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DEVELOPMENT OF THE COMPOSITION OF AQUEOUS EXTRACT OF LESPEDEZA BICOLOR AND GEL WITH EXTRACT AND MANGIFERIN

Maryana Yaromiy, Nataliia Bevz, Olha Mykhailenko, Volodymyr Kovalov, Nataliia Polovko, Yana Marchenko

The aim *of the work: research on the development and standardization of an aqueous extract of the aerial part of Lespedeza bicolor and the study of pharmacotechnological parameters of a gel with the extract and mangiferin.*
Materials and methods*: in the studies on obtaining an aqueous extract, the aerial part of Lespedeza bicolor, harvested in the Botanical Garden of the Ivan Franko National University of Lviv (Lviv, Ukraine) in the flowering phase, was used. Identification and quantitative content of biologically active compounds in the experimental samples of the extracts were carried out by chemical reactions, thin-layer chromatography and absorption spectrophotometry. The results obtained were used to standardize the selected aqueous extract of Lespedeza (AEL). In the development of the gel, AEL, mangiferin (China, X'ian Pincredit Bio-Tech Co., Ltd. QC004) and excipients of domestic and foreign production were used. The studies used physicochemical (pH value) and pharmacotechnological research methods (thermo- and colloidal stability, dispersion analysis, dialysis method through a semipermeable membrane).*
Results. *The conditions for obtaining AEL were experimentally substantiated. It was shown that the use of three-stage remaceration with infusion for 60 minutes at a temperature of 90–100 °C ensures maximum extraction of polyphenolic compounds. The TLC method identified the presence of rutin, quercetin, chlorogenic and caffeic acids, tannins and substances of polysaccharide structure in the extract. Methods for quantitative determination of polyphenolic compounds and flavonoids by absorption spectrophotometry were developed. Based on the results of microscopic analysis, study of the osmotic activity of the base and the dynamics of mangiferin release, the composition of a dermatological gel was proposed, containing AEL, mangiferin, PEO-400, ethanol 96 %, corn oil, tween 80, carbopol, trometamol, purified water.*
Conclusions. *The conditions for obtaining and standardizing the aqueous extract of Lespedeza bicolor were substantiated. The composition of the gel with mangiferin and AEL was proposed. It was shown that the introduction of 20 % PEO-400, 10 % ethanol 96 % and 1 % tween-80 into the gel base ensures its absorption capacity and increases the rate of mangiferin release*

Keywords: *aqueous extract of Lespedeza bicolor, extraction, mangiferin, dermatological agent, gel, thin-layer chromatography, absorption spectrophotometry*

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1. Introduction

The urgency of finding new effective pharmacotherapy for herpes infection is associated with the prevalence in the world, the ability of the virus to affect almost all organs and systems of the human body and cause various forms of infection – acute, latent and chronic recurrent [1]. Today, there are no means and methods of treatment that allow eliminating the herpes simplex virus from the human body, and the treatment of herpes infection is complex and not effective enough. Modern treatment methods are aimed at preventing the development of the virus or restoring those disorders that are caused by the activation of the herpes virus in the body. There are two main areas of herpes therapy – the use of etiopathogenetic antiviral therapy and a complex method that includes specific and nonspecific immunotherapy in combination with antiviral therapy [2].

One of the promising directions is the search for natural APIs and the creation of original domestic drugs based on them, which combine various mechanisms of antiviral protection, with additional pharmacological effects [3, 4].

In view of this, a promising raw material is mangiferin [5], which has numerous pharmacological effects [5, 6], primarily anti-inflammatory [7, 8], immunomodulatory [9], analgesic [10], antioxidant [11] and antibacterial [11, 12]. It has been experimentally confirmed that mangiferin exhibits antibacterial activity against *Staphylococcus aureus* [13], as well as antifungal (*C. albicans*, *A. niger* and *A. flavus*) [14] action. The antiviral activity of mangiferin obtained from Mangifera indica, as well as from the rhizome of *Anemarrhena asphodeloides*, against herpes simplex virus type 1 has been confirmed [15–17]. Mangiferin, with low solubility, trans-

dermal permeability, and bioavailability, has demonstrated the ability to penetrate the stratum corneum and pass into the epidermis and dermis [18]. The authors [18] confirmed the ability of mangiferin to cross the stratum corneum of the epidermis and penetrate the deeper layers of the epidermis and the dermis using *ex vivo* fluorescence microscopy. In addition to the ability to accumulate in the skin, mangiferin penetrates through the skin, which was confirmed by HPLC analysis with electrochemical detection of all layers of the skin and acceptor fluid [18]. Based on the study of the effect of mangiferin on the skin, the authors concluded that it could be used in dermatological and cosmetic products [18].

To expand the spectrum of pharmacological action, *Lespedeza bicolor* (Leguminosae) was chosen. It was experimentally established that Lespedeza extracts have antioxidant, reparative, anti-inflammatory [19, 20] and antimicrobial [21, 22] activity. Literature data and our own studies indicate that *Lespedeza* species extracts can be raw materials for the creation of new dermatological drugs [23]. In view of the above, the development of a soft dosage form with mangiferin and Lespedeza bicolor extract for cutaneous application is promising.

The aim of the research – study the development and standardization of an aqueous extract of the aerial part of Lespedeza bicolor and the study of the pharmacotechnological parameters of a gel with the extract and mangiferin.

2. Research planning (methodology)

The research methodology was based on two experimental stages. The first stage was the justification of the conditions for obtaining and standardization of the intermediate product – an aqueous extract of the aerial part of *Lespedeza bicolor*. The second stage included pharmacotechnological studies at the stage of developing a soft dosage form with the obtained extract and mangiferin [24]. The choice of the basis was based on the medical and biological requirements for SDF for the treatment of herpes infection. When developing the extract and SDF, physical, physicochemical and pharmacotechnological research methods were used. The obtained research results were interpreted and conclusions were drawn.

3. Materials and methods

The research was conducted at the National University of Pharmacy during 2024.

3. 1. Obtaining an aqueous extract of Lespedeza bicolor

To obtain the aqueous extract, the above-ground part of *Lespedeza bicolor* Turcz., Leguminosae, was prepared, consisting of a mixture of buds, flowers, leaves and stems. The raw material was prepared in the collection "Medicinal Plants" of the Botanical Garden of the Ivan Franko National University of Lviv (Lviv, Ukraine) in the phenophase of mass flowering. The samples were identified by Senior Researcher Skibitskaya M. I. and stored in the Herbarium (LW) of the Ivan Franko National University of Lviv (LW0056630), Ukraine. The raw material was dried at an ambient temperature of 20–24 °C.

The aqueous extract was obtained by the method of re-maceration with forced stirring at a temperature of 95–100 °C. The extraction temperature was selected based on the results of the analysis of the conditions for obtaining aqueous extracts [25]. As previously conducted studies have shown, purified water is an effective extractant for the extraction of polyphenolic compounds and tannins from the aerial part of Lespedeza bicolor. The infusion obtained according to the rules of manufacture in pharmacy conditions (15 min. infusion in a water bath and 45 min. infusion during cooling) contains 0.612±0.016 % polyphenolic compounds, 0.041±0.013 % flavonoids and 0.062±0.016 % tannins [26].

The number of extractive substances was determined by the SPhU method [27].

To identify the BAS extract, the TLC method was used. 0.1 ml of the extract is placed in a 5.0 ml volumetric flask and made up to volume with 70 % ethyl alcohol. A reference solution is prepared: 1 mg of rutin, 5 mg of quercetin, 5 mg of chlorogenic acid and 5 mg of caffeic acid are dissolved in 10 ml of methanol. On the starting line, a TLC plate with a layer of silica gel R is applied in strips of 10 µl of the test solution and 5 µl of the reference solution, placed in the solvent system butanol R – anhydrous acetic acid R – water R (4:1:2), when the solvent front has passed 10 cm, the plates are dried in air until the odour of the solvent disappears for 30 minutes, treated with a 5 % solution of aluminium chloride in methanol, dried in a stream of warm air and viewed in UV light at a wavelength of 365 nm.

The sum of polyphenolic substances was determined after reaction with phosphorus-molybdenum-tungsten reagent in a saturated sodium carbonate solution according to the SPhU method [27]. The blue-coloured reaction product is characterized by the presence of a rather shallow absorption maximum at a wavelength of 760 nm, which corresponds to the absorption maximum of the pyrogallol solution under these conditions.

The sum of polyphenols, in terms of pyrogallol, in milligrams, is calculated by the formula:

$$x, \text{mg} = \frac{625 \cdot A_1 \cdot m_2}{A_2 \cdot m_1},$$

where m_1 – mass weight of liquid extract, in grams; m_2 – mass of pyrogallol, in grams.

The content of the sum of polyphenols in terms of pyrogallol in 1.0 g of liquid extract should be at least 35 mg.

For the quantitative assessment of the sum of flavonoids, the method of absorption spectrophotometry in the visible region was used after interaction with a solution of aluminium (III) chloride in an acetic acid medium [26]. The optical density of the coloured test solution and the reference solution is measured 30 minutes after preparation at a wavelength of 408 nm relative to the compensation solution.

The content of the sum of flavonoids (X, mg) in terms of rutin is calculated by the formula:

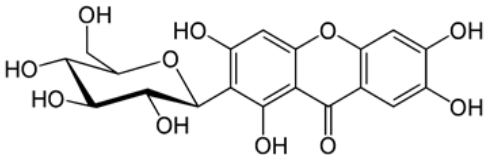
$$X=\frac{A\cdot m_0\cdot 5.0\cdot 1.0\cdot 100}{A_0\cdot V\cdot 50.0\cdot 25.0}=\frac{A\cdot m_0}{A_0\cdot V\cdot 2.5},$$

where A – optical density of the test solution; A_0 – optical density of the solution of the rutin standard sample; m_0 – mass weight of rutin, g; V – volume of extract taken for analysis, ml.

The content of the sum of flavonoids in terms of rutin in 1.0 g of liquid extract should be at least 0.5 mg.

3. 2. Justification of the gel composition

In the research on the development of the SDF composition, mangiferin was used – a light yellow fine-crystalline powder obtained from the leaves of the mango tree (*Mangifera indica*), (manufacturer X'ian Pincredit Bio-Tech Co., Ltd, China, batch number QC004). The substance contains 98.5 % of mangiferin:



1,3,6,7-Tetrahydroxy-2-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-9H-xanthen-9-one

The excipients selected for the development of a gel with an aqueous extract of the aerial part of Lespedeza bicolor and mangiferin are widely used in pharmaceutical technology. The composition of the experimental samples is given in Table 1.

Table 1

Composition of test samples

Ingredient name	Sample number/ingredient content, %						
	1	2	3	4	5	6	7
Mangiferin	5						
Lespedeza aqueous extract	5						
PEO-400	20	–	20	–	20	20	20
Ethanol 96 %	–	10	10	10	10	–	10
Corn Oil	–	–	–	–	–	5	5
Tween 80	–	–	–	1	1	1	1
Carbopol	0.8						
Trometamol	0.8						
Purified water	Up to 100						

The test samples of the mixture of mangiferin with solvents and gels were homogenized using a mixer MI-2 for liquid and soft dosage forms for 15 min at a speed of 100 rpm. The samples were evaluated for appearance, pH value, osmotic capacity of bases and release of mangiferin by dialysis through a semipermeable membrane.

Osmotic activity was studied at a temperature of 34±1 °C (the samples were kept in a thermostat TC-80M-2) in *in vivo* experiments by dialysis through a semipermeable cellophane membrane. The mass of the inner cylinder of the dialyzer was determined on an electronic balance every hour for 8 hours of the experiment. The amount of liquid absorbed by the test samples (with a weight of 10.0 g) was expressed as a percentage of the samples.

The determination of the kinetic parameters of the samples was carried out by dialysis through a semipermeable membrane of a dialyzer consisting of two chambers. A regenerated dialysis cellulose membrane Cuprophan (Medicell International Ltd., London, Great Britain) was placed on the first chamber with a diameter of 50 mm and an area of 1963 mm^2, a sample of the test sample (10.0 g) was placed, evenly distributed on the surface of the membrane and the dialyzer was collected. The dialysis fluid was introduced into the receptor chamber – phosphate buffer solution with pH 6.0 PI, which was prepared according to SPhU [27]. The dialyzer was placed in a TC-20 thermostat with a temperature of (34±1) °C. Sampling (aliquot) was carried out every hour for 5 hours of the experiment. The content of mangiferin in the selected samples was determined by absorption spectrophotometry in the ultraviolet region.

Microscopic stud*ies* were performed using a laboratory microscope Granum R-40 (China) with a built-in digital video camera DCM 310 and an achromatic objective of the DIN standard: 40x. In combination with a 10-fold eyepiece, a microscope magnification of 400x was obtained. For image visualization, the images were displayed on the screen and processed using the Toup View 3.7 program.

Statistical processing of the experimental data results was carried out in accordance with the requirements of articles SPhU 5.3. [27].

4. Research results

4. 1. Obtaining an aqueous extract of *Lespedeza bicolor*

When justifying the conditions for obtaining an aqueous extract, the influence of time and the number of stages of extraction of raw materials on the yield of BAS was determined. The extraction efficiency was assessed by the content of polyphenolic compounds and flavonoids, which were investigated in each portion of the extract, which was taken after 15 min for 120 min (Fig. 1).

As can be seen from Fig. 1, the content of both polyphenolic compounds and flavonoids in the extracts gradually increases during extraction during the first 60 minutes and then changes insignificantly. Therefore, this time is optimal for the extraction of the aboveground part of lespedeza. A more effective extraction method is remaceration, so it was necessary to determine the number of extraction stages at which the maximum depletion of the raw material occurs. The raw material was remacerated four times with incubating for 60 min at a temperature of 95–100 °C and forced mixing. The minimum amount of extractant for the first stage, at which a "mirror" was obtained, in relation to the raw material is 4:1, for the following stages of extraction the extractant was divided into equal parts. The content of polyphenolic compounds in terms of pyrogallol and flavonoids in terms of rutin, which was determined in each portion of the extract, is given in Table 2.

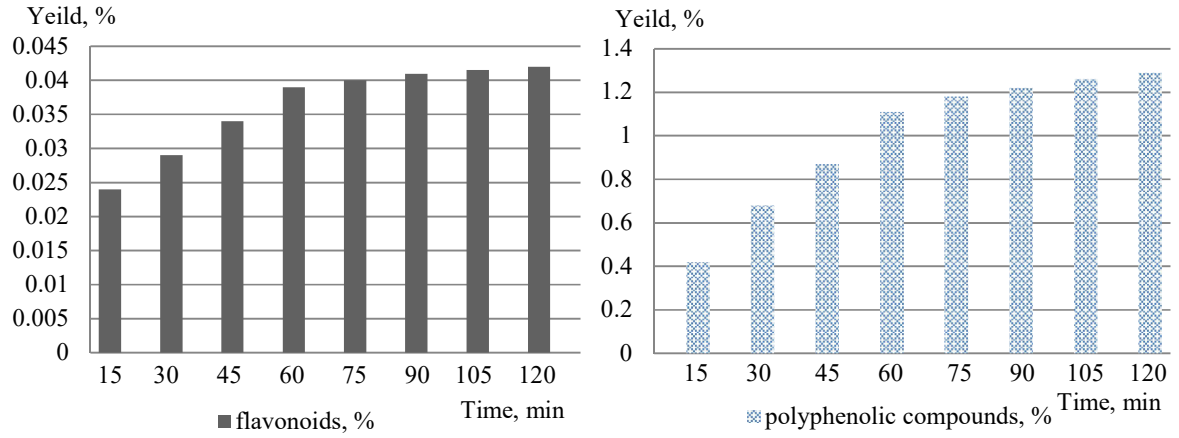


Fig. 1. Dependence of the content of polyphenolic compounds and flavonoids in the aqueous extract of lespedeza on the extraction time

The obtained results indicate that the first three stages of extraction are effective, during which intensive extraction of biologically active compounds is observed, while at the 4th stage of extraction a small amount of polyphenolic compounds and flavonoids is extracted. This allows us to conclude that the maximum depletion of the raw material is provided by three-stage remaceration with a total ratio of raw material: finished extract of 1:8. To obtain a liquid extract, the first extract was collected and its volume was measured, and the second and third extracts were combined, filtered and evaporated under vacuum in a rotary evaporator at a temperature of 90–100 °C to obtain a total ratio of raw material: finished product of 1:2. The condensed residue was mixed with the first extract, settled at a temperature not higher than 10 °C for 48 h and filtered.

For the manufacture of medicines and their further use in medical practice, the extract must be standardized. The standardization of the aqueous extract was carried out based on the results of experimental studies, in accordance with the requirements of the SPhU (Table 3) [27]. It was found that it is a transparent liquid of greenish-brown colour with a specific pleasant herbal odour.

According to the results of BAS identification in the liquid extract, the presence of substances of flavonoid structure, tannins and hydrocarbons was established using colour reactions [26].

TLC method identified phenolic compounds: flavonoids, hydroxycinnamic acids (rutin, quercetin, chlorogenic and caffeic acids), tannins and substances of polysaccharide structure [26] (Fig. 2).

The content of dry residue in AEL was within 18.8–20.2 %, tannins in terms of pyrogallol – 35.87–38.26 mg/ml, flavonoids in terms of rutin 0.58–0.65 mg/ml. The studied extract withstood tests for heavy metals, the content of which did not exceed 0.001 % (100 ppm). Based on the results of the study, a specification for AEL was compiled, which is given in Table 3.

Table 2

The content of polyphenolic compounds and flavonoids in lespedeza extract depending on the degree of extraction, *n*=3

Biologically active compounds	Extraction stages			
	1	2	3	4
Flavonoids, mg/ml	0.31±0.02	0.22±0.03	0.13±±0.02	0.05±±0.02
Polyphenolic compounds, mg/ml	1.92±0.09	0.86±0.06	0.69±±0.03	0.14±±0.02

Table 3

Specification for *Lespedeza bicolor* aqueous extract

Indicator	Permissible limits
Description	A greenish-brown liquid with a specific pleasant herbal odor
Identification: – polyphenolic compounds – flavonoids	The chromatogram of the test solution should show spots that correspond in Rf value and color to the spots of rutin, quercetin, chlorogenic and caffeic acids Other, less noticeable spots may be present
Heavy metals, %	Not more than 0.001
Dry residue, %	Not less than 18 %
Quantitative determination: – flavonoids, expressed as rutin – sum of polyphenols, expressed as pyrogallol	 Not less than 0.5 mg in 1.0 g of extract Not less than 35 mg in 1.0 g of extract

Pale blue fluorescence (caffeic acid)	Caffeic acid: pale blue fluorescence
Brownish yellow fluorescence (quercetin)	Quercetin: brownish yellow fluorescence
Yellow fluorescence (rutin)	Rutin: yellow fluorescence
Blue fluorescence (chlorogenic acid)	Chlorogenic acid: blue fluorescence
Test solution	Reference solution

Fig. 2. Scheme of chromatography obtained during the identification of flavonoid substances in the aqueous extract of lespedeza: 1 – test extract solution; 2 – reference solution (markers of caffeic acid, quercetin, rutin, chlorogenic acid)

4. 2. Justification of the gel composition

Based on the analysis of literature sources and the results of our own experimental studies, which confirmed the antimicrobial and anti-inflammatory activity, we introduced 5 % aqueous extract of lespedeza into the composition of the dermatological gel [28, 29]. Mangiferin, which has antiherpetic and antimicrobial activity, was also chosen as the API [17, 29].

Mangiferin is moderately soluble in ethanol and slightly soluble in water in the temperature range from 15 to 30 °C (0.18 g/100 ml at 15 °C and 0.19 g/100 ml at 30 °C in purified water and 0.71 g/100 ml at 15 °C and 1.25 g/100 ml at 30 °C in 96 % ethanol), practically insoluble in organic solvents used in pharmaceutical technology [30].

At the stage of determining the conditions for introducing mangiferin into SDF, the ability to disperse in various solvents of different nature was studied. Hydrophilic solvents were used as solvents – purified water, glycerin, PEO-400, ethanol 96 %, hydrophobic solvent – corn oil and surfactant – polysorbate 80 (Tween-80), which is a solubilizer and increases the solubility of poorly soluble compounds.

The results of the study showed that mangiferin is not soluble in the selected solvents at the maximum ratio (1:20) at a temperature of 20 °C, and at certain ratios (PEO-400 and ethanol 96 % – 1:2, Tween 80 and corn oil – 1:3, purified water – 1:3, glycerin – 1:6) it is wetted and then mixed with the solvent to form homogeneous dispersions of light yellow colour. Given that the substance is not soluble in the selected solvents, it should be introduced into the SDF in the form of a finely dispersed powder. The different ability to be wetted by solvents necessitated the need for a reasonable choice of a hydrophilic carrier substance for the introduction of mangiferin into the SDF. Microscopic studies showed that the mangiferin substance belongs to the monoclinic system (Fig. 3, *a*). The particles of the unground substance have an anisodiametric shape, with fragments on the surface, capable of agglomeration, the size of the majority is in the range of 0.4–0.1 μm. Microscopic studies of a suspension of mangiferin with PEO-400 and ethanol 96 % in a ratio of 1:2 and purified water 1:3 showed that the size of mangiferin particles in a suspension with water and PEO-400 does not change, a certain part of the API is in the form of agglomerates. In a suspension with 96 % ethanol, mangiferin is less prone to agglomeration, the particles are more homogeneous, the vast majority have a size of less than 0.1 μm (Fig. 3, *d*).

According to the organoleptic and physicochemical properties, the gel samples (Table 1) are a homogeneous mass of yellow-cream color with a weak herbal odor, thermo- and colloidally stable, with a pH value in the range of 6.24–6.68. The justification of the drug composition was carried out based on the results of microscopic studies of the samples, determination of their osmotic properties and the ability of mangiferin to be released from the bases.

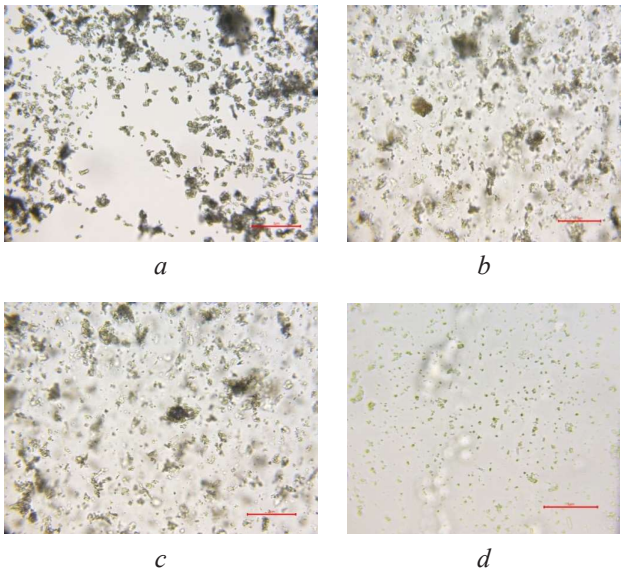


Fig. 3. Micrographs: *a* – mangiferin; suspensions of mangiferin with; *b* – water; *c* – PEO-400; *d* – 96 % ethanol (at 400x magnification)

Microphotographs of the samples (Fig. 4) demonstrated the tendency of mangiferin to agglomerate in an aqueous medium, however, preliminary dispersion with 96 % ethanol reduces the size of the agglomerates, and the introduction of surfactants prevents their formation (samples 4, 5) except for sample 6, which did not contain ethanol. However, the introduction of surfactants into sample 6 reduces the size of agglomerates compared to sample 1. In samples 6 and 7, the presence of an oil phase with a particle size of 0.14–0.22 μm is observed.

Studies (Fig. 5) have shown that gel bases with osmotically active non-aqueous solvent PEO-400 (sam-

ples 1, 3, 5) have pronounced osmotic activity and are able to absorb about 40 % of water within 8 hours, in contrast to gels that do not contain PEO-400 and absorb less than 10 % of water (samples 2 and 4). The introduction of oil and surfactant leads to a slight increase in the osmotic properties of model samples with PEO-400 (about 5 % for samples 6 and 7), which occurs due to a decrease in the amount of hydrophilic phase and, accordingly, an increase in the concentration of the osmotically active hydrophilic carrier substance in it.

An important stage in the justification of the composition of the SDF base is the study of the dynamics of the drug release by the *in vitro* method. The dependence of the release of mangiferin on the composition of the experimental gel bases was determined (Table 1). The quantitative determination of mangiferin in the acceptor liquid was investigated by the method of absorption spectrophotometry in the ultraviolet region. An aliquot of the experimental samples was taken every hour during the 5 hours of the experiment.

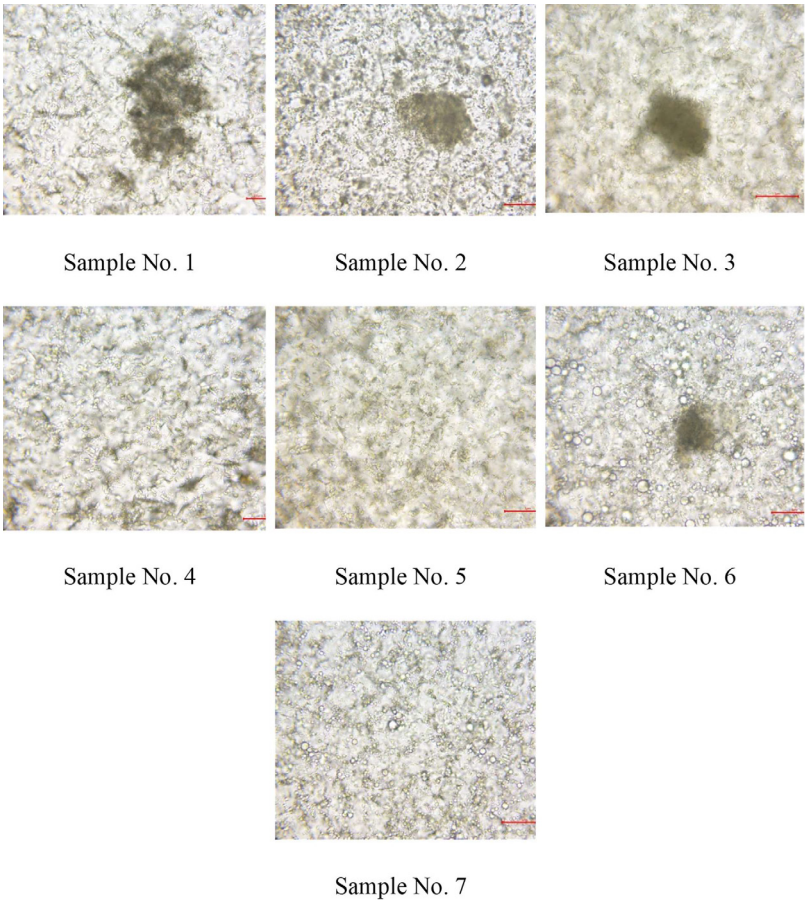


Fig. 4. Micrographs of test samples at 400× magnification

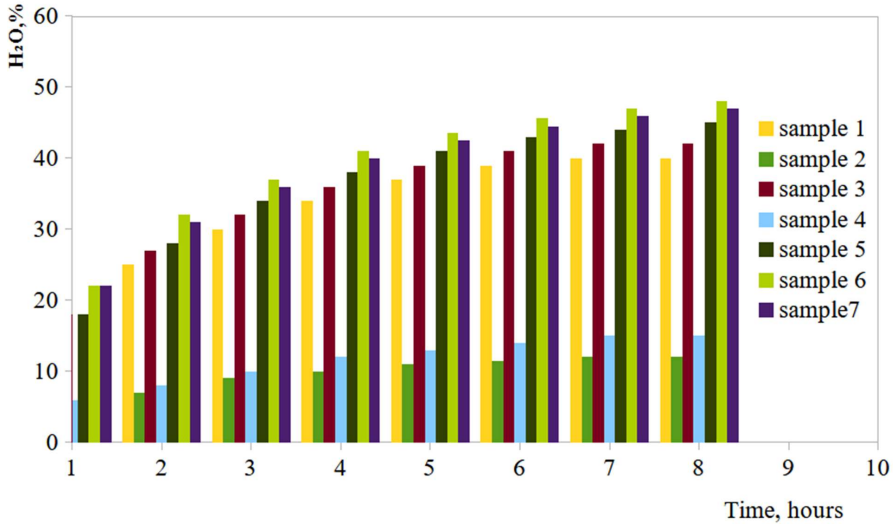


Fig. 5. Kinetics of water absorption by test samples

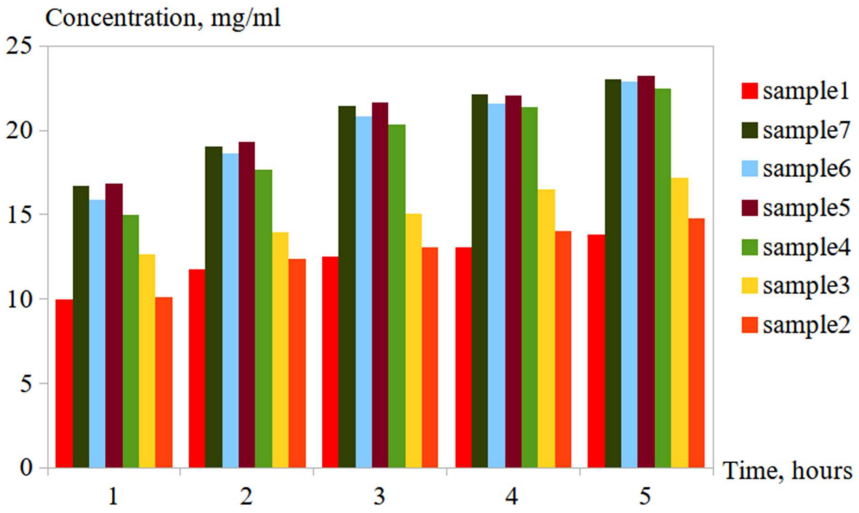


Fig. 6. Dynamics of mangiferin release from experimental bases

Studies of the quantitative content of mangiferin in the acceptor liquid showed its ability to be released into a buffer solution (Fig. 6). More intensive release from all bases occurs during the first 3 hours of the study, and then a slowdown in the release of the API was observed and, accordingly, the feasibility of conducting studies after 5 hours of the experiment disappeared. The completeness of the release depends on the composition of the base. The introduction of 96 % ethanol and PEO-400 into the base increases the release by about 20 % compared to bases containing only one of the indicated co-solvents (sample 3, compared to 1 and 2). However, Tween-80 has a more intensive effect on the rate and completeness of the release (samples 4–7), and the introduction of 5 % corn oil does not statistically significantly affect this process (sample 7 compared to 5).

5. Discussion of research results

5. 1. Obtaining an aqueous extract of *Lespedeza bicolor*

The selected conditions for the extraction of the aerial part of *Lespedeza bicolor* made it possible to obtain an aqueous extract containing almost five times more polyphenolic compounds in terms of pyrogallol and 50 % more flavonoids in terms of rutin compared to obtaining an infusion from the same type of raw material [26]. The obtained extract is promising for use in dermatological products [28, 29], which was confirmed in studies of the antioxidant activity of the aqueous extract of *Lespedeza cuneata* [31].

5. 2. Justification of the gel composition

The justification for the choice of the base was based on the biomedical requirements for a soft drug, the base of which should affect the course of the disease in different phases. Due to the osmotic properties provided by the introduction of 20 % PEO-400, the base will absorb exudate with viruses. Given the low solubility of mangiferin, the choice of a mixture of co-solvents (ethanol, PEO-400 and Tween-80) that prevent aggregation of mangiferin and ensure its release from the base is justified. The introduction of oil contributes to the softening effect of the base. The use of surfactants, ethanol, hydrophobic excipients corresponds to the main approaches to the development of SDF with mango extract, the main BAS of which is mangiferin [32, 33]. However, the use of liquid paraffin, petrolatum and other synthetic fat-like substances limits the release of API from the base, creates an occlusion effect due to which the absorption of exudate is reduced and, accordingly, the effectiveness of the drug is reduced. Conversely, the use of vegetable oil in the composition of the drug and the additional introduction of AEL will contribute to the healing of the skin defect.

Confirmation of the ability to be released from the SDF base is carried out by biopharmaceutical studies most often using the dialysis method through a semipermeable membrane. An important stage of the research is the choice of the acceptor medium and the method of quantitative determination of API in it [34]. When conducting biopharmaceutical studies, the use of the absorption spectrophotometry method for the quantitative determination of mangiferin has been justified. During the studies, it was found that in the range from 200 nm to 400 nm, a 0.016 % solution of the standard sample of mangiferin and a 0.016 % solution of the aqueous extract of lespedeza in phosphate buffer solution with pH 6 are characterized by the presence of absorption maxima at wavelengths of 242 nm, 257 nm, 318 nm and 369 nm. The dialysis fluid sample taken after diffusion of the gel test sample has a rather intense and flat maximum at a wavelength of 255 nm and maxima at wavelengths of 318 nm and 369 nm (Fig. 7).

The presence of an intense maximum at a wavelength of 255 nm in the dialysis fluid of gel extraction indicates the influence of gel excipients on the character of the spectrum (Fig. 7).

The amount of mangiferin transferred to the release medium was determined at a wavelength of 369 nm, at which there is a minimal effect on the optical density of the aqueous extract of Lespedeza (Fig. 8).

Therefore, the selected method can be used for the quantitative determination of mangiferin in the acceptor liquid.

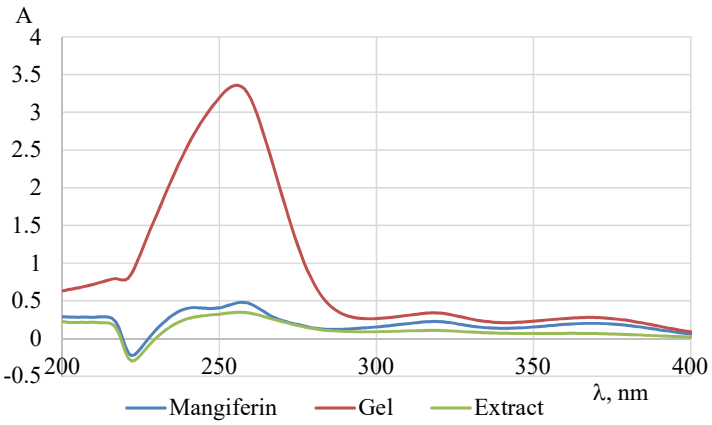


Fig. 7. Electronic absorption spectrum 1 – release from the gel; 2 – mangiferin solution and 3 – solution of aqueous extract of Lespedeza in phosphate buffer solution with pH 6.0

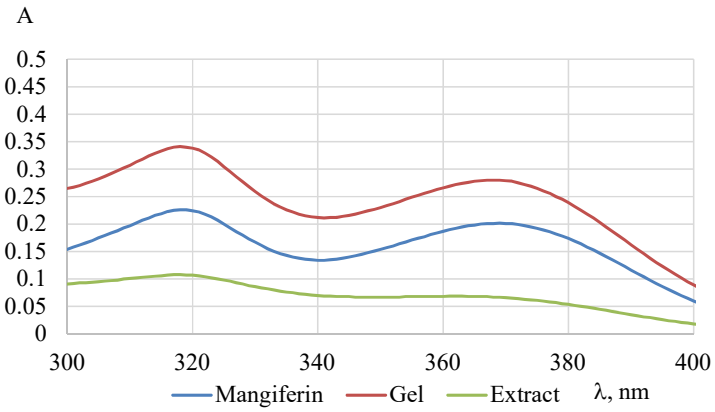


Fig. 8 Electronic absorption spectrum 1 – release from the gel; 2 – mangiferin solution and 3 – solution of aqueous extract of Lespedeza in phosphate buffer solution with pH 6.0

Practical significance. The results obtained will contribute to expanding the range of medicines for the treatment of skin diseases and expand the possibilities of further research and use of *Lespedeza bicolor* aqueous extract as an API.

Study limitations. The study we planned was completed in full, the results obtained were predictable and reproducible. The methods chosen within the framework of the planned study have no limitations. However, we did not conduct additional studies to determine the microbiological purity of the gel, the choice of preservative, which should be carried out using microbiological studies.

Prospects for further research. At the next stages of research, it is advisable to investigate the microbiological purity of the developed product, if necessary, justify the introduction of a preservative, justify the technological parameters of production, and standardize the drug.

6. Conclusions

The conditions for obtaining AEL were experimentally substantiated. It was shown that the use of three-stage remaceration with infusion for 60 minutes at a temperature of 90–100 °C ensures maximum extraction of polyphenolic compounds of tannins and flavonoids.

AEL was standardized. The presence of rutin, quercetin, chlorogenic and caffeic acids in the extract was identified by TLC. Methods for quantitative determination of the amount of polyphenolic compounds and flavonoids by absorption spectrophotometry were developed.

Based on the results of organoleptic, microscopic analysis, study of the osmotic activity of the base and the dynamics of mangiferin release, the composition of the gel with mangiferin and AEL was proposed. It was shown that the introduction of 20 % PEO-400, 10 % ethanol 96 % and 1 % Tween-80 into the gel base ensures its absorption capacity and increases the rate of mangiferin release.

The use of the absorption spectrophotometry method in determining the quantitative content of mangiferin in dialysis fluid during biopharmaceutical research is justified.

Conflict of interests

The authors declare that they have no conflict of interest regarding this study, including financial, personal, authorship or other, that could influence the study and its results presented in this article.

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Data availability

Additional data will be available upon reasonable request.

Use of artificial intelligence tools

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

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