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Composition and technology development of ear drops with cerumenolytic action (based on thick *Viscum album* L. leaves extract)

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Earwaxes lead to a decrease in the quality of life. A rational and effective way to eliminate earwaxes without a participation of medical personnel is to use ear drops and solutions to dissolve sulfur masses. In this regard, therapeutic and hygienic drops of a cerumenolytic action are of a particular relevance in the pharmaceutical market.

The aim of the work was to develop a composition and technology of ear drops of a cerumenolytic action based on thick *Viscum Album* L. leaves extract.

Materials and methods. The objects of the study were thick *Viscum Album* L. leaf extract, chitosan, sodium alginate, polyethylene oxide of various degrees of polymerization, propylene glycol, sodium hyaluronate, preservatives (benzalkonium chloride, nipagine and nipazole). At the screening stage, 9 experimental formulations were proposed. The cerumenolytic activity of the developed formulations was evaluated in a dissolution test of reproduced artificial earwax in comparison with a 3% hydrogen peroxide solution, TEA-cocoyl hydrolyzed collagen (A-Cerumen Plus, Gilbert Laboratories, France) and a 0.9% sodium chloride solution. Physical and chemical parameters (a degree of liquid coloring, turbidity and transparency, pH, density and viscosity) were determined according to of the State Pharmacopoeia of the Russian Federation (XV ed.). The microbiological study was performed using the agar diffusion method.

Results. In the course of the study, it was shown that the composition of ear drops of a cerumenolytic action based on thick *Viscum Album* L. leaves extract, exceeds the level of a lipolytic, proteolytic and general cerumenolytic kinds of activity of the compositions containing surfactants; in its effectiveness, it was comparable with the comparison drug – A-Cerumen drops. To obtain the optimal rate of the onset effect and its duration, it is advisable to use sodium hyaluronate in the amount of 0.2 g per 25 ml of drops as an adjuvant. The most preferred preservative was benzalkonium chloride. The developed ear drops met the requirements of the State Pharmacopoeia of the Russian Federation (XV ed.) for this dosage form, while the pH was 5.86± 0.1, and the viscosity was 4.2676±0.2 MPa×s.

Conclusion. The conducted research has shown the prospects for further work on the development and implementation of ear drops of a cerumenolytic action in practice. The recommended composition is the following: thick *Viscum Album* L. leaf extract, sodium hyaluronate, benzalkonium chloride, purified water.

Keywords: *Viscum Album* L. leaves; thick extract; ear drops; cerumenolytic effect; sulfur plugs earwaxes

Abbreviations: SP – State Pharmacopoeia; RF – Russian Federation; SRMR – State Register of Medicinal Remedies; IZ – inhibition zone; APS – active pharmaceutical substance.

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Разработка состава и технологии капель ушных церуменолитического действия на основе омелы белой листьев экстракта густого

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Ушные серные пробки приводят к снижению качества жизни человека. Рациональным и эффективным способом устранения пробок без участия медицинского персонала является использование ушных капель и растворов для растворения серных масс. В этой связи особую актуальность на фармацевтическом рынке имеют лечебно-гигиенические капли церуменолитического действия.

Цель. Разработка состава и технологии капель ушных церуменолитического действия на основе омелы белой листьев экстракта густого.

Материалы и методы. Объектами исследования были омелы белой листьев экстракт густой, хитозан, альгинат натрия, полиэтиленоксид разной степени полимеризации, пропиленгликоль, гиалуронат натрия, консерванты (бензалкония хлорид, нипагин и нипазол). На этапе скрининга было предложено 9 экспериментальных составов. Церуменолитическую активность разработанных составов оценивали в тесте растворения воспроизведенной искусственной ушной серы в сравнении с раствором перекиси водорода 3%, препаратом ТЕА-кокоилгидролизованого коллагена (А-Церумен Плюс, Лаборатории Жильбер, Франция) и 0,9% раствором натрия хлорида. Физико-химические показатели (степень окраски жидкости, мутности и прозрачности, водородный показатель, плотность и вязкость) определяли согласно ГФ РФ XV издания. Микробиологическое исследование выполнено по методу диффузии в агар.

Результаты. В ходе исследования было установлено, что состав капель ушных церуменолитического действия на основе омелы белой листьев экстракта густого превосходит по уровню липолитической, протеолитической и общей церуменолитической активности композиции, содержащие поверхностно-активные вещества, а также был сопоставим по эффективности с препаратом сравнения – каплями А-Церумен. Для получения оптимальной скорости наступления эффекта и его продолжительности в качестве вспомогательного вещества целесообразно использовать натрия гиалуронат в количестве 0,2 г на 25 мл капель. Наиболее предпочтительным консервантом являлся бензалкония хлорид. Разработанные ушные капли соответствовали требованиям, предъявляемым ГФ РФ XV издания к данной лекарственной форме, при этом pH составил 5,86±0,1, а вязкость 4,2676±0,2 мПа×с.

Заключение. Проведенное исследование показало перспективность дальнейших работ по разработке и внедрению в практику ушных капель церуменолитического действия состава: омелы белой листьев экстракт густой, гиалуронат натрия, бензалкония хлорид, вода очищенная.

Ключевые слова: листья омелы белой; экстракт густой; ушные капли; церуменолитическое действие; серные пробки

Список сокращений: ГФ – Государственная Фармакопея; РФ – Российская Федерация; ГРЛС – государственный реестр лекарственных средств; ЗЗР – зона задержки роста; АФС – активная фармацевтическая субстанция.

INTRODUCTION

One of the main factors leading to the development of the pharmaceutical market for eardrops is the growing trend of otorhinolaryngological diseases. As a result, many pharmaceutical companies are looking forward to developing new and improved formulations of eardrops that provide increased efficacy and safety. Such modern innovative developments include therapeutic–and hygienic medicines with a cerumenolytic action, designed to dissolve excess earwax. Earwax is a physiological body secret that can cause blockage of the ear canal for various reasons, often unrelated to hygiene. Hearing loss, dizziness, otalgia, and tympanic membrane infection are concomitant clinical manifestations that are caused by the presence of the earwax plugs, which can ultimately lead to a decreased quality of life and working capacity [1, 2].

Part of the ear's self-cleansing mechanism is earwax, which is naturally removed from the ear canal without

causing problems [3]. When this process is disrupted, sulfur masses (earwax plugs) are formed, which linger in the ear canal and can cause it to block up. In this case, medical attention may be required due to the patient's inability to remove the plug on one's own. Non-physiological accumulation of earwax contributes to the pathology of the ear canal; skin diseases in which the epidermis is renewed too quickly; increased cholesterol levels; irrational hygienic cleaning of the ear cavity with household objects (including matches); prolonged presence of a foreign body in the ear canal (hearing aid, in-ear earmuffs); professionally occupationally unfavorable factors (high dustiness of the workplace, increased temperature, humidity, atmospheric pressure); water sports (swimming, diving) [4, 5].

A rational and effective way to remove earwax plugs without the participation of medical personnel is the use of eardrops and solutions designed to lysis wax masses [6]. Eardrops have a number of advantages over other dosage forms, as they provide a local effect, which is important for drops that dissolve the earplug at the site of application: oil-based compounds (e.g., olive or almond oil); water-based compounds (e.g., sodium bicarbonate or a saline solution); a combination of the above compounds and solutions, as well as carbamide peroxide (a compound of hydrogen peroxide with urea) and glycerin [1].

Today, eardrops containing active pharmaceutical substances (APIs) that dissolve wax, such as choline salicylate, are a promising direction for elimination of wax plugs [7].

Analyzing the trade names, listed in the State Register of Medicinal Products (SRMPs) of the Russian Federation (RF), it can be concluded that the total number of eardrops is 25 trade names from 14 pharmaco-therapeutic groups.

The range of medicines for dissolving earplugs is presented in the form of liquid dosage forms: drops – 98% and solutions – 2% [8]. Thus, the development of new medicines of a cerumenolytic action can be considered an actual direction of modern medicine, and it is advisable to include various pharmacologically active compounds such as choline or choline-containing components, in the formulation of these drugs.

THE AIM of the work was to develop a composition and technology of cerumenolytic eardrops based on choline containing thick *Viscum Album* L. leaf extract.

MATERIALS AND METHODS

Study objects

The objects of the study were the leaves of *Viscum album* L. of the *Viscaceae* Batsch family of the *Santalales* Dumort. order, growing on a *Malus domestica* Borkh., collected in the territory of Tatarka village of the Shpakovsky district, Stavropol region (Russia), during the fruiting phase (16 June 2023). The analyzed plant raw materials were selected using the average sample method [9].

The choice of active components was based on the currently available cerumenolytic medicinal preparations of a cerumenolytic action. Compositions No. 1–8, 10 were obtained by dissolving the APIs in purified water. The choice of the compositions was based on the analysis of the medicinal preparations of a cerumenolytic action [8]. Thick *Viscum Album* L. leaves extract was chosen as an available source of choline [9, 10]. The studied compositions are presented in Table 1.

Thick extract production

Previously, it was found that the maximum choline content in extracts from the *V. album* leaves was observed during the extraction of raw materials with purified water [9]. Therefore, this extractant was used in further studies.

The analyzed extracts were obtained by an exhaustive extraction of raw materials with purified water in a flask with a reflux condenser on a boiling water bath ("Armed", Russia), for 60 min. The contents of the flask were filtered through a paper filter and the extraction was repeated twice by the above method. The filtrate was condensed in a vacuum rotary evaporator RE-52AA ("Biomer", Russia) to the state of a thick extract with the humidity of no more than 25% (production yield was 76.4%).

Modeling of earwax composition

To reproduce the earwax, the following composition (Table 2, selected according to the literature data [11].

Artificial earwax was obtained according to the following procedure: the amounts of epithelial cell suspension and fetal bovine serum indicated in Table 2, were weighed into a mortar, and dispersed with 5 drops of water to form a paste-like consistency. In a glass tube, the remaining components were dissolved in 5 ml of chloroform (Table 2) and added to the resulting paste. After the dispersion, it was incubated on a water bath ("Armed", Russia) at 37°C until the chloroform smell disappears. The reproduced sulfur (shelf life 7 days) was stored in the refrigerator for 24 h prior to the study [11].

Evaluation of cerumenolytic activity in vitro

A comparative study of the cerumenolytic activity of the analyzed samples was carried out by the *in vitro* method on the model of a reproduced earwax dissolution.

Before starting the experiment, the artificial earwax was heated to 35°C and the exact amounts (200 mg

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each) were measured, rolled by hand, and transferred into standard Wasserman centrifuge glass tubes. The test compositions in a volume of 1.3 mL were added to the same test tubes and incubated at the temperature of 24°C for 30 minutes (during the screening phase of the compositions) and after 0,5, 1, 5, 10, 20, 40 and 60 min (after selecting the most promising compositions). After that, the samples were transferred to clean test tubes, 3 ml of purified water was added and centrifuged at 3 500 rpm for 10 min (hereinafter referred to as CM-6M centrifuge, Elmi, Latvia). The optical density of the centrifugate was measured on an UV spectrophotometer SF-102 ("Aquilon", Russia) at 600 nm (lipolytic activity of the sample) and 280 nm (proteolytic activity of the sample) against purified water.

The cerumenolytic activity of the test samples was determined in lytic units according to the formula:

$$LU = \frac{A_s - A_c}{m_s},$$

где LU – Lytic Units; A_s – optical density of the test sample; A_c – optical density centrifuge; m_s – mass of the sample in grams.

The total cerumenolytic activity is an algebraic sum of the proteo- and lipolytic activities of the composition. The test for each sample was performed in repetitions [11].

Microbiological studies

Due to the need to meet the requirements for microbiological purity, the next stage of the work was to select the optimal preservative for the selected composition-leader of the active components of drops, and determine its concentration (Table 3). The antimicrobial activity evaluation of the studied samples of eardrops was carried out *in vitro* by an agar diffusion method. The determination of the sensitivity of microorganisms to the test compositions was carried out using the "wells" method [12].

The following strains were used in the work*:* *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 6538), *and Streptococcus pyogenes* (ATCC 19615). The test strains of microorganisms were provided free of charge by the staff of the Microbiology laboratory of Research Institute for Lepra Research in Astrakhan (Russia). Commercial reagent kits (nutrient media) were used for the microorganisms cultivation: nutrient broth for the cultivation of microorganisms dry (GRM-broth) produced by the State Research Center for Applied Microbiology and Biotechnology, Obolensk (State Research Center for Applied Microbiology and Biotechnology), RU No. FSR 2007/00002; nutrient medium for the isolation of staphylococci dry "Staphylococc agar" produced by the State Research Center for Applied Microbiology and Biotechnology, Obolensk. Scientific Center of Applied Microbiology and Biotechnology", Obolensk (State Scientific Center of Applied Microbiology and Biotechnology), RU No.FSR 2011/10007; dry trypton-soy agar produced by the State Scientific Center of Applied Microbiology and Biotechnology, Obolensk (State Scientific Center of Applied Microbiology and Biotechnology), RU No. RZN 2017/6544. The nutrient media had been prepared in accordance with the manufacturer's instructions.

The inoculum of test strains was prepared from a daily culture grown in the nutrient broth. The obtained cultures were centrifuged, washed with a saline solution, and the supernatant was taken. The dilution was prepared from the resulting precipitate according to the 0.5 McFarland turbidity scale (1.5×10^8 CFU/ml). Then, Petri dishes filled with the corresponding media of 20 ml per cup, were seeded by the "lawn" method with a swab soaked in a test culture solution, and dried in the thermostat for 30 min. A drill (d=6 mm) was used to drill holes ("wells") at a distance of 2.5 cm from the center of the Petri dish and at equal distances from each other, which were then filled with test objects in a volume of 1 ml. After that, the studied test Petri dishes were placed in a thermostat at 37°C for 18–24 h. At the end of this period, the diameter of the growth retardation zones around the studied objects (the "well", including the "well" itself) was measured [13]. Each seeding was carried out in six replicates.

Measurement of the pH index

The potentiometric pH determination was performed using the MARK 901 device ("VZOR", Russia) at the temperature of 20°C, in accordance with the methodology proposed in the State Pharmacopoeia of the Russian Federation of the XVth edition (SPh RF XV ed.) of the GPhM.1.2.1.0004. Ionometry[1].

Degree of liquids coloration

The color of liquids was determined visually by method No. 2, by comparison with the corresponding standards, guided by the SPh RF XV ed., GPhM.1.2.1.0006. Degree of liquids coloration[2].

Transparency and degree of turbidity

The test was performed under the illumination of a 40W frosted glass electric lamp located above the sample, viewing the solutions perpendicular to the vertical axis of the test tubes on a black background 5 minutes

[1] GPhM.1.2.1.0004 Ionometry. State Pharmacopoeia of the Russian Federation XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-1/ionometriya/. Russian

[2] GPhM.1.2.1.0006 Degree of liquids coloration. State Pharmacopoeia of the Russian Federation XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-1/stepen-okraski-zhidkostey/. Russian

after the preparation of the standard, according to the requirements of the SPh RF XV ed., GPhM.1.2.1.0007. Transparency and opalescence degree (turbidity) of liquids[3].

Density

The density determination of the tested eardrops was carried out using a pycnometer according to method No. 1 GPhM.1.2.1.0014 Density of SPh RF XV ed.[4].

Viscosity

The kinematic viscosity of the test solutions was determined using a glass capillary viscometer VPZH-1 ("Ekros Analitika", Russia), according to the GPhM method.1.2.1.0015 Viscosity[5]. Using the experimental values of kinematic viscosity and density of the tested compositions, the dynamic viscosity index was calculated according to the formula given in the pharmacopoeial monograph.

Statistical analysis

The results were processed by methods of variational statistics using the capabilities of the software package StatPlus 7.0 (AnalystSoft Inc., USA, License 16887385). The obtained data were checked for the normality of the distribution according to the Shapiro-Wilk test. Parametric ANOVA methods with the Newman–Keulse post-test and nonparametric statitic analysis methods – the Kruskal–Wallis test, were used to compare groups of averages. The differences were considered significant at $p < 0.05$.

Results

The research was carried out according to the developed design, which included 2 blocks of work at this stage: screening studies of the claimed compositions with the selection of the most active samples and development of the optimal composition, as well as the technology of eardrops.

Screening study of cerumenolytic activity

At the first stage of the experimental studies, a screening evaluation of the compounds known from the literature data and reference drugs of the cerumenolytic action was carried out in order to identify the leading composition.

9 samples (Table 1) and 3 reference drugs were analyzed: TEA-cocoyl hydrolyzed collagen (A-Cerumen Plus, Gilbert Laboratories, France), a 3% hydrogen peroxide solution and a sodium chloride solution, the activity of which was evaluated by the method of sequential lysis of the earwax obtained in laboratory conditions.

During the evaluation of the proteolytic activity (Fig. 1) of the analyzed samples, it was found that the samples under Nos. 4, 7, 8 and 10 significantly superior to both the reference preparation of a 3% hydrogen peroxide solution and saline sodium chloride. These formulations had also a proteolytic activity comparable to the reference drug (A-Cerumen).

A further evaluation of the lipolytic activity (Fig. 2) showed that all test samples, with the exception of sample 10, were inferior to the reference A-Cerumen, but superior to the reference preparations of a 3% hydrogen peroxide solution and a saline sodium chloride solution. The lipolytic activity of sample No. 10 containing thick white mistletoe extract was comparable to that of A-Cerumen.

The total cerumenolytic activity of the analyzed samples is shown in Fig. 3. During the evaluation of the total cerumenolytic activity of the tested samples, it was found that the highest activity level was observed for samples 8 and 10. That was comparable to A-Cerumen and exceeded the reference values of a 3% hydrogen peroxide solution and a saline sodium chloride solution.

The results obtained made it possible to confirm that the sample containing thick white mistletoe leaf extract, had a high cerumenolytic activity and could be used for the development of eardrops. In order to create an optimal composition that would provide efficacy and comfort in the application process, a set of the subsequent studies was conducted.

Results of cerumenolytic activity evaluation of leading compositions

Taking into account that some cerumenolytic activity was also provided by compositions based on the mistletoe white leaf extract with chitosan (samples 1, 2), sodium alginate (samples 7–9) and sodium hyaluronate (4–6), as well as to ensure its prolonged action due to the viscosity increase, 9 compositions were proposed and studied in the second stage of the research (Table. 3).

The technology consisted in the production of aqueous solutions using the "two cylinders" method: 0.05 g of white mistletoe leaf extract was weighed on a scale, dissolved in half the volume of the measured solvent (purified water). In the second half volume of the solvent, depending on the sample number, the auxiliary component – chitosan, sodium hyaluronate or sodium alginate – was dissolved.

[3] GPhM.1.2.1.0007 Transparency and degree of opalescence (turbidity) of liquids. State Pharmacopoeia of the Russian Federation XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-1/prozrachnost-i-stepen-opalestsentsii-mutnosti-zhidkostey/. Russian

[4] GPhM.2.1.0014 Density. State Pharmacopoeia of the Russian Federation XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-1/plotnost/. Russian

[5] GPhM.1.2.1.0015 Viscosity. The State Pharmacopoeia of the Russian Federation XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-2/1-2-1/vyazkost/. Russian

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Table 1 – Analyzed samples at screening stage

No.	Composition
1	Propylene glycol (INEOS Manufacturing GmbH, Germany) – 20 parts Distilled water – 80 parts
2	Polyethylene oxide– 400 (JSC “M–Khim”, Russia) – 20 parts Distilled water – 80 parts
3	Chitosan (JSC “OK” LTD, Russia) – 5 parts Distilled water – 95 parts
4	Chitosan – 10 parts Distilled water – 90 parts
5	Polysorbate – 80 (NeoFroxx, Germany) – 6 parts Distilled water – 94 parts
6	Sodium dodecyl sulfate (“Vekton”, Russia) – 5 parts Distilled water – 95 parts
7	Sodium hyaluronate (HTL SAS, France) – 1 part Distilled water – 99 parts
8	Sodium alginate (JSC “Arhangel’skie vodorosli”, Russia) – 2 parts Distilled water – 98 parts
9	Isotonic solution of sodium chloride 0,9% (JSC “Groteks”, Russia) – reference drug
10	Mistletoe leaf thick extract 0,1 part Distilled water – up to 100 parts
11	Hydrogen peroxide solution 3% (JSC “Jodnye tekhnologii i marketing”, Russia) – reference drug
12	A-Cerumen (GILBERT Laboratories, France) – reference drug

Table 2 – Composition of reproduced sulfur plug

No.	Ingredient	Quantity, g
1	Squalene	0.448
2	Cholesterol	1.463
3	Cholestrolpalmitate	0.336
4	Cholesterolstearate	0.336
5	Oleyloleate	0.336
6	Oleylstearate	0.336
7	Triolein	0.105
8	Oleic Acid	2.097
9	Stearic Acid	0.795
10	Cholesterol Sulfate	0.14
11	Dioleylphosphotidylcholine	0.131
12	Dysteroylphosphotidylcholine	0.131
13	Dipalmitoylphosphotidylcholine	0.131
14	Dimyristoylphosphotidylcholine	0.131
15	Egg yolk	0.104
16	Fetal bovine serum	2.800
17	Epithelial Cell suspension	4.200
Total		14.000

Note: all ingredients were provided by Sigma-Aldrich, Germany.

Table 3 – Compositions of model samples selected on the basis of screening study

Component	Model solution								
	1	2	3	4	5	6	7	8	9
Thick white mistletoe leaf extract, g	0,05								
Chitosan, g	1.0	0.5	–	–	–	–	–	–	–
Sodium hyaluronate, g	–	–	–	0.2	0.6	0.35	–	–	–
Sodium Alginate	–	–	–	–	–	–	1.0	2.0	3.0
Water, ml	Up to 50 ml								

Table 4 – Proteolytic activity of leading compositions

Model sample	Time, min						
	0.5	1	5	10	20	40	60
Composition 1	11.4±0.27*#Δ	25.8±0.01	28.7±0.8	27.7±0.23	16.7±0.65*#Δ	18.7±0.75	11.4±0.6
Composition 2	16.3±0.28	29.1±0.35	26.9±0.95	27.8±0.15	10.2±0.8*#Δ	16.6±0.56	13±0.41
Composition 3	19.3±0.55	26.3±0.34	27.4±0.31	29.9±0.26	11±0.35*#Δ	10.5±0.63	12.6±0.26
Composition 4	20.7±0.37	28.3±0.7	26.5±0.3	26.8±1	26.3±0.65	15.9±0.19	11.8±0.14
Composition 5	19.6±0.77	29.1±0.71	28.5±0.11	27.2±0.73	24.3±0.9	18.7±0.97	18.6±0.17
Composition 6	20.8±0.04	29.3±0.22	25.5±0.10	27.9±0.34	24.3±0.81	19.5±0.77	19.8±0.54
Composition 7	11.4±0.31*#Δ	29.2±0.97	27.5±0.92	29.1±0.13	14.2±0.87*#Δ	14.3±0.6	10.5±0.17
Composition 8	12.8±0.83*#Δ	27.1±0.43	26.8±0.04	25.1±0.94	11.4±0.01*#Δ	11.8±0.31	13.5±0.1
Composition 9	17.6±0.63	26.7±0.31	28.2±0.5	28.5±0.89	19.2±0.01*#Δ	19.1±0.34	17±0.89

Note: # – significant to sample 4 (Newman–Keulse test, p <0.05); * – significant relative to sample 5 (Newman–Keulse test, p <0.05); Δ – significant relative to sample 6 (Newman–Keulse test, p <0.05).

Table 5 – Lipolytic activity of leading compositions

Model sample	Time, min						
	0.5	1	5	10	20	40	60
Composition 1	15.8±0.74	34.2±0.92	33±0.43	33.6±0.49	18.5±0.81*#Δ	15.5±0.01	16.7±0.62
Composition 2	15.2±0.81	34.4±0.92	31.9±0.79	32.2±0.31	17±0.29*#Δ	16.5±0.19	19±0.13
Composition 3	17.6±0.64	30.3±0.13	33.6±0.03	32.9±0.26	19±0.12*#Δ	19.8±0.39	15.3±0.03
Composition 4	19.2±0.85	38.3±0.93	39.2±0.8	30.9±0.61	28.2±0.98	19.2±0.88	17.1±0.18
Composition 5	19.4±0.84	39.9±0.93	39.4±0.19	32.9±0.43	26.1±0.68	16.5±0.89	16.9±0.28
Composition 6	15.2±0.66	39.2±0.14	38.7±0.79	32.4±0.41	26.4±0.28	15.9±0.96	18±0.49
Composition 7	16±0.47	34.9±0.75	30.6±0.87	30.5±0.89	18.9±0.58*#Δ	15.3±0.31	18.3±0.26
Composition 8	15.2±0.56	30.8±0.41	31.5±0.39	34.7±0.27	18.7±0.08*#Δ	16.6±0.12	16.7±0.22
Composition 9	15.6±0.02	32.5±0.11	34.2±0.81	30.1±0.92	17.3±0.4*#Δ	16.2±0.41	17.8±0.9

Note: # – significant to sample 4 (Newman–Keulse test, p <0.05); * – significant relative to sample 5 (Newman–Keulse test, p <0.05); Δ – significant relative to sample 6 (Newman–Keulse test, p <0.05).

Table 6 – Total cerumenolytic activity of leading compositions

Model sample	Time, min						
	0.5	1	5	10	20	40	60
Composition 1	27.2±0.62 *#Δ	60±0.64	61.7±0.49	61.3±0.3	35.2±0.21 *#Δ	34.2±0.78	28.1±0.74
Composition 2	31.5±0.47 *#Δ	63.5±0.87	58.8±0.37	60±0.12	27.2±0.7 *#Δ	33.1±0.72	32±0.94
Composition 3	36.9±0.74	56.6±0.86	61±0.76	62.8±0.12	30±0.79 *#Δ	30.3±0.56	27.9±0.72
Composition 4	39.9±0.92	66.6±0.87	65.7±0.77	57.7±0.59	54.5±0.57	35.1±0.18	28.9±0.51
Composition 5	39±0.06	69±0.32	67.9±0.09	60.1±0.96	50.4±0.9	35.2±0.26	35.5±0.84
Composition 6	36±0.87	68.5±0.57	64.2±0.65	60.3±0.48	50.7±0.05	35.4±0.39	37.8±0.78
Composition 7	27.4±0.93 *#Δ	64.1±0.02	58.1±0.55	59.6±0.46	33.1±0.52 *#Δ	29.6±0.11	28.8±0.78
Composition 8	28±0.53 *#Δ	57.9±0.59	58.3±0.87	59.8±0.02	30.1±0.99 *#Δ	28.4±0.91	30.2±0.62
Composition 9	33.2±0.91	59.2±0.5	62.4±0.25	58.6±0.48	36.5±0.6 *#Δ	35.3±0.46	34.8±0.94

Note: # – significant to sample 4 (Newman–Keulse test, p <0.05); * – significant relative to sample 5 (Newman–Keulse test, p <0.05); Δ – significant relative to sample 6 (Newman–Keulse test, p <0.05).

Table 7 – Compositions of model eardrops samples with preservatives

Component	Model solution								
	1	2	3	4	5	6	7	8	9
Thick white mistletoe leaf extract	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Sodium hyaluronate	0.2	0.2 0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Benzalkonium chloride	0.0025	0.005	0.0075	–	–	–	–	–	–
Nipagin	–	–	–	0.0125	0.025	0.0625	–	–	–
Nipazole	–	–	–	–	–	–	0.0025	0.005	0.0125
Distilled water	Up to 25 ml								

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Table 8 – Results of antimicrobial activity study of model eardrop mixtures

Test cultures	Dimensions of growth retardation zone of model solution, mm								
	1	2	3	4	5	6	7	8	9
Pseudomonas aeruginosa	–	–	–	–	–	–	–	–	–
Staphylococcus aureus	19.17±1.9999	19.33±1.31	20.83±1.85	–	–	–	–	–	–
Streptococcus pyogenes	11.83±1.18	14.00±1.13	15.67±1.31	8.17±3.59	5.33±4.40	7.83±4.31	–	–	–

Table 9 – Results of determination of physical and chemical parameters of the leading model composition

Physical and chemical parameters	Test result
Degree of liquids coloration	color did not exceed the color intensity of the standard Y4
Transparency and turbidity	opalescence did not exceed the opalescence of the standard I
pH	5.86±0.1
Density	1.0029±0.00001
Viscosity	4.2676±0.2 MPa×s

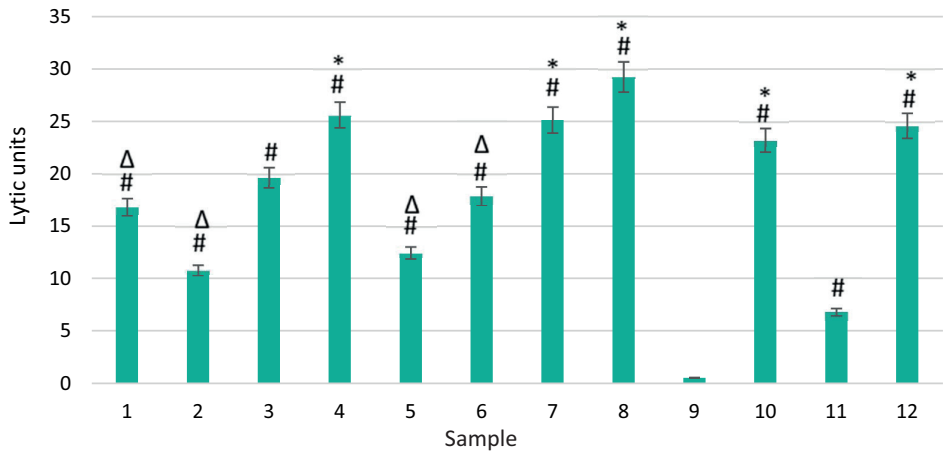

Figure 1 – Proteolytic activity of test samples

Note: # – significant relative to sample 9 (Newman–Keulse test, p <0.05); * – significant relative to Sample 11 (Newman–Keulse test, p <0.05); Δ – significant relative to sample 12 (Newman–Keulse test, p <0.05).

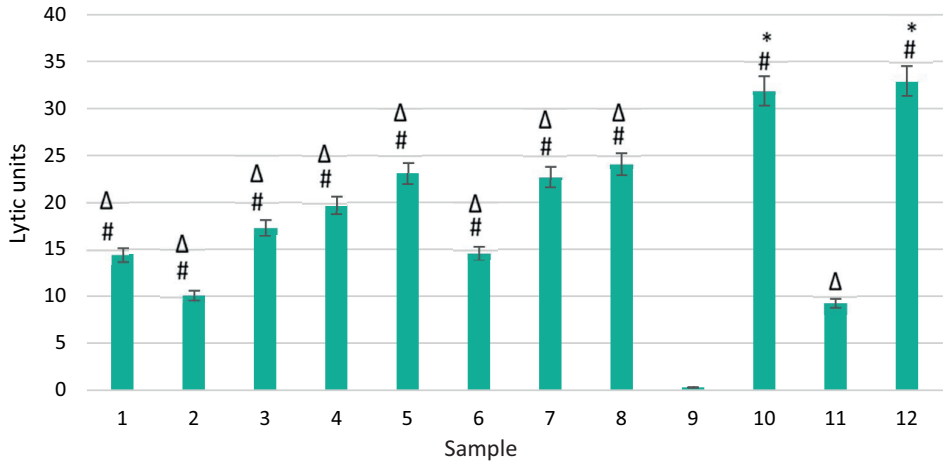

Figure 2 – Lipolytic activity of test samples

Note: # – significant relative to sample 9 (Newman–Keulse test, p <0.05); * – significant relative to Sample 11 (Newman–Keulse test, p <0.05); Δ – significant relative to sample 12 (Newman–Keulse test, p <0.05).

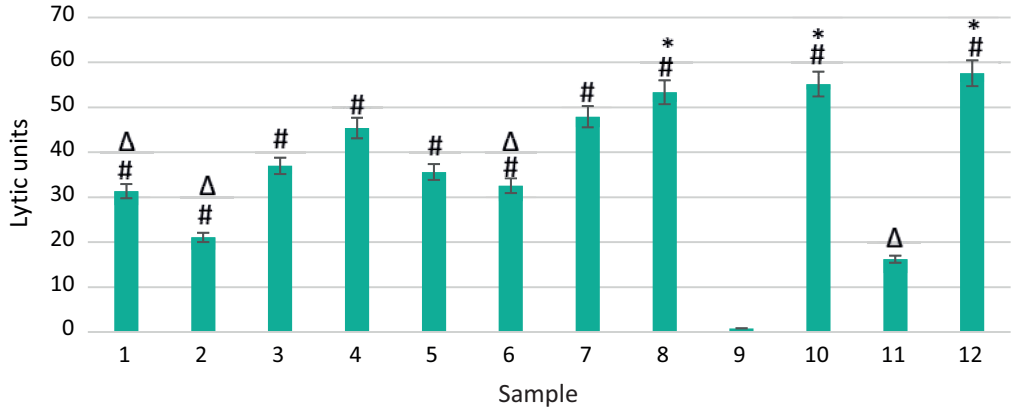

Figure 3 – Total cerumenolytic activity of test samples
Note: # – significant relative to sample 9 (Newman–Keulse test, p <0.05); * – significant relative to Sample 11 (Newman–Keulse test, p <0.05); Δ – significant relative to sample 12 (Newman–Keulse test, p <0.05).

For the obtained 9 samples, the cerumenolytic activity was also evaluated over time. Different time intervals were chosen in order to select the optimal mode of the developed eardrops administration.

During the evaluation of the cerumenolytic activity of the analyzed samples, it was found that the highest proteolytic activity is possessed by samples No. 4–6 with a stable level of activity for 20 min. Samples No. 1–3, 7–9 were inferior to samples 4–6 in terms of the proteolytic activity The results are presented in Table 4.

The results of determining the lipolytic activity are presented in Table 5. The highest lipolytic activity is observed in samples 4, 5 and 6, which exceeded that of the other samples (p <0.05).

The total cerumenolytic activity is shown in Table 6.

The highest total cerumenolytic activity was observed in samples Nos 4–6.

A comparative assessment of the cerumenolytic effect of 9 experimental samples showed that samples No. 1–3, 7–9 had a weak declared activity in the interval of 20 min from the beginning of the experiment, while samples No. 4–6 (thick white mistletoe leaf extract+sodium hyaluronate in various ratios) showed a high level of its effectiveness.

Based on the obtained results, the composition of thick white mistletoe leaf extract with sodium hyaluronate was selected for further work.

Results of microbiological studies

Based on the analysis of the literature data [8] and the requirements of the current pharmacopoeial monograph[6], it was established that a wide range of antimicrobial agents are used in the development of liquid medicinal products: nipagin, nipazole, and most widely – benzalkonium chloride (all substances are provided by Sigma-Aldrich, Germany) [1–4]. An antimicrobial preservative is introduced into the composition of eardrops to ensure its microbiological stability throughout the shelf life. The samples of model solutions of eardrops with the preservatives listed in Table 7, were prepared, the concentrations had been chosen based on the literature data [14].

According to the methodology of microbiological research, the following results were obtained (Table 8).

The obtained experimental data indicate that all the studied objects did not have an inhibitory effect on the test strain *of Pseudomonas aeruginosa.*

Objects No. 4–9 had no antibacterial effect on *the Staphylococcus aureus* test strain.

Objects No. 7–9 had no antibacterial effect on *the Streptococcus pyogenes* test strain.

With regard to the *Staphylococcus aureus* test culture, the studied objects 1–3 were highly active, where in objects 1 and 2, the growth retardation zone was 19 mm on average, and in object 3 it was more than 20 mm.

High antibacterial activity of the studied objects 1–3 was observed against the *Streptococcus pyogenes* test strain. From the table data, it can be concluded that objects 2 and 3 were more active than object 1, which indicates their antibacterial effect. In the course of the experiment, objects 4–6 showed a very low activity, all of these objects had a GRZ smaller than 10 mm.

In general, the antibacterial activity of the studied samples, based on the obtained data, was most pronounced against gram-positive microorganisms (*Staphylococcus aureus, Streptococcus pyogenes*), where a high activity was observed in objects 1-3, with a complete absence of GZR in the *Pseudomonas aeruginosa* test strain.

Results of physicochemical tests

For the reference sample, in accordance with the recommendations of the SPh RF XV ed., the degree

[6] GPA Drops. The State Pharmacopoeia of the Russian Federation XV edition [Electronic resource] – Access mode: https://static-0.minzdrav.gov.ru/system/attachments/attaches/000/063/449/original/ОФС_Капли.docx ?1690467979. Date of application 09/10/2023

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of the solution coloration, its transparency and turbidity, viscosity, pH of the medium, density, were determined [15]. The results are presented in Table 9.

As a result of testing, it was found that the proposed composition has a yellow tint, not exceeding the color intensity of reference Y_4. Transparency and turbidity degrees do not exceed the turbidity of reference I.

The pH value (5.86±0.1) and the pH shift to the acidic side relative to the neutral value, were determined. The literature indicates that the pH from 4.0 to 6.0, is optimal for ear plugs [4]. Given that the purpose of the drops is to dissolve earplugs, discomfort or irritation should not occur when using the drops, which will be determined in further studies. From the consumer point of view, eardrops should have sufficient viscosity to ensure the absence of leakage from the auricular cavity. So, it can be considered that the proposed composition with a dynamic viscosity index of 4.2676±0.2 MPa×s is promising for a further study [15].

DISCUSSION

The auditory canal is an environment with complex homeostatic mechanisms of self-purification and protection. The fundamental basis for the ear canal homeostasis is the migration of cells from the exfoliating layers of the keratinizing epithelium that lines the entire auditory canal and tympanic membrane. This movement leads to a «conveyor belt» effect, in which dead epithelial cells are moved from the bony ear canal to the cartilaginous part, where it is removed by glandular secretions and canal hairs, forming “earwax” or “cerumen”. Earwax has numerous protective properties and is essential for maintaining the optimal functional state of the outer ear. The protective properties of cerumen are determined by its chemical properties and the composition of the microbiota, which is usually represented by saprophytic, commensal, and symbiotic microorganisms [16]. However, an excess of earwax is negative.

Earwax accumulation occurs more frequently in the elderly and those who use hearing aids or earmuffs. For example, Radford JC has shown that in the UK, up to 44% of nursing home residents with dementia have problems with earwax discharge and cerumenolysis [17]. Maharjan M. et al. they also demonstrated that the impaired earwax discharge leads to hearing impairment in children. In addition to hearing impairment, in some cases there is an imbalance in the mental state of a person with a social maladaptation [18]. All of the above makes it necessary to develop new strategies, including pharmacological ones, aimed at clearing the ear canal of excess earwax. Along with the classical mechanical method of earwax removal from the ear canal, a relatively new and effective method of removing excess cerumen, cerumenolysis, during which cerumenolytic agents are used, has recently been identified [19]. As pointed out by Anh N.Q. et al., currently available cerumenolytics demonstrate a different level of efficacy, with the greatest activity of preparations containing surfactants and compounds that soften earwax [20]. The latter include choline itself or its various sources [1]. In the previous studies, it was found that a high content of choline and its esters was observed in the aqueous extract of white mistletoe leaves [10], which can be considered a promising cerumenolytic.

The study showed that the model composition of a cerumenolytic action, containing a thick mistletoe leaf extract, is superior in activity to the compositions based on surfactants (propylene glycol, polyethylene oxide, chitosan, polysorbate, sodium dodecyl sulfate, sodium hyaluronate and sodium alginate), herewith, this extract showed more pronounced lipolytic properties, while the proteolytic effect remained moderate.

It should be noted that an important aspect of effective cerumenolysis is the rate of the effect onset and its duration, which, in turn, can be ensured by selecting the optimal ratio of excipients [21]. It was found that it is advisable to use sodium hyaluronate as an auxiliary agent to increase the cerumenolytic effect of the composition based on thick white mistletoe leaf extract. Its inclusion in the composition provided the onset of the cerumenolytic action in 0.5–1 min and its prolongation up to 20 min. In this case, taking into account the comparability of the obtained data on the proteolytic, lipolytic and general cerumenolytic effects of the formulations containing sodium hyaluronate in the amount of 0.2, 0.35 and 0.6 g, the optimal content of sodium hyaluronate can be considered 0.2 g, which is economically more profitable.

An important component of eardrops, which ensures their microbiological stability and possible antibacterial activity, is a preservative. In this study, benzalkonium chloride, nipazole, and nipagin were selected as potential preservatives, which are included in most of the existing drugs of a cerumenolytic action [22]. At the same time, the strains of microorganisms most frequently causing infectious lesions of the middle ear were selected for microbiological research [23]. Based on the data obtained, the most optimal preservative can be considered benzalkonium chloride in the amount of 0.0075 g. It provides a high level of the antibacterial activity and microbiological stability.

As a result, the conducted complex of studies made it possible to determine the following optimal composition of eardrops of a cerumenolytic action:

Thick white mistletoe leaf extract	0.0250 g
Sodium hyaluronate	0,2000 g
Benzalkonium chloride	0.0075 g
Purified water	up to 25 ml

It is important to note that the developed composition met the requirements of the State Pharmacopoeia of the Russian Federation XV edition to drops, on the basis of which this composition can be considered promising for a further, including clinical, study and a possible implementation in clinical practice.

Study limitations

The study does not present the technological scheme of production in the conditions of large pharmaceutical plants, since at this stage, there are no final results of stability and, accordingly, the choice of the type of packaging that ensures the safety of the drug during the declared shelf life (these studies are at the experimental stage). In the future, it is also expected to assess the local irritant action of the developed eardrops.

CONCLUSION

The development of cerumenolytic ear drops is of interest due to the increasing demand. The results obtained in this comprehensive study suggest the relevance of further investigation of the developed eardrops, which have thick mistletoe leaf extract as the main active ingredient.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

All the authors made an equivalent contribution to the preparation of the publication. All authors confirm that their authorship meets the ICMJE international criteria (all authors have made a significant contribution to the development of the concept, research and article preparation, read and approved the final version before publication). Anastasia E. Pozdnyakova – research concept, conducting the experiment, preparing the manuscript; Similla L. Adzhiakhmetova – conducting the experiment, preparing the manuscript; Elena O. Sergeeva – conducting the experiment, preparing the manuscript; Dmitry I. Pozdnyakov – data analysis, preparing the manuscript; Ekaterina A. Yurtaeva – conducting the experiment, preparing the manuscript; Irina O. Borodina – data analysis, preparation of the manuscript; Dmitry V. Kompantsev – preparation of the manuscript.

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