Korea-Kazakhstan International Medical Forum

18/11/2015, Al-Farabi Kazakh National University

Limonium gmelinii plant-derived pharmaceuticals

Prof. Galiya E. Zhussupova,
Dr. Aizhan I. Zhussupova

Preambule

Full provision of domestic pharmaceutical market in Kazakhstan with the local original mainly plant-derived pharmaceuticals is one of the main priorities of socio-economic policy of the Government of Kazakhstan, as well as of the current state program of import substitution and increase of locally produced medicines to 40-50 %.

Limonium gmelinii Mill (Plumbaginaceae family)

- wide distribution on the territory of the Republic;
- appropriate plant features;
- simple, economically and environmentally efficient technology of the substance extraction in the form of a dry extract;
- remains after the extraction, can be used to store apples, wool and fur products;
- its roots were introduced into medicine as a promising domestic industrially significant plant raw material in 2002 and into the State Pharmacopoeia of the Republic of Kazakhstan (RK) in 2009.

DISTRIBUTION of Limonium Mill plant species in KAZAKHSTAN

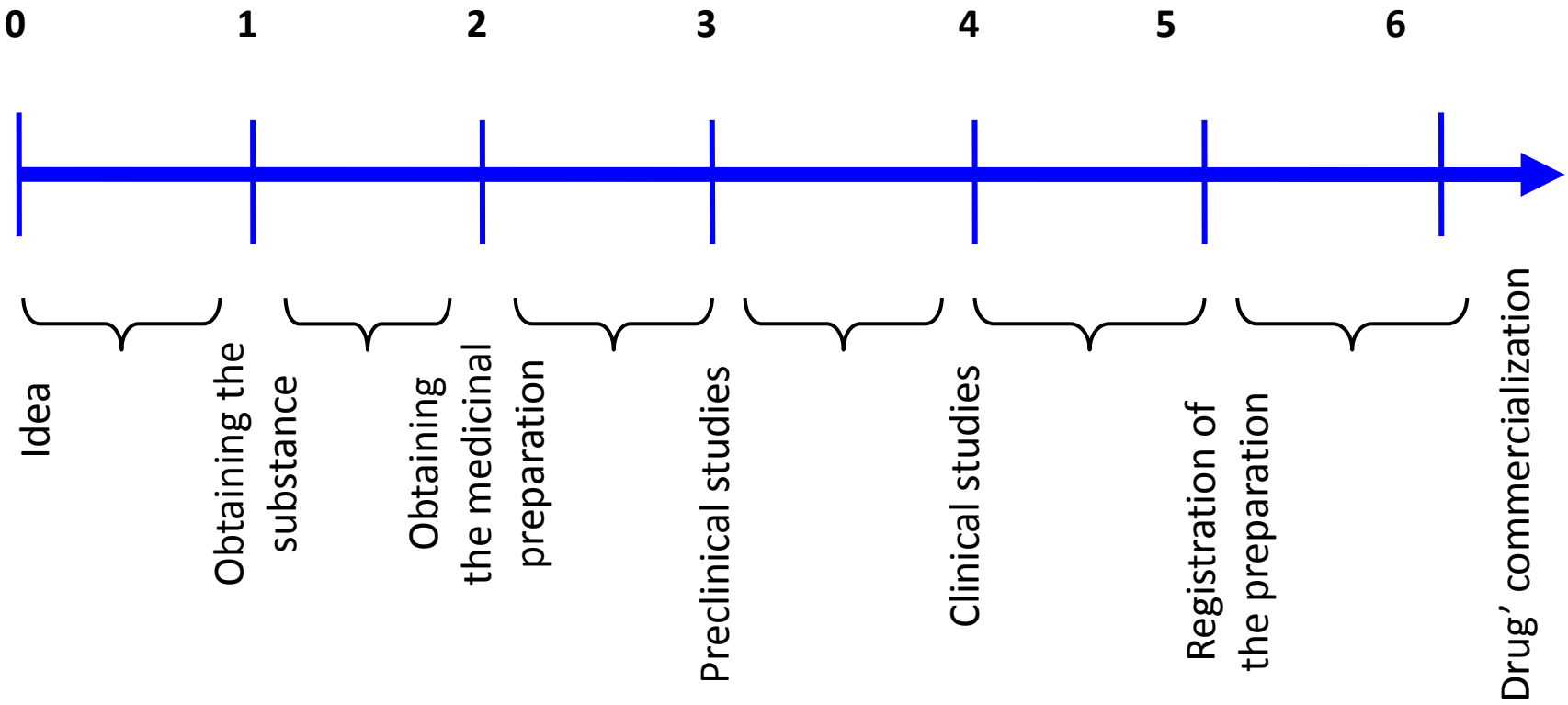
SCALE

100 0 100 200 300 400 500 км

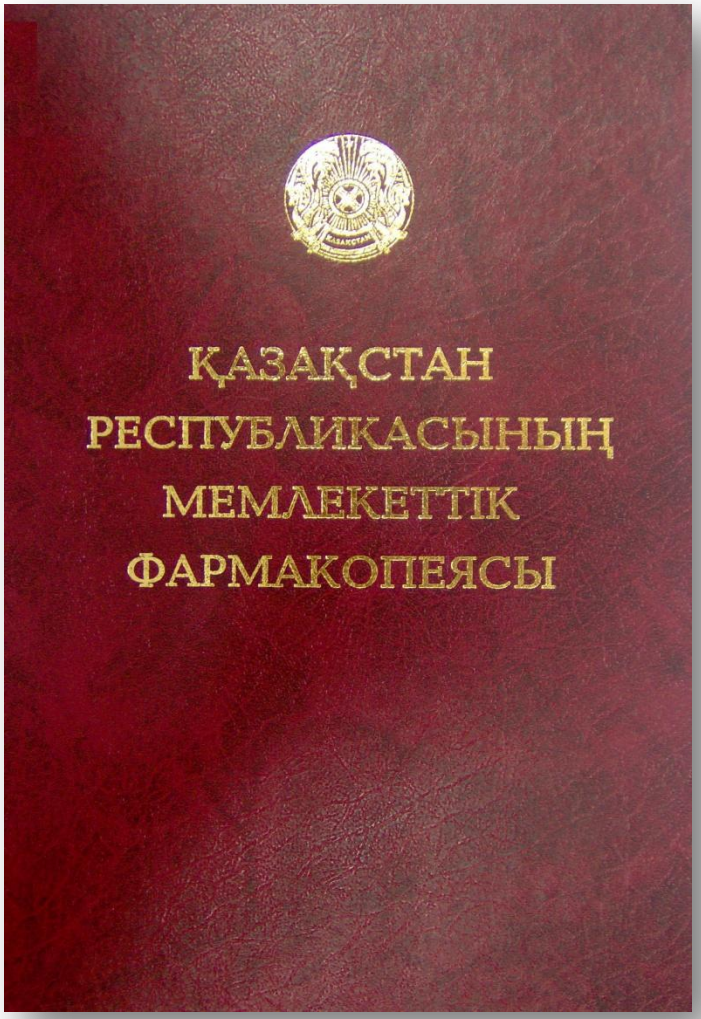
Symbols: ○- *Limonium gmelinii*, □ - *Limonium myrianthum*, △ - *Limonium otolepis*,

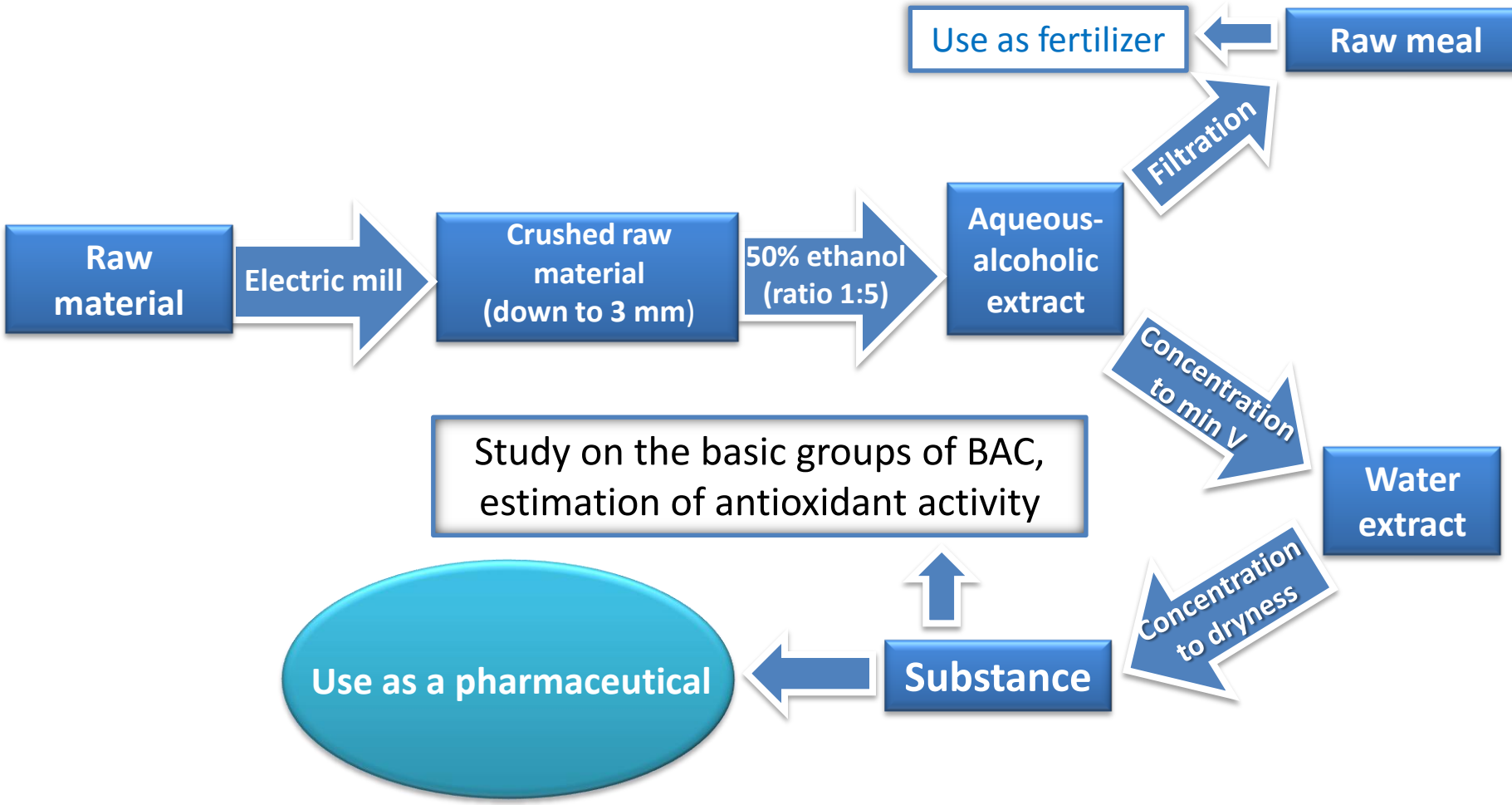
▭ - *Limonium Popovii*, ⏢ - *Limonium sufroticosum*, ◊ - *Limonium leptophyllum*

Stages of implementation of the drug



Study of raw materials quality in accordance with the rules governing the world's leading Pharmacopoeia, first and foremost, the European Pharmacopoeia and the State Pharmacopoeia of the Republic of Kazakhstan.





The scheme for obtaining the substance "Limonidin"

Physical, chemical and technological characteristics of the substance “Limonidin”

Characteristics	Test results				
Physical and chemical characteristics					
Color	reddish-brown with patches of darker particles				
Taste	bitter, astringent				
Odor	specific (faint)				
Shape of the particles	nonisodiametric crystal structure in the form of plates, the surface is complex and diverse				
Polarizability	only the smallest powder particles tend to polarize				
Solubility	soluble in 30% and 50% ethyl alcohol, insoluble in benzene, chloroform				
	Name of solvent	Temperature, ° C			
		25	40-50	100	120
	Purified water	MS	S	ES	-
	Glycerol	IS	VSS	-	ES
	Dimethyl sulfoxide	ES	-	-	-
	Propylene glycol	ES	-	-	-
	Polyethylene glycol 400	IS	IS	-	-
	Sunflower oil	IS	IS	IS	IS
	Note: ES - easily soluble, S - soluble, MS - moderately soluble, VSS - very slightly soluble, IS - insoluble.				
Wettability	Partial				
Hygroscopicity	Hygroscopic				
Technological characteristics					
Fractional composition, %	Not classified				
Bulk density, g/cm3	1.03±0.05				
Flowability, g/s	2.7±0.5				
Angle of repose, °	57.7±1.3				
Moisture content, %	12.0 ± 2.0				

The substance is:

- crystalline solid material with a brown color, bitter, astringent taste and a specific odor;

- soluble in aqueous alcohol solutions;

- characterized by low flowability due to strong hygroscopicity.

Dispersity of the substance was not classified, as this parameter is set technologically.

“Limonidin” substance:

Contains:

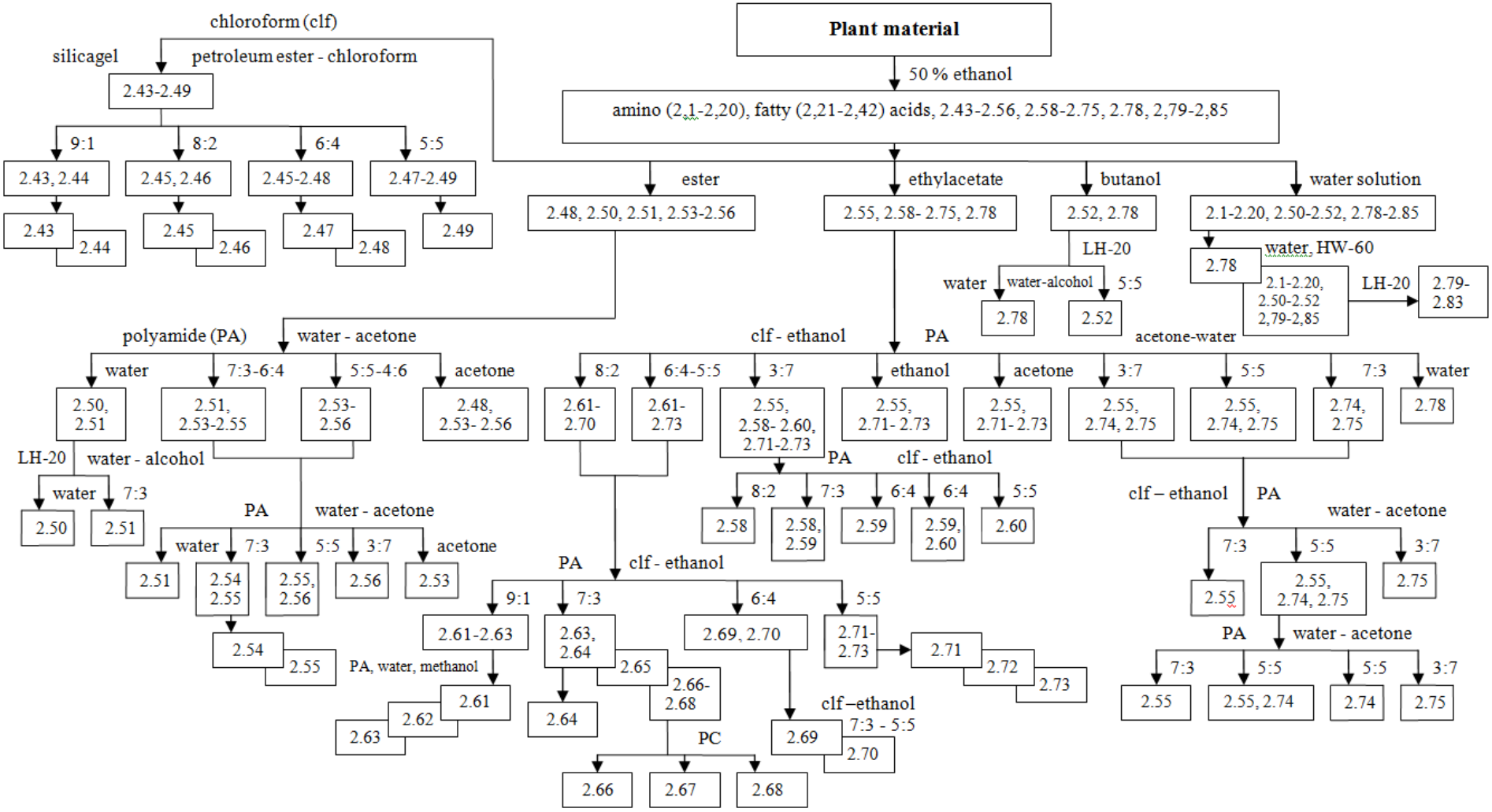
- phenols;
- phenolic acids;
- flavonoids;
- tannins;
- microelements;
- vitamins;
- amino acids;
- uronic acids;
- oligosaccharides

Recommended as:

antitumor,
hepatoprotective,
antioxidant,
astringent,
anti-inflammatory,
antiviral remedy and
immunomodulator

Registered and recommended by the Ministry of Health, RK for the industrial production and use in the medicinal practice (Pharmacopeia article 42-1243-04, RK-MP-3-No.008963 from 02.06.04)

During the chemical study, 82 compounds were found. The study has shown the presence of 34 microelements, vitamins (C, E and ß-carotene) and xanthophylls. 38 compounds were isolated and identified. Out of them the following compounds have not been previously described: campesterol-3-O-β-D-glucopyranoside, 3,5,7,3',4',6'-hexahydroxyflavone, myricetin-3-O-α-L-(2''-galloyl)-arabinopyranoside, 3,5,7,3',4',6'-hexahydroxyflavane and (-)-epigallocatechin-(4β→8)-(-)-3,5,7,3',4',6'-hexahydroxyflavane.



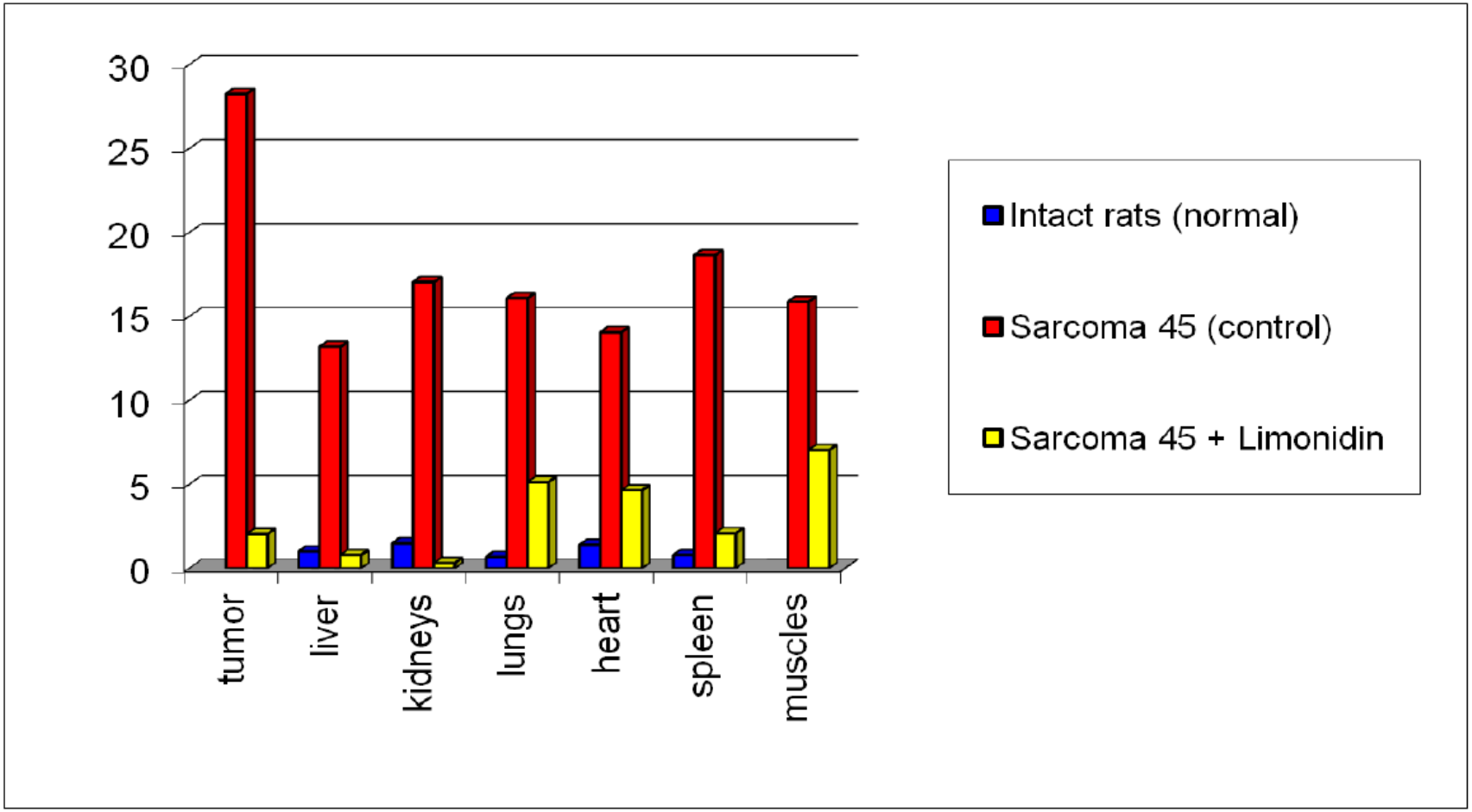
Scheme of separation of mixture of biologically active compounds, isolated from *Limonium* Mill genus plants

Preclinical study of the “Limonidin” substance

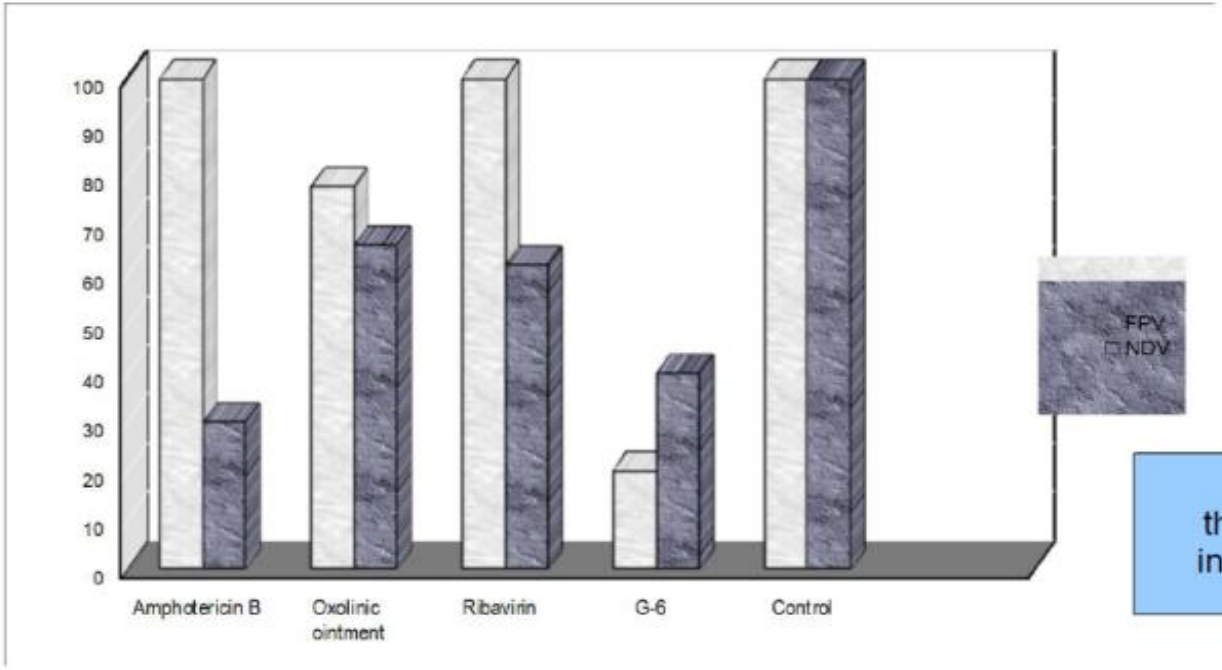
Results of study of the hepatoprotective activity of the "Limonidin“ substance

№	Test conditions	LHP (convent.un/g)	MDA (мM/g)	SOD (convent.un/g)	Catalase (convent.un/g)
1	Control	23.9±0.6	1.9±0.1	147.2±3.2	302.2±8.4
II	CCl_4	37.9±0.3	4.2±0.1	21.5±4.6	162.7±4.2
III	CCl_4+ Limonidin (100 mg/kg)	29.2±4.1	2.5±0.2	119.2±2.8	175.4±1.5
IV	CCl_4+ Silibor (200 mg/kg)	28.6±2.8	2.7±0.1	122.3±2.2	172.5±3.0

"Limonidin" action on content of the lactic acid
in tumor and basic organs of rats with sarcoma 45 mmol/g



Comparative analysis of antiviral activity of the “Limonidin” substance with commercial preparations



The effect of antiviral drugs on the level of microviruses reproduction: comparative study

Note:
the vertical axis - virus inhibitory activity (in %)

The substance Limonidin is indicated as G6, other medical products are Amphotericin B, Oxolinic ointment and Ribavirin. Mixoviruses used are A/FPV/Rostock/34 and NDV (La Sota).

The substance Limonidin had a higher level of suppression of the influenza and parainfluenza viruses reproduction in comparison with commercial drugs (Oxolinic ointment and Ribavirin) and its effect is comparable with that of Amphotericin B. The sensitivity of the paramyxovirus was greater than that of the orthomyxovirus. Comparison of the level of virus reproduction, depending on concentration of Limonidin, has shown that its reduction led to the decline of the antiviral activity of the preparation: at the concentration of 0.025 % suppression of the influenza virus replication was 80 % and that of Newcastle disease virus – 60%.

Action of the substance “Limonidin” (in %)
on the model of ortho- and paramyxoviruses

Concentration of the preparation	Avian influenza virus (strain A/FPV/Rostock/34)	Newcastle disease virus (strain La Sota)
2.5%	100	100
0.25%	100	100
0.025%	80	60
0.0025%	20	20

МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ И СОЦИАЛЬНОГО РАЗВИТИЯ РЕСПУБЛИКИ КАЗАХСТАН

РЕГИСТРАЦИОННОЕ УДОСТОВЕРЕНИЕ

РК-ЛС-3№021523

В соответствии с Кодексом Республики Казахстан «О здоровье народа и системе здравоохранения» настоящее удостоверение выдано:

Наименование владельца регистрационного удостоверения	**Химфарм АО**
Страна владельца регистрационного удостоверения	**КАЗАХСТАН**

в том, что лекарственное средство Зарегистрировано и разрешено к применению в медицинской практике на территории Республики Казахстан.

Информация о зарегистрированном лекарственном средстве

Торговое наименование препарата	**Лимонидин**
Международное непатентованное название (при наличии)	
Лекарственная форма	**Порошки**
Дозировка (концентрация)	
Фасовка	**По 0.1 кг, 0.5 кг, 1 кг, 1.5 кг, 2 кг, 5 кг, 10 кг, 15 кг в двойном пакете из полиэтилена. Субстанция**
Код АТХ	
Состав активных веществ	**Лимонидин**
Срок хранения	**3 года**
Порядок отпуска (по рецепту, без рецепта)	**Без рецепта**

Информация о производителе лекарственного средства

Тип организации или участок производства	Наименование организации	Страна
Производитель	**Химфарм АО**	**КАЗАХСТАН**

Дата регистрации (перерегистрации) 21.07.2015 г.
Действительно до 21.07.2018 г.
Номер приказа от 21.07.2015 г. №559

Ф.И.О. руководителя государственного органа
(или уполномоченного лица)

М.П.

УТВЕРЖДЕН
Директор по качеству
АО «Химфарм»
_____ Л.М. Бочкунова
«07» 06 2015 г.

ЭКСПЕРТИЗА ПРОВЕДЕНА
РГП на ПХВ «Национальный центр экспертизы лекарственных средств, изделий медицинского назначения и медицинской техники» МЗСР РК
_____
«10» 07 2015 г.

ПРИКАЗ
Комитета контроля медицинской и фармацевтической деятельности МЗСР РК
«21» 07 2015 г.
№ 559

ВРЕМЕННЫЙ АНАЛИТИЧЕСКИЙ НОРМАТИВНЫЙ ДОКУМЕНТ

Наименование лекарственной субстанции

Limonidinum

Лимонидин

МНН:

Наименование и страна организации - производителя

АО «Химфарм», Республика Казахстан

Наименование и страна владельца регистрационного удостоверения

АО «Химфарм», Республика Казахстан

Наименование и страна организации - упаковщика

АО «Химфарм», Республика Казахстан

Предполагаемое применение

Для изготовления нестерильных лекарственных препаратов

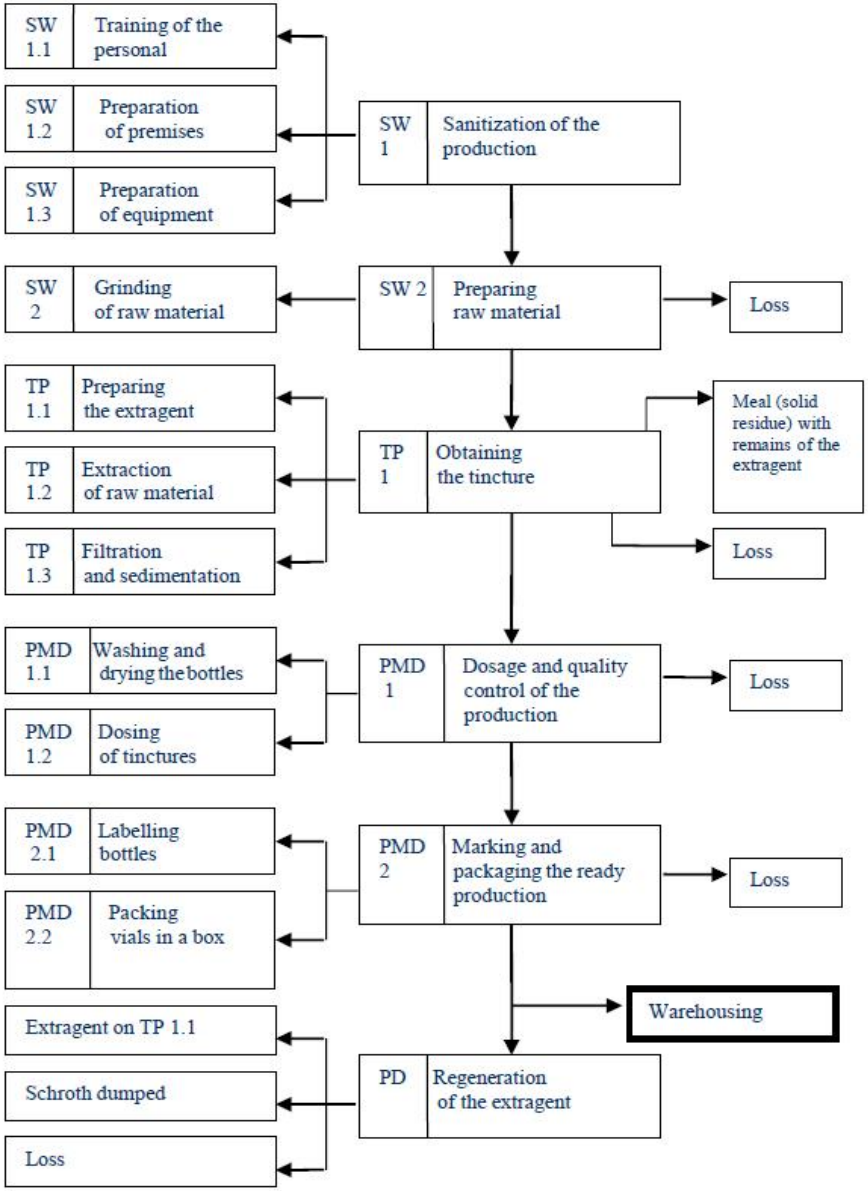
ВАНД РК 42-673-14

Вводится впервые

Срок введения установлен с
«21» 07 2015 г.
Срок действия до
«21» 07 2018 г.

ИЗДАНИЕ ОФИЦИАЛЬНОЕ **ПЕРЕПЕЧАТКА ВОСПРЕЩЕНА**

САРАПТАМА ЖҮРГІЗІЛДІ



Technological scheme of tincture "Limonidin"

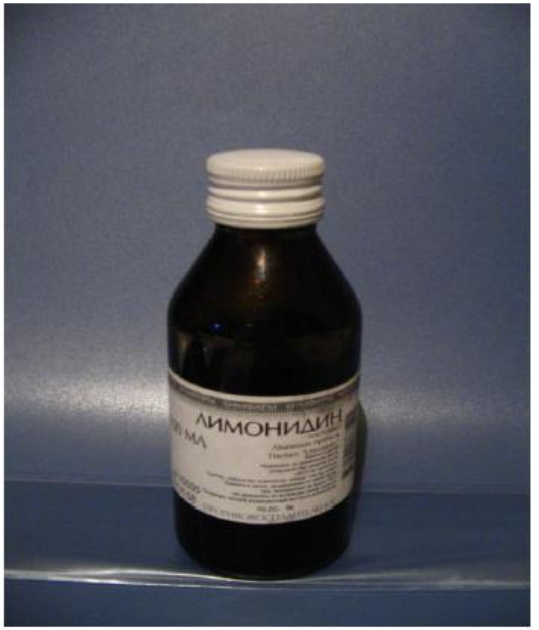
Tincture "Limonidin"

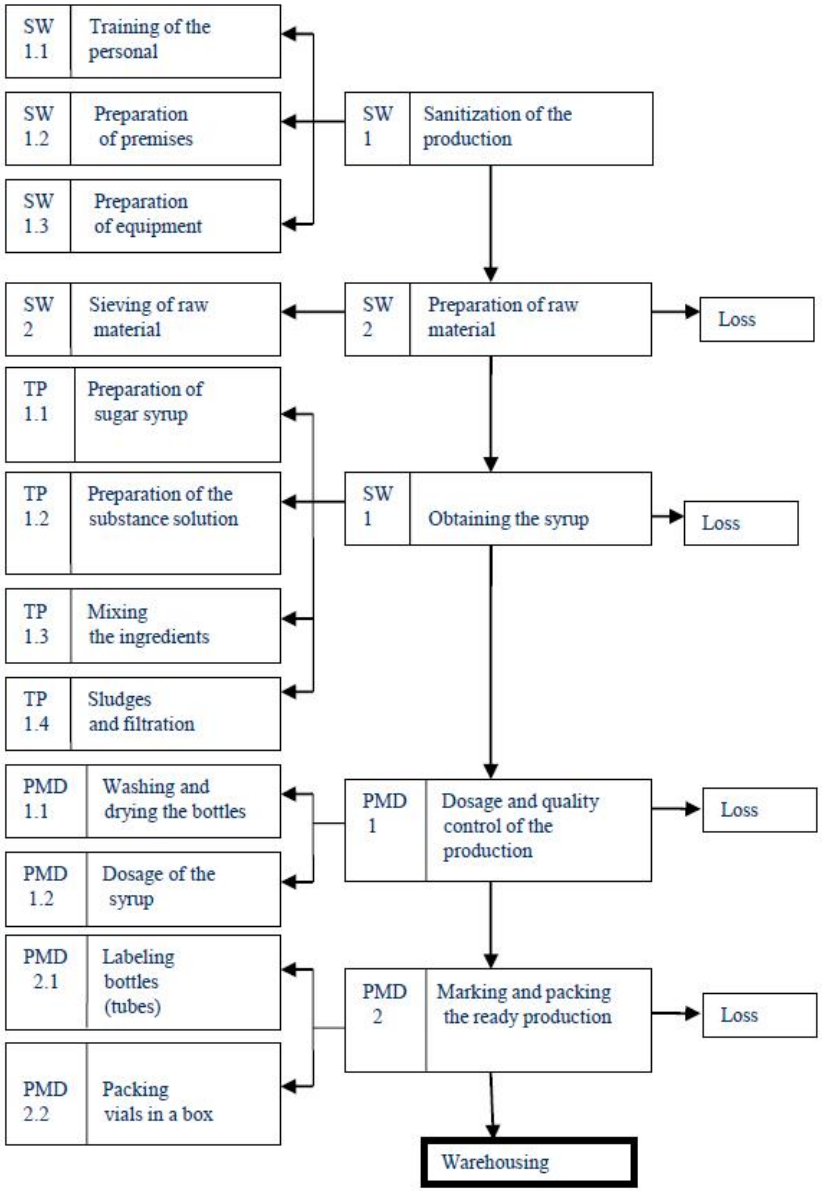
✓ I and II phases of clinical trials were conducted, which have been approved by the Pharmacological center;

✓ temporal pharmacopeia article of syrup "Limonidin" was considered and adopted by the Pharmacopeia center on 17.03.05;

✓ industrial regulation on production of tincture No. 288 was approved at the Ministry of Health on 29.05.06;

✓ application: anti-inflammatory agent.

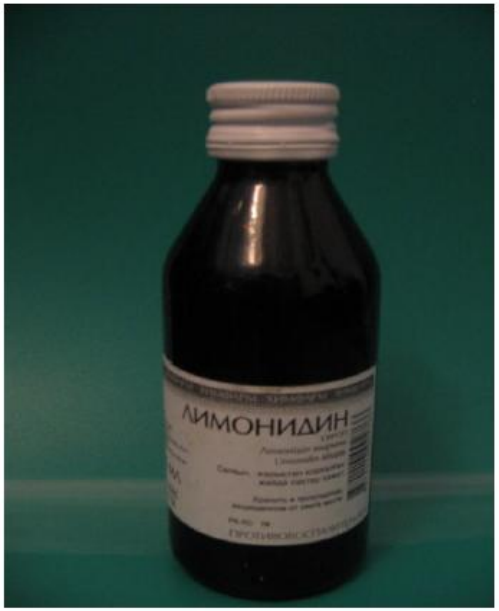


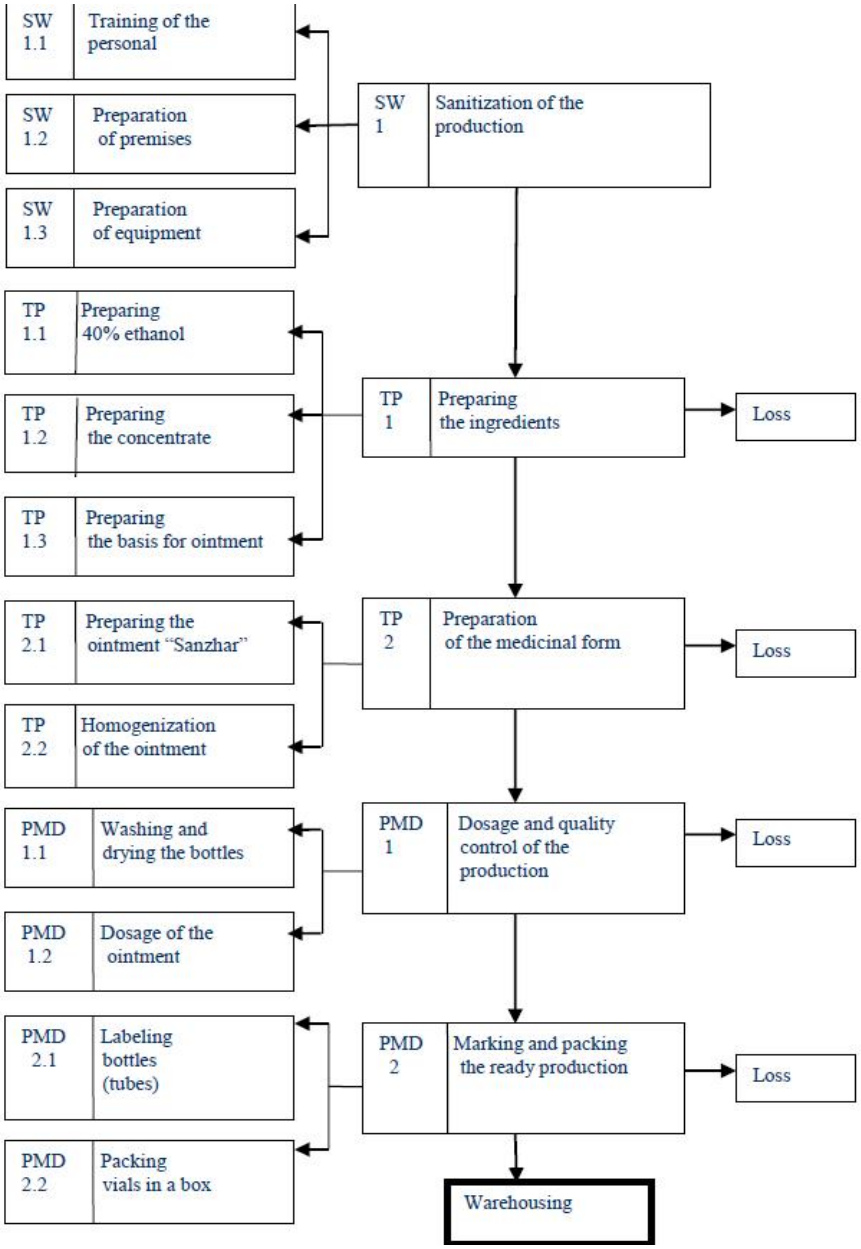


Technological scheme of syrup "Limonidin"

Syrup "Limonidin"

- ✓ I and II phases of clinical trials were conducted, which have been approved by the Pharmacological center;
- ✓ temporal pharmacopeia article of syrup "Limonidin" was considered and adopted by the Pharmacopeia center on 17.03.05;
- ✓ industrial regulation on production of syrup No. 289 was approved at the Ministry of Health on 29.05.06;
- ✓ application: anti-inflammatory agent.





Technological scheme on production of ointment "Limonidin"

Ointment "Limonidin"

- ointment "Limonidin" is included into the State register of the medicinal preparations of the Ministry of Health (PA RK 42-1277-08, Reg. RC-MP-5-No.008739 from 24.11.2008);
- temporal pharmacopeia article of ointment "Limonidin" was approved in MHCSDRK on 05.10.06;
- application: anti-inflammatory and antiviral agent.

Comparative cost of the ointment “Limonidin” and foreign ointments, used as references during its clinical trials

No.	Minimum retail price of ointments used as standards in clinical trials of “Limonidin”	Retail price of the ointment “Limonidin”
1.	"Bepanten" (France) - 790 tenge (30 g) (anti-inflammatory preparation)	Original plant domestic ointment “Limonidin” – 225 tenge (30 g) (anti-inflammatory and antiviral agent)
2.	Butadion ointment (Germany) - 270 tenge (20 g) (anti-inflammatory preparation)	
3.	Ointment Epigen (Spain) - 2500 tenge (5 ml) (antiviral agent)	

Road map

- Validation of analytical procedures, used for the quality control of the substance "Limonidin" and medicinal preparations, obtained on its basis
- Validation of the technological scheme of production of the substance "Limonidin" and medicinal preparations, obtained on its basis

Final product

- **Industrial manufacturing of the product for introduction into market**
- **Commercialization**

  Substance "Limonidin" and medicinal preparations, obtained on its basis have a high competitive ability in the field of price, quality and efficacy of the product

Conclude a license agreement with PC "Romat"

Patents

1. **A method for producing an anti-inflammatory, wound healing ointment, based on vegetable raw material. Preliminary patent RK No. 14987**;

2. **Agent for the treatment of periodontal disease, erosive and ulcerative lesions of the oral mucosa. Preliminary patent RK No. 14419**;

3. **Anti-inflammatory, wound healing pharmacological agent. Preliminary patent RK No.15257**;

4. **Pharmaceutical agent that is used in obstetric practice. Preliminary patent RK No. 16151**;

5. **Hepatoprotective agent. Preliminary patent RK No. 15679**;

6. **Antioxidant agent. Preliminary patent RK No. 15680**;

7. **A method for producing a total polyphenol complex roots of *Limonium*. Preliminary patent RK No. 14418**;

8. **A method for producing tincture of the roots of *Limonium gmelinii*. Preliminary patent RK No. 17339**;

9. **Use of the syrup from the roots as *Limonium gmelinii* agent having antiinflammatory activity. Preliminary patent RK No. 17338**.

Thank you
for your attention!