SELECTION OF ANTIMICROBIAL SUBSTANCES IN THE RECTAL CREAM COMPOSITION CONTAINING THICK EXTRACT OF CARROT AND RUTIN

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Today, the reduction of global pollution and the introduction of sustainable development in all spheres of life should be the priority for any manufacturer. The development strategy for the planet and environment preservation consists of many directions, and one of the most important ones is responsible production and consumption. They should be used in the correct proportion so that they do not dominate the pharmacological action of other active pharmaceutical ingredients (APIs). The application of preservatives is regulated by the relevant articles of the National Pharmacopoeia of the countries throughout the world, legislative authorities, the International Guideline for Harmonization (ICH), which provide guidance as for the medicinal products containing new ingredients requirements. And is revised, from time to time, to correspond to the WHO and US FDA protocol lines as for their compliance with regulatory obligations to protect public health [1,2].

There is a lot of literature data related to the application of natural substances, derivatives of phenolic compounds, which are beneficial for human health and minimize the risk of side effects due to their antioxidant properties as antimicrobial agents. Therefore, in pharmaceutical technology there is a growing interest and demand for natural components with antimicrobial properties, so-called "green" preservatives, the basis of which are identical to natural components. Biopreservatives also attract a lot of attention, because they are of natural origin and are much safer and more useful than synthetic preservatives. Natural products obtained from plant raw materials are widely used not only to provide antimicrobial action, but also to mask the unpleasant smell and appearance of APIs [3,4,6,7].

A rectal cream with a thick extract of sowing carrot and rutin is being developed at the Department of Industrial Technology of Drugs, National University of Pharmacy, Kharkiv. It is known that the properties of flavonols and their glycosides are actively studied for their antimicrobial activity [3,4]. Rutin has been found to inhibit the growth of *Escherichia coli*, inhibit the vital activity of *Proteus vulgaris*, *Shigella sonnei* and *Klebsiella* sp., and has antimicrobial activity against *Pseudomonas auruginosssa* and *Bacillus subtilis*. Due to inhibition of DNA isomerase IV, it inhibits the activity of *E. coli*. Rutin synergistically enhances the antibacterial activity of other flavonoids against *Bacillus cereus* and *Salmonella enteritidis*, and has antifungal activity against *Candida gattii* strain with a minimum inhibitory concentration of 60 μg/ml [8].

Therefore, the aim of this work was selection of effective antimicrobial preservatives in rational concentrations for the composition of rectal cream with rutin and thick carrot extract.

Objects. The following antimicrobial substances were studied - synergistic mixtures of multifunctional "green" preservatives, which are not harmful to human health and have a wide spectrum of antimicrobial activity and pH: Microcare PE (Phenoxyethanol) 1%, Sharomix 702 (Dehydroacetic Acid, Benzoic Acid, Phenoxyethanol) (1.35%), Sharomix MCI II (Methylchloroisothiazolinone, Methylisothiazolinone and Benzyl Alcohol) 0.1%, Sharomix 300 (Me, Pr-paraben, Bronopol, Phenoxyethanol) 0.7%, Sharomix Amplify AM-25 (Phenoxyethanol, Chlorphenesin, Caprylyl Glycol, Didecyldimonium chloride) 0.5%, Sharomix EG10 (EthylHexylGlycerin, Phenoxyethanol) 1.1%, SharoSENSE Plus 181 (Maltol, Polyquaternium-80) 0.5%. A sample of rectal cream without added preservatives was also studied because, based on written data, it was established that rutin has antimicrobial activity against many strains of microorganisms.

Materials and methods

Tests to establish the minimum inhibitory concentration (MIC) for the *E. coli* culture were carried out using the method of serial dilutions (Fig. 1) [9,10,11]. 2 ml of a nutrient medium, 2 ml of the studied preservative of a given concentration and 2 ml of the microorganism's suspension were introduced into the test tube. A test tube for control was prepared, into which all components, except for the preservative, were added: 2 ml of nutrient medium, 2 ml of the microorganism's suspension and 2 ml of water. When measuring the optical density, as "0" point was taken a mixture of 4 ml of nutrient medium and 2 ml of water. Thus, the total volume of each test tube was 6 ml and the initial concentration of the preservative was reduced in three times. Therefore, with the aim of calculations facilitation, all initial concentrations were taken in multiples of three. All experiments were performed in duplicate to ensure reproducibility. A decrease in the solution optical density indicates that the preservative has fulfilled its function and inhibited the growth of microorganisms. The preservative concentration, at which the optical density of the suspension to be studied is equal to or close to zero was considered to be MIC. The amount of preservative was adjusted to the required concentration with water. It was mixed well and the necessary amount was taken for the preparation of solutions of lower concentrations. Each test tube was marked and first a preservative was added in a given concentration, water (where necessary), then a nutrient medium, and finally a bacterial suspension was added. All test tubes were placed in a thermostat at a temperature of 37 ºC for 24 hours. The next day, the optical density was measured by FEC and microorganisms were inoculated into Endo medium [13].

Testing of the antimicrobial preservatives effectiveness was carried out according to the procedure in accordance with SPhU 2.3 (*5.1.3*) [14]. The study was performed under aseptic conditions of a laminar flow cabinet (biological safety cabinet AS2-4Ye1 Esco, Indonesia).

Soyabean Casein Digest Agar and Sabouraud-dextrose agar were used as nutrient media, and buffer solution with sodium chloride and peptone pH = 7.0 containing 50 g/l polysorbate-80, 5 g/l lecithin and 1 g/l histidine hydrochloride was used as solvent.

Staphylococcus aureus ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404 were used as microorganism test cultures, and the inoculum was prepared according to SPhU 2.3 (*5.1.3*).

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According to SPhU requirements, growth properties of nutrient media (soybean-casein nutrient media - for growing bacteria and Sabouraud-dextrose media without adding an antibiotic - for growing fungi) were checked and suitability of the method for determining the total number of viable cells was checked. Control in determining the growth properties of the medium is a standard medium with guaranteed growth properties, on which the quantitative and qualitative growth of microorganisms (morphology of colonies) is correctly detected. The results of verification of growth properties of culture media are given in Table 1.

Requirements and criteria for the selection of preservatives: The preservative properties of the preparation are adequate if, in the conditions of the test, there is a significant fall or no increase, as appropriate, in the number of microorganisms in the inoculated preparation after the times and at the temperatures prescribed. The acceptance criteria, in terms of decrease in the number of microorganisms with time, vary for different types of preparations according to the degree of protection intended depending on the requirements for the finished medicinal product [15].

Results and discussion

Comparison of the minimum inhibitory concentrations of the studied preservatives is presented in Fig. 1.

The results shown in fig. 1 indicate that the most effective were SharoSENSE Plus 181 and Sharomix Amplify AM-25, which showed an inhibitory effect at 480 ppm and 500 ppm concentrations, respectively, Sharomix 300, Sharomix MCI II, Microcare PE with 1000 ppm, 1120 ppm and 4000 rrm indicators. The least effective were Sharomix 702 and Sharomix EG10, with MICs of 9000 ppm and 11000 ppm, respectively. Therefore, SharoSENSE Plus 181 and Sharomix Amplify AM-25, which had the lowest MIC index and belong to "green" preservatives, were chosen for further research.

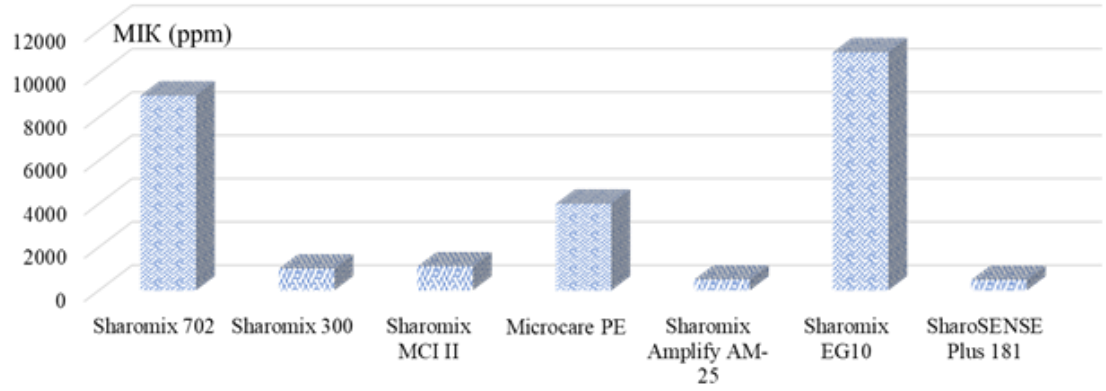

Fig. 1 Minimum inhibitory concentrations of the studied preservatives

Data given in table 1 demonstrate that the nutrient media corresponded to the growth properties test in accordance with the SPhU 2.0 (*2.6.12*) requirement, and the test microorganisms corresponded to the taxonomic characteristics, the morphology of the colonies on the media and the morphology of the cells during microscopy were typical for the respective strain.

The suitability of the method for determining the total number of viable cells checking consists in comparing the results of counting the number of test microorganisms in the tested preparation and control cultures.

Table 1 Growth properties of nutrient media

Test microorganisms	Test microorganisms	Microorganisms test		Conclusions
		temperature, °C	duration, hours	
Staphylococcus aureus ATCC 6538	Soyabean Casein Digest Agar	30-35	18-24	The morphology of colonies and cells is typical
Pseudomonas aeruginosa ATCC 9027	Soyabean Casein Digest Agar	30-35	18-24	The morphology of colonies and cells is typical
Candida albicans ATCC 10231	Sabouraud-dextrose agar	20-25	48-72	The morphology of colonies and cells is typical
Aspergillus brasiliensis ATCC 16404	Sabouraud-dextrose agar	20-25	120-168	The morphology of colonies and cells is typical
-	Negative control test	35	24-72	There is no growth of microorganisms

To do this, a suspension of a test strain of one of the

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types of microorganisms, which contains about 100 colony-forming units (CFU) per millilitre, was introduced into test tubes with a dilution of the preparation prepared for each test microorganism separately. Control test microorganisms were prepared. The inoculated samples were thoroughly mixed. 1 ml of the preparation dilutions and control dilutions, separately for each test strain, containing no more than 100 CFU, were inoculated by the surface method on dense nutrient media: Soyabean Casein Digest Agar for bacteria and Sabouraud-dextrose agar for fungi. The results of the validity verifying of the methodology for calculating the number of living cells are shown in Table 2.

Table 2 Results of the methodology testing suitability

Sample	Average number of CFU in 1 ml of sample			
	S. aureus	*P. aeruginosa*	*C. albicans*	*A. brasiliensis*
Suspension of microorganisms + rectal cream	78	64	75	68
Control inoculation	89	76	69	65

The results given in table 2, obtained during the counting of each of the test microorganisms in the presence and absence of the test sample, differ by no more than 1.2 times, which meets the acceptance criterion (no more than two times). That is, the method of surface inoculation into cups using a standard solvent is suitable for the number of microorganism's determination in the preparation and can be used to test the effectiveness of antimicrobial preservatives.

To test the efficacy of the selected antimicrobial preservatives, each rectal cream sample container was inoculated with a freshly prepared suspension with one of the test microorganisms, while simultaneously providing a microbial load of 10^5 to 10^6 CFU in 1 ml of sample, thoroughly mixed to distribute them evenly in the sample volume and stored at a temperature of 20 to 25 ° C in a light-protected place.

Immediately after inoculation and at regular intervals (14 and 28 days), 1 ml of sample was taken from each sample and the number of viable microorganisms was determined by plate count.

The criterion for the effectiveness of the preservative in the dosage form was a decrease in the number of viable cells of test microorganisms in the preparation for a certain period of time after its contamination. In accordance with the requirements of the SPhU for rectal preparations, *log* reduction in the number of viable bacteria after 14 days should be at least 3, after 28 days the number of viable cells should not increase; *log* reduction in the number of viable fungal cells after 14 days should be at least 1, after 28 days the number of viable cells should not increase. Results of the study of antimicrobial effectiveness of preservatives are given in Table 3.

Table 3 The results of the antimicrobial preservatives effectiveness in the rectal cream samples

Test culture of microorganisms	Preservative (concentration, %)	lg of number of viable microorganisms immediately after inoculation, lg CFU/ml	lg reduction of viable microorganisms number,lg CFU/ml (SPhU requirements 2.3 / results obtained)	
			14 діб	28 діб
Staphylococcus aureus ATCC 6538	Rectal cream without the preservative	4,28	3/2,83	NI/2,71
	SharoSENSE Plus 181	4,59	3/3,97	NI/ND
	Sharomix Amplify AM-25	4,38	3/3,37	NI/ND
5Pseudomonas aeruginosa ATCC 9027	Rectal cream without the preservative	4,44	3/2,74	NI/2,50
	Sharomix Amplify AM-25	4,44	3/4,5	NI/NI
	SharoSENSE Plus 181	4,52	3/ND	NI/ND
Candida albicans ATCC 10231	Rectal cream without the preservative	4,74	1/0,76	NI/0,61
	Sharomix Amplify AM-25	4,54	1 / 3,86	NI/ND
	SharoSENSE Plus 181	4,68	1/ ND	NI/ND
Aspergillus brasiliensis ATCC 16404	Rectal cream without the preservative	4,57	1/ 0,65	NI/0,51
	Sharomix Amplify AM-25	4,59	1 / ND	NI/ND
	SharoSENSE Plus 181	4,66	1/ND	NI/ND

Notes: NI –no increase in the number of microorganisms compared to the number of viable microorganismsat the previous control point;ND –no viable cells of microorganisms were detected in the experiment

The results presented in Table 3, indicate that the rectal cream sample without the preservatives does not meet the SPhU requirements, since the lg reduction of viable bacteria (*Staphylococcus aureus* and *Pseudomonas*

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aeruginosa) was less than 3.0 after 14 days and 28 days of storage, respectively. For fungal cells, on day 14 the lg reduction of cells in samples without the preservatives was 0.76 for *Candida albicans* and 0.65 for *Aspergillus brasiliensis* (minimum according to requirements – 1.0), which also does not meet the requirements. That is, the results obtained prove the need to add antimicrobial substances to the composition of the developed rectal cream.

Samples of rectal cream with Sharomix Amplify AM-25 showed antimicrobial activity against *Staphylococcus aureus*. Thus, on day 14, the lg reduction of bacterial cells number was 3.37 (minimum according to requirements – 3.0); on day 28, no cells were detected. For the *Pseudomonas aeruginosa*, on day 14, the lg reduction of bacterial cells number was 4.5. On day 28, there was no increase in the number of microorganisms compared to the previous control point. For *Candida albicans* in samples with Sharomix Amplify AM-25 on the 14th day of observation, the lg reduction of the number of cells was 3.86, and for *Aspergillus brasiliensis*, no cells were detected. On the 28th day of the experiment, no cells of these test microorganisms were detected.

The following results were observed for samples with SharoSENSE Plus 181: on day 14, lg reduction of *Staphylococcus aureus* cell was 3.97; *Pseudomonas aeruginosa* cells were not detected. For *Candida albicans* and *Aspergillus brasiliensis*, on day 14 the number of cells were not detected. On day 28, no cells of all test microorganisms were detected.

Visual observations of samples of rectal cream with different preservatives showed that all preservatives provide the necessary microbiological stability of the drug during storage: the samples remained homogeneous for a long time, without stratification, changes in odor and color and other signs of the harmful effects of microorganisms.

Summarizing the results of Table 3, it can be noted that the data obtained meet the SPhU requirements for rectal preparations.

Taking into account the requirements of safety, economy and physicochemical properties, SharoSENSE Plus 181 at a concentration of 0.5 % was chosen as the most acceptable preservative.

Conclusions. The minimum inhibitory concentrations of the preservatives for *E. coli* were determined by the method of serial dilutions. It was found that the most effective were SharoSENSE Plus 181 and Sharomix Amplify AM-25, which showed an inhibitory effect at a concentration of 480 ppm and 500 ppm, respectively.

The efficacy of antimicrobial preservatives was tested, the growth properties of culture media and the suitability of the methodology for determining the total number of viable cells were checked. It was found that the growth media met the SPhU requirements 2.6 *(2.6.12*), and the test microorganisms met the taxonomic characteristics - the morphology of colonies on the media and the morphology of cells during microscopy were typical for the corresponding strain.

The efficacy of SharoSENSE Plus 181 and Sharomix Amplify AM-25 in the composition of rectal cream was investigated. The results obtained were found to be in compliance with the SPhU requirements for rectal preparations. Taking into account the requirements of safety, efficiency and physicochemical properties, SharoSENSE Plus 181 at a concentration of 0.5 % was chosen as the most acceptable preservative.

Selection of antimicrobial substances in the rectal cream composition containing thick extract of carrot and rutin
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Introduction. Synthetic preservatives can significantly extend the shelf life of a perishable product, although they have many side effects that are harmful to human health. In view of this, there is a current trend towards the use of preservatives made from natural plant products. According to the literature, natural substances are widely used as effective preservatives. Regulatory authorities that control the quality, safety and efficacy of medicines pay great attention to natural substances that can be used in the pharmaceutical industry, which will reduce the risk of side effects. A rectal cream with a thick extract of sowing carrot and rutin is being developed at the Department of Industrial Technology of Drugs, National University of Pharmacy, Kharkiv. It is known that the properties of flavonols and their glycosides are actively studied for their antimicrobial activity. Rutin has been found to inhibit the growth of *Escherichia coli*, inhibit the vital activity of *Proteus vulgaris*, *Shigella sonnei* and *Klebsiella* sp., and has antimicrobial activity against *Pseudomonas auruginosssa* and *Bacillus subtilis*. Due to inhibition of DNA isomerase IV, it inhibits the activity of *E. coli*. Rutin synergistically enhances the antibacterial activity of other flavonoids against *Bacillus cereus* and *Salmonella enteritidis*, and has antifungal activity against *Candida gattii* strain with a minimum inhibitory concentration of 60 μg/ml. Samples of rectal cream with rutin and thick carrot extract without and with the addition of various antimicrobial agents were tested. **Material & methods.** The objects of the study were : Microcare PE (Phenoxyethanol) 1%, Sharomix 702 (Dehydroacetic Acid, Benzoic Acid, Phenoxyethanol) (1.35%), Sharomix MCI II (Methylchloroisothiazolinone, Methylisothiazolinone and Benzyl Alcohol) 0.1%, Sharomix 300 (Me, Pr-paraben, Bronopol, Phenoxyethanol) 0.7%, Sharomix Amplify AM-25 (Phenoxyethanol, Chlorphenesin, Caprylyl Glycol, Didecyldimonium chloride) 0.5%, Sharomix EG10 (EthylHexylGlycerin, Phenoxyethanol) 1.1%, SharoSENSE Plus 181 (Maltol, Polyquaternium-80) 0.5%. A sample of rectal cream with and without added preservatives was studied. **Results & discussion.** The minimum inhibitory concentrations of the studied preservatives for *E. coli* culture were determined using the method of serial dilutions. The efficacy of antimicrobial preservatives was tested, the growth properties of culture media were checked, and the suitability of the method for determining the total number of viable cells was verified. The efficacy of SharoSENSE Plus 181 and Sharomix Amplify AM-25 in the composition of rectal cream was investigated. The compliance of the obtained results with the requirements of the SFU for drugs for rectal use was established. **Conclusions.** It was found that the most effective were SharoSENSE Plus 181 and Sharomix Amplify AM-25, which showed an inhibitory effect at a concentration of 480 ppm and 500 ppm, respectively. It was found that the culture media were suitable for growth properties in accordance with

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the requirements of SFU 2.6 (2.6.12), and the test microorganisms corresponded to the taxonomic characteristics - the morphology of colonies on culture media and cell morphology during microscopy were typical for the corresponding strain. Taking into account the requirements of safety, economy and physicochemical properties, SharoSENSE Plus 181 at a concentration of 0.5% was chosen as the most acceptable preservative.

Keywords: antimicrobial substances, rectal cream, composition, thick extract of carrot, rutin, minimum inhibitory concentrations, effectiveness of antimicrobial preservatives

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