, specification, cheilitis.

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