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EXPERIMENTAL RESEARCH ON THE DEVELOPMENT OF COMPOSITION OF COMPLEX ACTION OINTMENT BASED ON PHYTOCOMPLEX

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The aim of the work *is to develop the composition of a complex action ointment based on active pharmaceutical ingredients of natural origin for the treatment of cheilitis of various etiologies.*
Materials and methods. *The development of the ointment base by selecting active pharmaceutical ingredients and auxiliary substances determined by organoleptic, physicochemical, rheological and microbiological research methods.*
Results. *Studies of the antimicrobial activity of experimental samples have established that the optimal ointment base is a water/oil emulsion, which provides better API release rates than absorption bases.*
According to the results of structural and mechanical studies, the introduction of the API complex reduces the structural viscosity of the ointment base. Furthermore, the ability of the ointment to thin out with an increase in the gradient of the shear rate is shown, which will contribute to the uniform distribution of the API during the technological process and the easy application of the ointment to the skin.
The study of textural properties of the experimental ointment samples (cohesion, adhesion, and elasticity) also confirmed the satisfactory spreadability of the sample. The optimal container for its consistent properties was chosen according to the results of the ointment's structural, mechanical and textural properties.
A test of the effectiveness of antimicrobial preservatives was conducted, resulting in the minimum effective concentration of sodium benzoate in the amount of 1 % was substantiated. Furthermore, based on the results of a complex of studies, the composition of the emulsion ointment was developed, which includes vaseline (liquid paraffin) 20 %, emulsifiers glycerol monostearate 5.5 %, polysorbate-80 3.5 %, viscosity regulator of the aqueous phase hydroxyethyl cellulose 3 %, phytocomplex API "Phytol" (concentrated aqueous extract of burdock root: oak bark: pot marigold flower in the ratio 5:1:1.5, respectively) and essential oils of tea tree 1 % and geranium 1.5 %, purified water.
Conclusions. *Based on organoleptic, physicochemical, rheological and microbiological studies, the composition of the ointment of complex action was developed based on active pharmaceutical ingredients of natural origin for the treatment of cheilitis of various etiologies.*
Keywords: *ointment base, emulsion ointment, composition, multicomponent phytoextract, tea tree essential oil, geranium essential oil*

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1. Introduction

Inflammatory lesions of the mucous membrane of the mouth, including inflammation of the red border, mucous membrane, and skin of the lips and tongue, were combined under the name cheilitis (from the Greek *cheila* – lip). Cheilites significantly reduce the quality of all areas of the patient's life. Conventionally, these lesions are divided into actual cheilitis and symptomatic cheilitis, the occurrence of which is characterised as a symptomatic manifestation of other skin diseases, for example, atopic or contact dermatitis, eczema, allergic reaction to solar radiation, etc.

Clinical manifestations of cheilitis are swelling with redness and peeling of skin tissues, acute and chronic inflammation of a catarrhal, ulcerative, erosive nature, determined by the type of irritant, intensity and time of its exposure, and immunological reactivity of the body [1].

Despite the significant prevalence, cheilitis, as a pathology of the oral cavity, is considered insufficiently studied to this day, as evidenced by the clinical and epidemiological studies of some authors, who established the frequency of their occurrence in a fairly wide range (from 6.8 to 30 %). Meteorological (44 %) and exfoliative cheilitis (40 %) are the most common, and chronic cracked lips (12 %) and eczematous cheilitis (4 %) are less common [1–3].

The tactics of cheilitis treatment, which depends on the causes of the lesion, in addition to etiotropic treatment, immunocorrection and diet therapy, also include local therapy, which, according to a number of authors, has a leading place in the modern treatment of this pathology [2, 3].

The purpose of local therapy is to eliminate inflammation, local moisturizing, emollient, antibacterial, anti-

mycotic and reparative action, and restore the hydrolipid mantle of the skin. Among local remedies, ointments, creams, and gels have become widespread, the main components of which are corticosteroids, antibiotics, keratolytic agents, antihistamines, and tissue regeneration stimulators [3–5].

Treatment of cheilitis with existing topical means is quite long and often ineffective, which makes it urgent to develop new means of treatment for this pathology with the use of natural drugs, which are not inferior to synthetic drugs in terms of effectiveness, are suitable for long-term use, and have significantly fewer side effects.

The aim of the work is to develop the composition of a complex-acting ointment based on active pharmaceutical ingredients of natural origin for the treatment of cheilitis of various etiologies.

2. Planning (methodology) of the research

The pharmaceutical market of topical soft drugs for the treatment of cheilitis is mostly represented by emollients and protective agents based on zinc oxide, a mixture of hydrocarbons and fats, urea preparations, salicylic acid, glycerin, etc. Other drugs that promote the healing of skin lesions include dexpanthenol, methyluracil, zinc hyaluronate, and proteolytic enzymes. The range of medicinal products based on natural raw materials is insufficiently presented, more often they contain propolis, the extraction of biologically active substances from Kalanchoe, pot marigold, comfrey [6, 7]. However, in the symptomatic treatment of cheilitis, it is most often recommended to use multicomponent medicinal products that have a complex effect: they stimulate tissue regeneration and epithelization processes, have anti-edematous and anti-inflammatory effects. The extraction of biologically active substances (BAS) from the root of burdock root, the bark of the common oak, and the flowers of the medicinal marigold could be promising for the creation of such a phytocomplex [8, 9].

It is known that burdock root extract reduces the development of inflammatory processes, restrains the processes of lipid peroxidation, normalizes the antioxidant-prooxidant balance in the body, eliminating manifestations of the inflammatory process. Due to the high content of tannins, the extract of common oak bark has an anti-inflammatory, antimicrobial, astringent effect, thanks to the latter, it eliminates the contamination of pathogenic bacteria in the affected area, preventing the occurrence of secondary infection [8, 9].

The extract of calendulas has an anti-inflammatory, wound-healing, bactericidal effect, accelerates the processes of tissue regeneration and epithelization due to the presence of flavonoids, saponins, resinous and tannic substances, inulin in its chemical composition [10].

Essential oils of tea tree and geranium have antiseptic, antioxidant, reparative properties, and also improve the consumer properties of the composition by correcting the smell [11, 12].

Thus, the combination of active pharmaceutical ingredients (APIs) from plant raw materials in the form of multicomponent extract “Phytol” and essential oils made it possible to create a powerful phytocomplex, where the components would potentiate the therapeutic effect of each other in the treatment of cheilitis, and the risk of side effects would be negligible. The concentration of the components of the phytocomplex was selected based on literature data followed by experimental microbiological studies. To substantiate the quantitative content of plant components in the composition of phytocomplexes, the antimicrobial activity of samples with different ratios of MPRM was studied. The composition of model samples of the phytocomplex of burdock root was introduced in the amount of 5 parts, and other components – up to 2 parts. A sample containing a 1:1:1 ratio of components served as a comparison. The results of the experiment showed that all samples of the phytocomplex containing 5 parts of burdock roots have higher indicators of antimicrobial activity. Increasing the content of oak bark to 1.5 and 2 parts did not lead to an increase in antimicrobial activity compared to the sample containing 1 part extract. An increase in the content of medicinal flower buds to 1.5 parts led to an increase in antimicrobial activity, and an increase to 2 parts did not lead to an increase in antimicrobial activity.

Thus, according to the results of this series of studies on the determination of antibacterial and antifungal activity of phytocomplex samples, the experimental sample with a ratio of components in the composition of the multicomponent extract – large burdock root:oak bark:pot marigold flower buds is considered the best, 5:1:1.5, respectively.

The concentration of phytocomplex in the composition of the ointment was determined by its antimicrobial effect by the diffusion method in agar in the range from 5 to 10 % with a step of 0.5. Among those studied, they stopped at the working concentration of 7.5 %, which showed the highest values of antimicrobial activity among concentrations from 5 % to 7.5 %, and concentrations above 7.5 % did not show a significant increase in antimicrobial properties.

The choice of the base of the ointment is based on medical and biological requirements (condition of the skin and mucous membrane, the course of the pathology), as well as pharmaceutical aspects of creating ointments (physicochemical properties of active pharmaceutical ingredients and auxiliary substances) [13–18].

The analysis of available information sources made it possible to choose classic ointment bases for the experiment: water/oil emulsion and absorption bases [13, 15, 16].

Therefore, the design of our research consisted in the planning of experimental studies with the aim of developing the optimal composition of the ointment based on active pharmaceutical ingredients of natural origin for the treatment of cheilitis of various etiologies. Elaboration of the composition of the ointment began with the substantiation of the base-carrier, which should contribute to ensuring a more complete achievement of the therapeutic effect. The main goal when choosing the base was to create a hydrophobic protective film on the surface of the skin, which would prevent damage and cracking of the granulation tissue,

soften the affected area and ensure a comfortable and painless application.

The research design will include the study of structural-mechanical and textural properties of experimental ointment samples (cohesion, adhesion and elasticity), the study of the antimicrobial activity of experimental samples, as well as the testing of the effectiveness of antimicrobial preservatives in order to justify their minimum effective concentration.

3. Materials and methods

The composition of the ointment was developed using petroleum jelly (liquid paraffin), anhydrous lanolin, Span 60 emulsifiers, glycerol monostearate, cetyl stearyl alcohol, polysorbate-80, Lanette sx, a viscosity regulator of the aqueous phase – hydroxyethyl cellulose.

Phytocomplex "Phytol" and essential oil of tea tree and essential oil of geranium were used as APIs in order to enhance the antimicrobial effect (they have antiseptic, antioxidant, reparative properties, and also improve the consumer properties of the composition by correcting the smell) [11, 12], who chose a total of 10 %, which is a classic approach in the manufacture of extemporaneous drugs in pharmacy practice.

Phytocomplex "Phytol" is an aqueous thick extract of medicinal plant raw materials (burdock root, common oak bark, pot marigold flower buds in a ratio of 5:1:1.5, respectively), obtained by us in laboratory conditions by the method of three-stage remaceration with a total DER value of 1:10 according to extraction temperature of 95–100 °C and condensed in a water bath to residual moisture of no more than 25 %.

Elaboration of the composition of the ointment began with the substantiation of the optimal composition of the base-carrier, which should contribute to ensuring a more complete achievement of the therapeutic effect. The main goal when choosing the base was to create a hydrophobic protective film on the surface of the skin, which would prevent damage and cracking of the granulation tissue, soften the lesion, and ensure comfortable and painless application [17, 18].

Experimental samples were made, one of the components of which was medical petroleum jelly, which is poorly absorbed by the skin and therefore has emollient properties. Vaseline is stable to oxidation, able to meet the relevant medical and biological requirements, and will not cause allergic and sensitizing manifestations after application [19]. Due to its properties, petroleum jelly is a traditional component of ointment bases, emollient creams for local use, and non-stick gauze medical bandages containing APIs. It is widely used in pharmacy technology.

Since the composition of the ointment must include both a hydrophilic component – phytocomplex "Phytol" and hydrophobic ones (essential oil of geranium, essential oil of tea tree), absorbent bases were prepared with the classic ratio of lanolin and vaseline in pharmaceutical drug technology. A number of emulsifiers capable of ensuring the stability of the fat base of the ointment and increasing the absorption of API from it were also used [20, 21] (Table 1).

Table 1

Experimental samples of ointments on absorption bases

Component	Sample/content, %						
	1	2	4	5	6	7	8
Vaseline	54.0	72.0	81.0	81.0	81.0	81.0	81.0
Anhydrous lanolin	36.0	18.0	9.0	–	–	–	–
Span 60	–	–	–	4.5	–	–	–
Glycerol monostearate	–	–	–	4.5	4.5	–	–
Cetylstearyl alcohol	–	–	–	–	4.5	4.5	9.0
Lanette sx	–	–	–	–	–	4.5	–
Phytocomplex «Phytol»	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Tea tree essential oil	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Geranium essential oil	1.5	1.5	1.5	1.5	1.5	1.5	1.5

Experimental samples of emulsion bases were made using a laboratory homogenizer Daihan Homogenizer with Direct Controller HG-15A (Daihan Scientific, Korea). When preparing samples with glycerol monostearate and the Lanette sx emulsifier, the emulsifier was melted in a porcelain cup in a water bath, petroleum jelly, phytocomplex "Phytol" were added, mixed and homogenized until homogenous at a speed of 40 rpm.

To choose the optimal basis of the ointment, samples were also prepared on emulsion bases, which have a lower viscosity than absorption ones, are easily applied and washed off from the surface of the skin, are more convenient to use, which is important in the presence of pain in cheilitis [18, 22].

The number of emulsifiers was varied in the composition of the samples, and hydroxyethyl cellulose (HEC) was used as a viscosity regulator of the aqueous phase (Table 2).

Table 2

Experimental samples of emulsion-based ointments

Component	Sample/content, %						
	9	10	11	12	13	14	15
Vaseline	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Polysorbate-80	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Glycerol monostearate	3.0	3.5	4.0	4.5	5.0	5.5	6.0
Hydroxyethyl cellulose	–	–	–	1	2.0	3.0	4.0
Phytocomplex «Phytol»	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Tea tree essential oil	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Geranium essential oil	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Purified water	up to 100.0						

Experimental samples were prepared according to the rules to produce emulsion systems, considering the melting point of the components: emulsifiers were melted in a porcelain cup No. 1 in a water bath, petroleum jelly was added, and mixed until homogeneous. Sodium benzoate, phytoextract was added to the required amount of purified water in porcelain cup No. 2, mixed with the calculated amount of hydroxyethyl cellulose and infused at room temperature for about 2 hours, stirred until it was completely dissolved. Mixtures No. 1 and 2 were transferred to a container of a homogenizer heated to 50 °C, and essential oils were added and homogenized at 30 rpm until a homogeneous mixture was obtained. After cool-

ing the mixture to 35–40 °C, essential oils of tea tree and geranium were added, and mixed until homogeneity.

Experimental samples of ointments on absorption and emulsion bases were subjected to organoleptic and physicochemical examination in accordance with the recommendations and methods given in SPhU 2.0, section "Soft medicinal products for dermal application" [14, 23].

One of the therapeutic effects, thanks to which recovery from cheilitis occurs, is antibacterial. Therefore, the first stage of the work was the study of the antimicrobial activity of the investigated ointment samples on absorption and emulsion bases. Antimicrobial activity of experimental samples was studied in vitro by the method of diffusion in agar in the modification of "wells" [14, 23, 24].

The following pure cultures were used as test microorganisms: gram-positive – *Staphylococcus aureus* ATCC 6538, gram-negative – *Escherichia coli* ATCC 25922, spore – *Bacillus subtilis* ATCC 6633, yeast-like fungus *Candida albicans* ATCC 885-653 [14, 23]. Soy-casein agar and Sabouraud-dextrose agar were used as nutrient media, and a buffer solution with sodium chloride and peptone pH=7.0 was used as a solvent. During the experiments, one-day suspensions of bacteria and two-day culture of a yeast-like fungus were used. The microbial load was 1×10^7 CFU/ml (colony-forming units in 1 ml of medium).

Determination of activity was carried out on two layers of dense nutrient medium poured into Petri dishes. 10 ml of melted "starved" non-inoculated medium was added to Petri dishes. After solidification of the lower layer of agar, 3–6 thin-walled stainless steel cylinders (diameter – 8 mm, height – 10 mm) were placed on its surface at an equal distance from each other and from the edge of the cup. The upper layer consisting of melted and cooled to 45 °C agar was poured around the cylinders, into which the seed dose of the daily culture of the microorganism was introduced. After solidification of the upper layer of the medium, the cylinders were removed with sterile tweezers, and the same dose of the studied samples was introduced into the formation of the wells, considering the volume of the wells. Petri dishes were kept for 30–40 minutes at room temperature and placed in a thermostat for 18–24 hours.

The results were calculated by measuring the zone of inhibition of the growth of microorganisms, including the diameter of the holes. Measurements were carried out with an accuracy of 1 mm, while focusing on the complete absence of visible growth. The absence of zones of inhibition of the growth of test cultures around the wells and the retention zone with a diameter of up to 10 mm indicates the insensitivity of microorganisms to the drug; zones of growth retardation of test cultures with a diameter of 10−15 mm indicate a low sensitivity of the culture; a zone with a diameter of 15−25 mm is estimated as an indicator of the sensitivity of the microorganism to the drug; and zones above 25 mm – high sensitivity of microorganisms to drug samples.

Control of dispersion of emulsions was carried out by the method of microscopy [23]. The research was carried out using a triocular digital usb microscope "NIKON DS-Fi1" (Japan) with a built-in camera (objective 40 X/0.65 160/0.17; eyepiece WD 0.56) with 100x magnification. The diameter of the dispersed particles was determined by measuring in the NIS-Elements L program. Comfrey ointment (Dr. Theiss, Germany) was used as a comparison drug.

The study of the structural and mechanical properties of the experimental samples was carried out on an Alpha series rotary viscometer (Fungilab, Spain) with an L4 spindle in the range of shear rates of 1.5–100 rpm at room temperature [25–27].

The study of textural properties of the samples was carried out at the Department of Drug Technology and Social Pharmacy of the Lithuanian University of Health Sciences (Kaunas, Lithuania) using a TA.XT Plus texture analyzer (Stable Micro Systems Ltd., Surrey, Great Britain). Textural properties of experimental ointment samples (cohesion, adhesion, and elasticity) were determined by spreading and reverse extrusion tests. Spreadability studies were carried out in compliance with the following parameters: the speed of movement of the piston with a disk – 3 mm/s; the distance (the depth of the disc insertion into the sample) is 20 mm. Setting the reverse extrusion parameters was carried out at a speed of movement of the piston with a disk of 2 mm/s and a depth of the disk embedding into the sample of 10 mm.

4. Research results

The results of the study of the antimicrobial activity of the experimental samples are shown in the Table 3.

Table 3

Results of antimicrobial activity of samples (n=5, P=95 %)

Sample	Cultures of microorganisms			
	S. aureus ATCC 25923	*E. coli* ATCC 25922	*B. subtilis* ATCC 6633	*C. albicans* ATCC 885-653
	Diameters of the growth retardation zone of microorganisms, mm			
1–8	–	–	–	–
9	21.0±0.3	13.4±0.5	–	18.2±0.5
10	21.3±0.4	13.4±0.5	–	18.2±0.3
11	21.3±0.3	13.8±0.3	–	18.7±0.5
12	22.8±0.1	16.7±0.2	11.1±0.2	19.4±0.1
13	23.0±0.5	16.8±0.2	11.1±0.1	19.5±0.1
14	24.2±0.3	17.2±0.5	12.0±0.4	20.2±0.3
15	24.3±0.1	17.5±0.7	12.3±0.2	20.0±0.2

Note: "–" – absence of growth retardation zones of microorganisms around the well or retardation zone with a diameter of up to 10 mm

Table 3 results showed that the experimental samples of ointment on absorbent bases No. 1–8 did not show antimicrobial activity against the tested test strains – the zones of inhibition of the growth of microorganisms around the wells were absent or were less than 10 mm. Samples of emulsion–based ointment No. 9–15 showed low or medium antimicrobial activity against cultures of *S. aureus* (diameter of growth retardation zones is 20–25 mm), *E. coli* (diameter of growth retardation zones

is 13–18 mm), *C. albicans* (diameter of zones of growth retardation is 17–21 mm). Moreover, for samples No. 12–15 with HPC content from 1 % to 4 %, the antimicrobial effect was slightly higher with the maximum zones of growth retardation in this study for samples No. 14, 15. In relation to the culture of *B. subtilis*, samples of emulsion–based ointment No. 9–11 did not show antimicrobial activity against the tested test strains (the zones of inhibition of the growth of microorganisms around the wells were less than 10 mm), and No. 12–15 – showed a low antimicrobial activity (zones microorganism growth delays were in the range of 10–13 mm).

Thus, studies of the antimicrobial activity of experimental samples of ointments on absorption and emulsion bases made it possible to choose a water / oil type emulsion ointment for further work, among which the best in this series of studies were the samples with HPC content of 3 and 4 %.

The results of organoleptic and physicochemical control of experimental ointment samples are shown in Table 4.

It was found that samples No. 9–12 passed the thermal stability test, but did not pass the colloidal stability test and delaminated, so we did not use them in further studies.

Therefore, in further studies we used samples of ointments No. 13–15.

An important parameter of emulsion technology is their dispersion and fractional composition, as these characteristics affect both viscosity characteristics and stability. It is known that the optimal particle size of emulsion ointments is 1–2 μm [23]. Photomicrographs of experimental ointment samples are shown in Fig. 1.

Microscopic examination of experimental ointment samples showed a uniform distribution of irregularly shaped particles. For further research, sample No. 14 was chosen, which showed the most uniform distribution of particles of the dispersed phase in a continuous medium and its identical fractional composition.

Studying the rheological properties of soft drugs is an important segment of technological research, which allows you to establish the optimal composition and predict a number of characteristics related to their flow: extrusion, spreading, uniformity of distribution on the surface of the skin, and even the flow rate of the finished product during production.

In the studies, comfrey ointment Dr. Theiss (Germany) was used as a comparison drug, one of the indications for its use is dryness, skin cracks, wounds and ulcers that do not heal for a long time [7]. The ointment also has similar organoleptic properties.

The results of the study of the structural and mechanical properties of the experimental samples are shown in the graph of the dependence of viscosity on the shear rate gradient (Fig. 2).

As the results of the research showed (Fig. 2), with an increase in the shear rate, thinning of the ointment is observed (from the structural viscosity value of 25768 at D_r 0.025, the indicator decreases almost 13 times), which indicates the possibility of the same thinning at the homogenization stage, which will contribute to uniform distribution of API in the base, and will also ensure the ease of applying the product to the affected area of the skin of the lips. The obtained results of the study also make it possible to predict the provision of high-quality transportation of the ointment after production by the reactor-packaging apparatus.

It should be noted that the indicators of the structural viscosity of the ointment and its base are significantly different (58717 and 25768 mPa·s at D_r 0.025), which indicates a significant effect of API on the rheological properties of the complex. However, as shown in Fig. 2, the dynamics of rarefaction of the base and ointment have a certain similarity, and the final result of the rarefaction curves of both samples reach almost the same values (about 2000 mPa·s at D_r 1.667). A similar pattern is observed in the comparison drug.

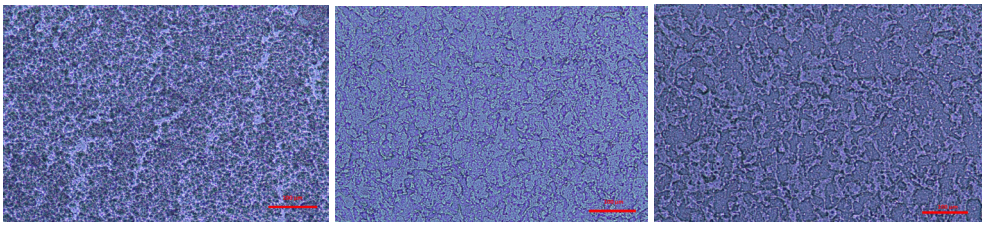
The study of textural (Fig. 3) properties of experimental ointment samples (cohesion, adhesion and elasticity) allows us to predict the consumer properties of the ointment (ease and convenience of application, penetration depth) and to choose the optimal container for the consistent properties of the ointment.

The results of studies on the spreading of experimental samples of the ointment, the base carrier and the comparison drug (Fig. 3) showed reliably similar indicators of the force required to destroy (displace) the system during application, and

Table 4

Results of research on the properties of experimental ointment samples

Indicators	Sample number						
	9	10	11	12	13	14	15
Organoleptic properties	A thick homogeneous ointment-like mass of beige colour with a characteristic smell of essential oils						
Emulsion type	Water/Oil						
Thermal stability	Stable	Stable	Stable	Stable	Stable	Stable	Stable
Colloidal stability	Not stable	Not stable	Not stable	Not stable	Stable	Stable	Stable
pH	5.08±0.04	5.09±0.04	5.03±0.05	5.01±0.04	5.08±0.03	5.09±0.04	5.09±0.03



Sample No. 13 Sample No. 14 Sample No. 15

Fig. 1. Microphotographs of experimental ointment samples

the consistency indicators indicate a rather dense structure of the ointment base (Table 5). However, compared to the base, the ointment sample has a slightly thinner consistency and is more easily deformed, which confirms the effect of the API complex on the texture characteristics of the ointment. The obtained data allow us to assume that the ointment will keep a uniform layer on the surface of the skin, and make it possible to choose a container for packaging that is suitable for the consistency – a wide-mouthed container, since at room temperature the extrusion capacity is quite low and makes it impractical to package the ointment in aluminium tubes.

Table 5

The results of the study of textural properties of experimental samples (n=5)

Sample	The studied parameters			
	Elasticity, g	Cohesion, g	Viscosity index, g/s	Consistency, g/s
Ointment	231.75± ±6.01	–292.51± ±3.93	–258.76± ±3.56	1499.58± ±11.82
Base	581.53± ±9.72	–630.26± ±11.03	–437.81± ±32.14	3719.58± ±14.86
Dr. Theiss ointment	1425.79± ±9.23	–1449.71± ±4.20	–638.67± ±40.13	9498.00± ±4.62

The results of the reverse extrusion measurement shown in Fig. 4, showed the deformation of the ointment with its reverse extrusion. It was established that the experimental samples of the ointment, its base and the reference preparation were characterized by similar thixotropic properties.

To study the effectiveness of the preservative in the selected ointment sample, series were developed using sodium benzoate preservative in concentrations of 0.1 %, 0.15 % and 0.2 %. At the beginning of the study, the sterility of the nutrient media, the solvent, the growth properties of the nutrient media, and the suitability of the determination method were checked. The nutrient medium met the growth properties and passed the sterility test in accordance with the requirements of SPhU 2.0, clause 2.6.12., and the test microorganisms met the taxonomic characteristics – the morphology of the colonies on the medium and the morphology of the cells under microscopy were typical for the respective strain. Also, the conducted test showed that the method of surface seeding with the dilution of samples using a typical neutralizing solvent meets the acceptance criteria and can be used in this case when testing the effectiveness of antimicrobial drugs. The results of the evaluation of the effectiveness of the selected preservative are shown in Table 6.

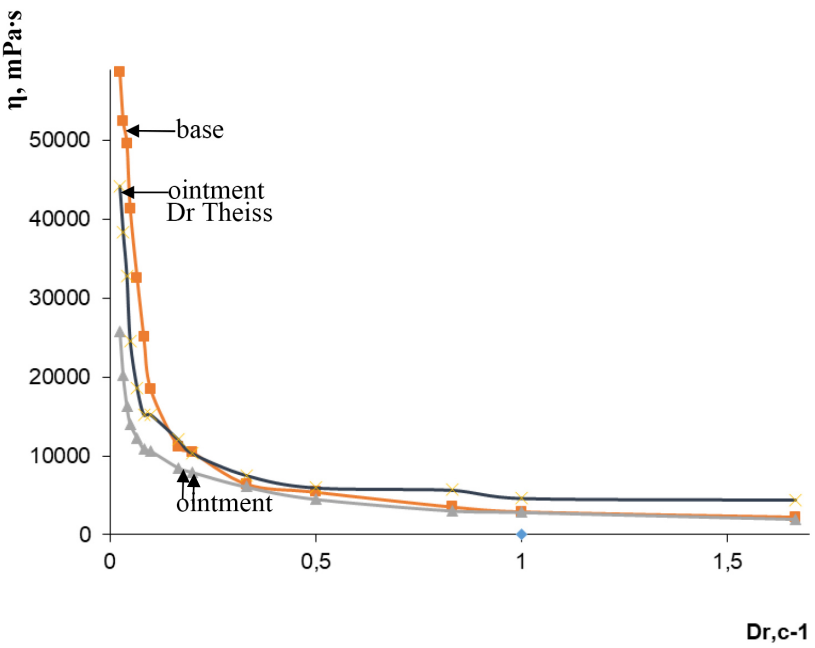


Fig. 2. Graph of the dependence of the structural viscosity of the experimental ointment sample (ŋ, mPa·s), the base and the comparison drug on the shear rate gradient (D_r, 1/s)

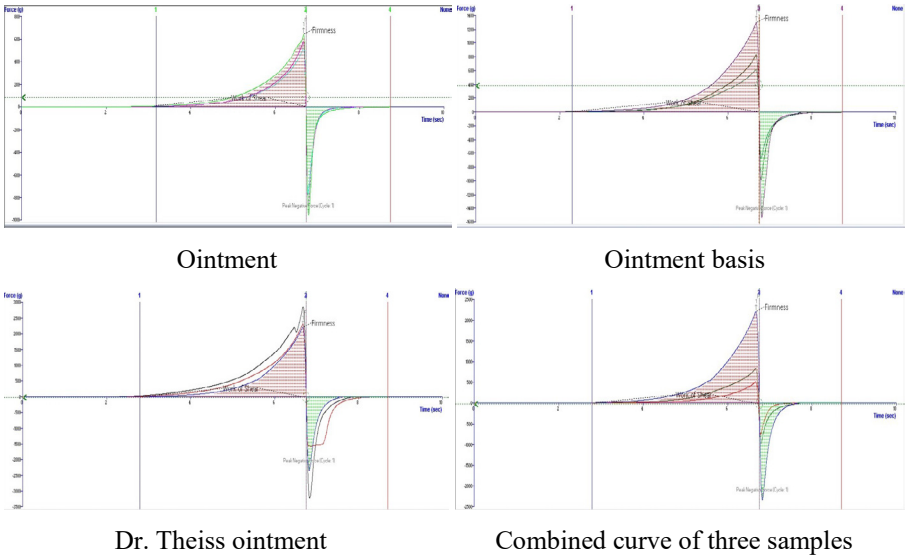


Fig. 3. Force/time curves of the samples obtained using the test TTC Spreadability Rig (HDP/SR)

The results shown in Table 6, indicate that after 2 days of storage of inoculated samples with preservative *lg*, the decrease in the number of viable bacteria was more than 2 and was at a concentration of 0.1 % for *S. aureus* 2.93, for *Ps. aeruginosa* – 2.74; at a concentration of 0.15 % for *S. aureus* was equal to 2.95, for *Ps. aeruginosa* – 2.79; at a concentration of 0.2 % for *S. aureus* was 3.05, for *Ps. aeruginosa* – 2.87.

After 7 days of storage of inoculated samples with preservative lg, the decrease in the number of viable bac-

teria was more than 3 and amounted to 3.34 for *S. aureus* at a concentration of 0.1 %, for *Ps. aeruginosa* – 3.70; at a concentration of 0.15 % for *S. aureus* was equal to 3.35, for *Ps. aeruginosa* – 3.78; at a concentration of 0.2 % for *S. aureus* was 3.42, for *Ps. aeruginosa* – 3.78.

For the cells of *C. albicans* fungi on the 14th day of *lg*, the decrease in the number of viable cells in samples with a preservative of 0.1 % was 2.79 (according to the requirements of at least 2), from 0.15 % – 2.78, from 2 % – 2, 91. For culture *As. brasiliensis* on the 14th day with a preservative of 1 % lg the decrease was 3.8, with 1.5 % – not registered, with 2 % – 3.89. On the 28th day, viable cells of bacteria and fungi were not detected in the samples with the preservative of the selected concentrations.

5. Discussion of research results

Microbiological studies have established that absorbent bases do not provide sufficient release of API, while emulsion-based samples have proven a satisfactory release process and antimicrobial effect of the phytocomplex included in the composition. The release process was investigated and the antimicrobial effect of experimental samples of ointment with API: multicomponent extract "Phytol", tea tree essential oil, geranium essential oil against *S. aureus* ATCC 25923, antifungal properties against *C. albicans* ATCC 885-653 was proven.

The processed experimental samples of the ointment are thick, uniform, beige-coloured ointment-like masses with a characteristic smell of essential oils. They pass tests for thermal stability and colloidal stability; the pH value is in the range of 5.08–5.09.

The study of dispersological characteristics of the proposed composition of ointments made it possible to choose for further experimentation the sample of ointment No. 14, which showed the most uniform distribution of particles of the dispersed phase in a continuous medium and its identical fractional composition.

Studies of the structural and mechanical properties of the experimental samples of the ointment showed practically the same dynamics of dilution of the base and ointment with an increase in the gradient of the shear rate, which allows us to assume a uniform distribution of the API during the technological process and the ability of the ointment to be easily applied to the skin.

The study of textural properties of the ointment made it possible to establish the parameters of cohesion –292.51±3.93 g, elasticity 231.75±6.01 g, consistency 1499.58±11.82 g/s and viscosity index –258.76±3.56 g/s, which allow predicting the consumer properties of the ointment (lightness and convenience when applying) and choosing the optimal container for the consistent properties of the ointment – a wide-mouthed container.

As a result of the study of the effectiveness of the preservative, sodium benzoate was chosen in the minimum effec-

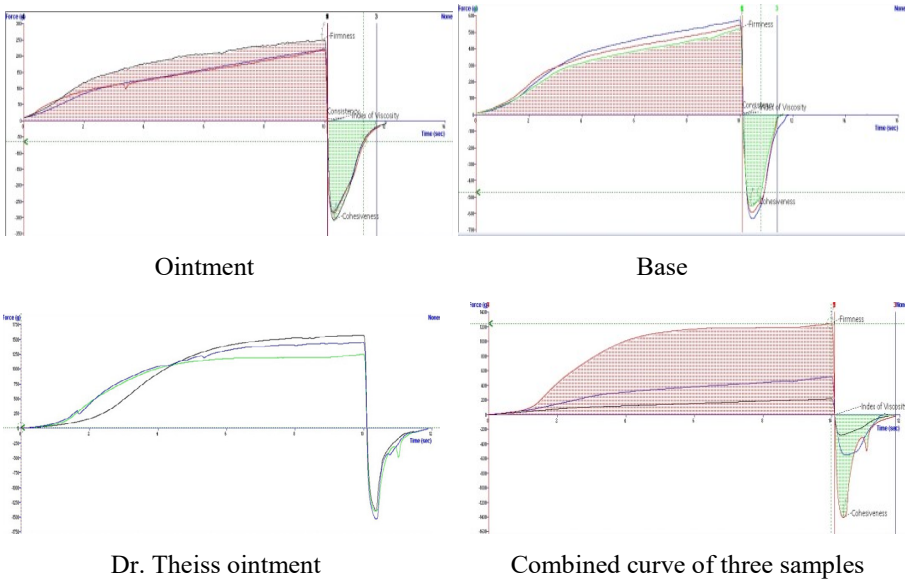


Fig. 4. Force/time curves of samples obtained using the test Back Extrusion Cell (A/BE)

Table 6

Results of antimicrobial preservative effectiveness in experimental samples

Concentration of sodium benzoate in the sample, %	lg number of viable microorganisms immediately after inoculation, lg CFU/ml	*lg* decrease in the number of viable microorganisms, lg CFU/ml (requirements of SPhU 2.3/obtained results)			
		2 days	7 days	14 days	28 days
Staphylococcus aureus ATCC 6538					
0.1	5.40	2/2.96	3/3.34	–	NI/NV
0.15	5.38	2/2.92	3/3.35	–	NI/NV
0.2	5.40	2/3.05	3/3.42	–	NI/NV
Pseudomonas aeruginosa					
0.1	5.44	2/2.74	3/3.70	–	NI/NV
0.15	5.48	2/2.79	3/3.78	–	NI/NV
0.2	5.50	2/2.87	3/3.78	–	NI/NV
Candida albicans ATCC 10231					
0.1	5.57	–	–	2/2.79	NI/NV
0.15	5.57	–	–	2/2.78	NI/NV
0.2	5.59	–	–	2/2.91	NI/NV
Aspergillus brasiliensis ATCC 16404					
0.1	5.40	–	–	2/3.80	NI/NV
0.15	5.42	–	–	NV	NI/NV
0.2	5.39	–	–	2/3.89	NI/NV

Note: NI – there is no increase in the number of microorganisms compared to the number of viable microorganisms at the previous control point; NV – no viable cells of microorganisms were detected in the experiment

tive concentration of 1 % for the experimental samples of the ointment.

Study limitations. Considering the multicomponent nature of the phytocomplex, the contribution to the overall result of each active pharmaceutical ingredient of the developed ointment remains unexplored, which is of interest for further work.

Prospects for further research consist in the development of methods for the identification and quantitative determination of API, the study of the stability of the developed emulsion ointment.

6. Conclusions

Organoleptic, physico-chemical, structural-mechanical, microbiological properties of experimental ointment samples were studied. Microscopic examination of ointment samples showed a uniform distribution of dispersed phase particles in a continuous medium.

Studies of the antimicrobial activity of experimental samples have established that the optimal basis of the ointment for the treatment of cheilitis is a water/oil emulsion, which provides better indicators of API release compared to absorption bases.

According to the results of structural and mechanical studies, it was established that the introduction of the API complex reduces the structural viscosity of the ointment base. The ability of the ointment to thin out with an increase in the gradient of the shear rate is shown, which will contribute to the uniform distribution of the API during the technological process and easy application of the ointment to the skin.

The study of textural properties of the experimental ointment samples (cohesion, adhesion and elasticity) also confirmed the satisfactory spreadability of the sample. The structural-mechanical and textural properties of the ointment made it possible to choose the optimal container for its consistent properties.

According to the results of the research, the composition of the emulsion ointment for the treatment of cheilitis was developed, which includes petroleum jelly (liquid paraffin) 20 %, emulsifiers glycerol monostearate 5.5 %, polysorbate 80 3.5 %, viscosity regulator of the aqueous phase hydroxyethyl cellulose 3 %, phytocomplex of AFI "Fitol" and essential oils of tea tree 1 % and geranium 1.5 %, purified water.

A test of the effectiveness of antimicrobial preservatives was conducted, because of which the minimum effective concentration of sodium benzoate in the amount of 1 % was substantiated.

Conflict of interests

The authors declare that they have no conflict of interests in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results presented in this article.

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Data availability

The manuscript has no associated data.

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