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Argumentation of the composition of new semi-solid medicinal preparation on the basis of complex soft extract of *Hypericum* and *Calendula* flowers

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Abstract
The composition of a new semi-solid medicinal preparation was developed on the basis of complex soft extract of tutsan (*Hypericum*) and marigold (*Calendula*) flowers for the treatment of skin wound process in phases II and III and for the local treatment of lesions of the oral mucosa. The optimal concentration of the soft extract was validated and the composition of the basis was chosen taking into consideration the medical and biological requirements for a medicinal preparation and physicochemical properties of the active pharmaceutical ingredient.

Keywords: Soft extract, tutsan, marigold flowers, antimicrobial preservatives, gels.

1. Introduction
The problem of wound healing in dermatology and dentistry remains relevant despite the continuous improvement of wound process treatment methods. The main contribution among medicinal preparations (MP) for local treatment of these medical conditions is been done by semi-solid medicinal preparations (SMP) [1]

Analysis of the nomenclature of SMP for the treatment of skin wound process in phases II and III and inflammatory diseases of the oral mucosa (OM) showed that the composition of this group of MPs includes APIs that relate to various pharmacotherapeutic groups. However, despite the wide-ranging nomenclature of SMP based on synthetic APIs, the popularity of herbal preparations is growing steadily due to a milder treatment effect and minimal side effects. Therefore it is of vital importance to develop and introduce into production highly efficient SMP based on substances of plant origin with wound healing, anti-inflammatory, antiviral properties, and minimal side effects manufactured according to the latest technologies [2, 3].

Complex soft extract of tutsan and marigold flowers was used as an API in the development of the SMP composition. The main biologically active substances (BAS) of the extract are flavonoids with significant antimicrobial, anti-inflammatory effect; marigold flowers also contain isorhamnetin - flavonoid with significant wound healing, antiulcer and antiviral properties [4]. Given the composition of the extract, its use is reasonable in SMP for the treatment of II and III phases of wound process and inflammatory diseases of the OM.

The concentration of the soft extract made from tutsan and marigold at a ratio of 1:10 was chosen based on literature data and data analysis of the State Register of Medicinal Products of Ukraine. It was established that herbal preparations based on tutsan and marigold flowers are the part of the composition of MP for external use at a concentration of 0.5% to 10%. We have selected the optimal concentration of the soft extract of hypericum and marigold flowers at 2% [2].

To achieve the desired therapeutic effect not only the pharmacological properties of APIs must be considered, but also the properties of excipients such as ointment base. At the pharmaceutical market of Ukraine SMP with the extract of *Hypericum perforatum* and *Calendula officinalis* are in the form of hydrophobic ointments, creams, and ointments on the basis of emulsion type O/W. However, hydrophobic ointments have an occlusive effect, poor rinseability, and do not mix with exudate. When used in dentistry the concentration of active substances in emulsion ointments quickly reduces due to the saliva dilution, and the outwashing of the medicinal substances into the lower gastrointestinal tract takes place, which is not acceptable in the case of inflammation of OM. Therefore, the development of SMP on hydrophilic base in gel form is of immediate interest since it is the most suitable for

application to the wound surface or mucous membrane [2, 5-8].

Materials and methods.

Complex soft extract of tutsan (hypericum) and marigold (calendula) (dissolution 1:10) was used as an active pharmaceutical ingredient (API) of the presented medicinal preparation. The optimum composition of gel basis was determined on the basis of the experimental studies. Poloxamer 407 and guar gum were selected as the gelling agents, and propylene glycol was added to provide moisture-retaining capacity of the preparation.

The antimicrobial activity of preservatives in the samples was examined in compliance to the State Pharmacopoeia of Ukraine (SPU) 2.0., 5.1.3. The method of evaluating the effectiveness of antimicrobial preservatives is based on addition of a certain number of test-microorganisms into the test samples and determining their amount after a certain period of time (2, 7, 14, 28 days for topically-applied preparations). According to the requirements of SPU, *Staphylococcus aureus* ATCC 6538, *Pseudomonas asaeruginosa* ATCC 9027, *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC 16404 were used as test-microorganisms. One-day bacterial cultures previously cultivated on solid nutrient medium, such as tryptic soy agar were used in the research.

Results and discussion. Gelling agents: Carbomer 974P, Poloxamer 407, guar gum, hydroxymethyl cellulose (HMC) and sodium carboxymethylcellulose (NaCMC) have been chosen as the objects of the research. For the purpose of selecting the optimal gelling agent and its concentration we have manufactured 15 gel samples with different concentrations of 5 gel forming agents. All gel compositions were injected with propylene glycol in the amount of 5%, which shows high solubility, promotes the absorption of API, shows osmotic properties, has antioxidant effect, prevents mold formation, and provides moisture-retaining capacity in the SMP. The gel compositions are shown in Table 1.

The results of the research indicate that at the manufacturing of gel samples number 1, 3, 10-12 and 13-15 viscous liquids are formed that does not meet the gel requirements.

Table 1: Composition of gels with soft extract of tutsan and marigold without preservatives

Component name	№ 1	№ 2	№ 3	№ 4	№ 5	№ 6	№ 7	№ 8	№ 9	№ 10	№ 11	№ 12	№ 13	№ 14	№ 15
Tutsan and marigold extract (1:10)	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0
Carbomer 974 P	0,1	0,4	1,0												
Poloxamer 407				15,0	30,0	50,0									
Guar gum							1,0	1,7	2,4						
HMC										5,0	7,0	10,0			
NaCMC													4,0	5,0	6,0
Propylene glycol	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0	5,0
Ammonium hydroxide	Up to pH 6,0	up to pH 6,0	up to pH 6,0												
Purified water	up to 100,0	up to 100,0	up to 100,0	up to 100,0	up to 100,0	up to 100,0	up to 100,0	up to 100,0	up to 100,0	up to 100,0	up to 100,0	up to 100,0	up to 100,0	up to 100,0	up to 100,0

Samples number 2, 4-6 and 7-9 were stable homogeneous gels, however, they differed in viscosity and not all of them have satisfactory sensory properties (ease of application, the rate and extent of absorption, skin condition after application etc).

Therefore, samples number 2, 5 and 9 have been selected for further research since they showed optimal viscosity and are suitable for application to the skin and mucous membranes. The other gel compositions based on given gel forming agents did not meet the requirements since it is either too thick (sample number 6) or have liquid consistency (samples number 4, 7 and 8).

Manufactured gel samples were packaged in plastic containers and dark glass jars and kept in a cool, dark place at a temperature 8-15 ˚C for 3 months. During the observation period there were changes in organoleptic properties of gels and mold formation in gel samples packed in plastic containers that indicates the need for preservatives in the gel composition and unfitness of plastic containers for gel storing.

The next stage of our research included the choice of antimicrobial preservatives. Antimicrobial preservatives were added into the composition of gels number 2 (based on carbomer 0,4%), № 5 (based on poloxamer 30%) and number 9 (based on guar gum 2.4%) for provision of microbiological purity during storage. Sodium benzoate, methyl and propyl parahydroxybenzoate mixture (3:1) were used as preservatives, in concentrations equal to half the maximum allowed value of the composition of cosmetic products. The composition of the studied gels is shown in Table 2.

Table 2: Composition of gels with complex soft extract of tutsan and marigold

Component name	№ 2.1	№ 2.2	№ 5.1	№ 5.2	№ 9.1	№ 9.2
Tutsan and marigold soft extract (1:10)	2,0	2,0	2,0	2,0	2,0	2,0
Carbomer 974 P	0,4	0,4				
Poloxamer 407			30,0	30,0		

Guar gum					2,4	2,4
Propylene glycol	5,0	5,0	5,0	5,0	5,0	5,0
Parabens (Nipagin+Nipasol) (3:1)	0,25		0,25		0,25	
Sodium benzoate		0,25		0,25		0,25
Ammonium hydroxide	up to pH 6,0	up to pH 6,0				
Purified water	up to 100,0	up to 100,0	up to 100,0	up to 100,0	up to 100,0	up to 100,0

Manufactured gel samples were packaged in dark glass jars and kept in a cool, dark place at a temperature 8 – 15˚C. Organoleptic characteristics showed that immediately after gels production there was destruction of gel structure in composition № 2.2 (preservative sodium benzoate), and after 3 months there was a change in the consistency of gel number 2.1, therefore, carbomer-based gels were not used for further research. After 6 months of storage gel samples № 5.1, 5.2 and 9.1, 9.2 had satisfactory organoleptic characteristics (color, smell, visual appearance). These samples were evaluated to determine the effectiveness of antimicrobial preservatives.

SPU 2nd edition establishes the acceptance criteria (A, B) for the effectiveness of antimicrobial preservatives for MP for topical use as a logarithm (lg) of the decrease in the number of viable microorganisms (MO) with respect to the definite initial number of MO. Acceptance criteria A states that after two days lg of the decrease must be no less than 2, after 7 days – no less than 3, further the number of viable cells of bacteria should not increase. lg of the decrease of the number of viable cells of fungi after 14 days must be no less than 2, further on the number of viable fungi cells should not increase. Criterion A corresponds to the recommended effectiveness. If its justified that criterion A can not be achieved, MP have to meet the criterion B. In accordance with criterion B requirements for preparations for topical application, lg of the decrease of the number of viable cells of bacteria after 14 days must be no less than 3 and further the number of viable cells of bacteria should not increase. Lg of the decrease of the number of viable cells of fungi after 14 days should be no less than 1 and further the number of viable cells of fungi should not increase (Table 3) [9].

The research on gels without preservatives was conducted as a control. The results of the research indicated that they do not meet neither criterion A nor criterion B and will not provide adequate microbiological purity during production, packing, storing, distribution and usage.

The results of the research on the effectiveness of antimicrobial preservatives in the manufactured SMP with different preservatives are presented in Tables 4 - 5.

Table 3: SPU requirements to the effectiveness of antimicrobial preservatives (Medicinal preparations for topical use)

		lg **of the decrease**			
		2 days	**7 days**	**14 days**	**28 days**
Bacteria	A	2	3	-	NI
	B	-	-	3	NI
Fungi	A	-	-	2	NI
	B	-	-	1	NI

NI –the number of microorganisms does not increase compared to the number of viable microorganisms in the preceding point of reference.

Table 4: Results of the research on the effectiveness of antimicrobial preservatives in gels with complex soft extract of tutsan and marigold

Incubation days	**Number of CFU/ml (logarithm of the decrease of the number of viable cells)**			
	Staphylococcus aureus ATCC 6538	***Pseudomonas aeruginosa ATCC 9027***	***Candida albicans ATCC 10231***	***Aspergillus brasiliensis ATCC 16404***
Sample 5.1				
Primary isolation/ Initial load	$4 \cdot 10^6$ (6,6)	$8 \cdot 10^6$ (6,90)	$9 \cdot 10^5$ (5,96)	$7 \cdot 10^5$ (5,84)
2 days	ND	$4 \cdot 10^4$ (4,60)(lg decr. 2,30)	ND	$6 \cdot 10^4$ (4,77)(lg decr. 1,07)
7 days	ND	ND	ND	$1 \cdot 10^3$ (3,0) (lg decr. 2,84)
14 days	ND	ND	ND	$1 \cdot 10^3$ (3,0) (lg decr. 2,84)
28 days	ND	ND	ND	$2 \cdot 10^3$ (3,3) (lg decr. 2,54)
Sample 5.2				
2 days	ND	ND	ND	$8 \cdot 10^4$ (4,9) (lg decr. 0,94)
7 days	ND	ND	ND	$9 \cdot 10^2$ (2,96) (lg decr. 2,88)
14 days	ND	ND	ND	$1 \cdot 10^3$ (3,0) (lg decr. 2,84)
28 days	ND	ND	ND	$2 \cdot 10^3$ (3,3) (lg decr. 2,54)
Sample 9.1				
2 days	ND	ND	ND	ND
7 days	ND	ND	ND	ND
14 days	ND	ND	ND	ND
28 days	ND	ND	ND	ND
Sample 9.2				
2 days	ND	$5 \cdot 10^4$ (4,70) (lg decr. 2,20)	$1,4 \cdot 10^3$ (3,20) (lg decr. 2,76)	$4 \cdot 10^5$ (5,60) (lg decr. 0,24)
7 days	ND	ND	$5,3 \cdot 10^2$ (2,72) (lg decr. 3,24)	$1,1 \cdot 10^5$ (5,04) (lg decr. 0,80)
14 days	ND	ND	$1 \cdot 10^2$ (2,00) (lg decr. 3,96)	$1 \cdot 10^5$ (5,00) (lg decr. 0,84)
28 days	ND	ND	ND	$2 \cdot 10^2$ (2,30) (lg decr. 3,54)

ND – microorganisms were not detected.

The calculation of lg of the decrease / reduction of the number of viable cells was performed by subtracting the data obtained from the data of the primary isolation / initial load. As it is shown in Table 4, the combination of parabens (Nipagin +Nipasol) in the ratio 3: 1 (samples 5.1 and 9.1) showed viable antimicrobial activity in relation to bacteria and fungi. Furthermore, there was rapid bacterial destruction with the use of sodium benzoate (samples 5.2 and 9.2).

Table 5: Effectiveness of antimicrobial preservatives in gels with complex soft extract of tutsan and marigold flowers

Test samples	*Staphylococcus aureus ATCC6538*	*Pseudomonas aeruginosa ATCC9027*	*Candida albicans ATCC10231*	*Aspergillus brasiliensis ATCC16404*	Accordance to the criterion A or B
№5.1	+	+	+	+	Criterion A
№5.2	+	+	+	+	Criterion A
№9.1	+	+	+	+	Criterion A
№9.2	+	+	+	-	Does no meet the criteria A and B

As it is shown in Tables 4 and 5 examined gels № 5.1, 5.2 and 9.1 meet criterion A and the requirements of SPU to the effectiveness of antimicrobial preservatives in samples for topical use. Sodium benzoate at a concentration 0.25% in the composition of guar gum-based gel in a sample number 9.2 does not ensure efficiency as a preservative against *Aspergillus brasiliensis* ATCC 16404, i.e. does not meet pharmacopoeial acceptance criteria, thus this sample will not be used for further research.

Conclusions.

1. The study validated the optimal composition of gels with complex soft tutsan and marigold flowers extract for the treatment of skin wound process in phases II and III and for the local treatment of lesions of the oral mucosa on synthetic and natural bases.
2. The published data and analysis of the State Register of Medicinal Products of Ukraine were used as guidelines for determining the optimum concentration of complex soft extract (2% as an active pharmaceutical ingredient).
3. The results of microbiological research confirmed the effectiveness of antimicrobial preservatives (Nipagin : Nipasol (1 : 3) and sodium benzoate) in samples of gels based on carbomer 974 P, guar gum and poloxamer.

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