Akaki Tsereteli State University
Faculty of Technological Engineering



**Tinatin Kopaleishvili**

# Development of Formula and Technology of Soft Dosage Forms with Wound-Healing Activity Containing Tea Leaf Lipid Complex

**The Author's Abstract**
of the Doctoral Thesis Nominated for Ph Doctor Degree
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Scientific Advisor: Doctor of Biology, Professor **Aleko Kalandia**

Reviewers: **Temur Revishvili** Doctor of Science of Technics, Professor.
**Ketevan Kintsurashvili** Doctor of Science of Technics, Professor, Akademician of Abkhazia Science Engineering Academy.

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## General description of Research

### Topicality of Research

The intense scientific interest in the issue of wound pathogenesis and treatment is explained by an increase in injuries, natural disasters and terrorism.

In recent years, in modern medical practice, use of plants has been becoming increasingly important that is explained by the combined influence of biologically active substances contained in medicinal plants on the human body, the practical absence of toxic effects and by the sharp increase in complications caused by synthetic therapeutic agents.

Preparations containing vegetable lipid complex or the compositions of fat-soluble A and E vitamins are currently being widely used for treatment of wounds, burns and skin lesions of different etiology. Various vegetable oils and the preparations based on them are often used in the combination therapy of benign and malignant tumors, whose high therapeutic effect is explained by the existence of various biologically active substances in them.

The lipid-type biologically active substances such as fat-soluble vitamins, polyunsaturated fatty acids and phospholipids participate in the building and repair of the new cells, constitute a source of bioregulators and have a substantial impact on normalizing blood formation occurring in the human body, as well as on the improvement of the conditions of the skin and mucous coats.

The modern pharmaceutical world market is saturated with means of treatment and prevention of skin diseases. A significant proportion in the arsenal of the medical doctors specializing in skin diseases is accounted for by symptomatic medications – antibiotics and spirituous compositions, to which the microflora of the skin coating is gradually becoming resistant. The alcohol dries out the skin too much that is accompanied by the complete destruction of the lipid barrier of the skin.

So far, basic and applied research works have been stepped up on a broad front, which are focused on a comprehensive examination of natural, lipophilic biologically active compounds.

The pharmaceutical industry is committed to making available the selection of raw material resources and the development of technology for producing the natural lipid preparations for treatment of the damaged skin, containing an optimal ratio of the optimal micro- and macroelements, vitamins and indispensable fatty acids with a perfect formula, as different from the synthetic chemical compounds.

t should be noted that despite a comprehensive study of tea culture, interest in it has not been diminished so far, because tea leaf lipid complex obtained from it – extractive oil, shows much higher content of biologically active components valuable for the organism, compared to "classical" tea extracts or other plant-based medicinal oils.

High pharmaco-therapeutic effectiveness of medication based on tea leaf extractive oil, a severe shortage of cheap, practically unused, a little coarse tea green raw materials and medications for treatment of wounds, against a background of recovery of "Georgian tea", is a prerequisite for the cost-effective production.

Consequently, we believe that it is relevant and promising to develop the main aspects of the production of tea leaf extractive oil, especially since in the case of rational selection of organic solvent and processing regime, there will be created good conditions for increasing the yield capacity of oil.

At the same time, the development of formulas and technologies of the wound healing ointment and suppositories containing tea leaf lipid complex is relevant, promising and represents a defense medical counter-measure during extreme situations.

**Goal and objectives of research**

The goal of research is to develop formulas and technologies of tea leaf extractive oil and suppositories containing extracts obtained from environmentally pure medical plants of flora of Georgia, and ointments for healing of wounds of different etiology.

In order to achieve that goal, the following objectives have been defined:

1. Analytical literature survey and formulation of the main areas of scientific research. Search and installation of the research equipment.
2. An influence of conditions of the extraction of tea leaf extractive oil on the quality and quantity characteristics of target product.
3. Selection and justification of medicinal vegetable ingredients of ointments with wound-healing activity.
4. Development of technologies for producing stinging nettle leaf and sweet (unvinified) grape-stone hydrophilic extracts and plantain juice.
5. Development of technologies for producing suppositories containing tea leaf extractive oil.
6. Justification of the optimal vegetable composition of medical ointment containing tea leaf extractive oil for treating wounds of different etiology, and developemnt of their technological schemes.
7. Pharmacological study of the developed medical ointment.
8. Developing the methods for controlling the quality of the developed medical oinment and suppositories.



**Research novelty of the work** is conditioned by the fact that based on the analysis of literature sources, there have been justified the possibility of creating the soft therapeutic preparations having wound-healing activity from medicinal plant raw materials. There have been theoretically justified and experimentally confirmed a pattern of the tea leaf extraction with chloroform, as well as optimal conditions of its implementation. The developed technologies of tea laef extractive oil ensure maximal transfer of biologically active substances from tea laef to the extract.

- There have been justified the properties of the phytocomponents of stinging nettle and sweet (unvified) grape-stone hydrophilic extracts and plantain juice containing in ointiment with wound-healing activity. By using the standard, generally recognized and modified organoleptic and physico-chemical methods of research, including high-pressure liquid chromatography, spectrometry and fluorometry, there have been investigated their chemical compositions and developed the rational technologies for their production.
- There have been developed the compositions and technological schemes for producing ointment and suppositories containing tea leaf extractive oil.
- There have been carried out pharmacological studies of the mentioned soft dosage forms having wound-healing activity, based on their toxocological studies and investigation of wound-healing action.

**Practical bearing of work.**

There have been developed and practically implemented the rational technologies for producing tea leaf extractive oil, stinging nettle and sweet (unvinified) grape-stone thick hydrophilic extracts and plantain juice. The optimal parameters of technological processes for each of them have been also selected.

There have been developed formulas and technollogical schemes for producing ointment and suppositories containing tea laef extractive oil intended for treatment of wounds.

Pharmacological studies of ointment containing tea leaf extractive oil have been carried out in the training-testing laboratory of physiology at the Akaki Tserete State University's Biology Department.

**Approbation of work.** The main results of research were presented at the sessions of the Akaki Tserete State University's Department of Chemical and Environmental Technologies (2013-2017). The results of research were discussed and published in the proceedings of the following international scientific conferences:

- International Scientific-Practical Conference "Actual Problems and Modern Food Production Technologies", Kutaisi, ATSU, 2015.
- International Scientific-Practical Conference "Prospects for Integration of Science and Practice", Stavropol, 2014.
- International Scientific-Practical Conference "Innovative Technologies for the Production of Functional Foods", Kutaisi, ATSU, 2015.
- International Scientific-Practical Conference "Modern Engineering Technologies and Environmental Protection", Kutaisi, ATSU, 2016.

**Publications.** 8 scientific articles around the topic of the thesis work have been published in Georgian and international high-rated periodicals.

**Volume and structure of work.**

The thesis work comprises 122 computer printing pages. It includes Introduction part, 5 chapters, the main conclusions and list of references with 135 titles of literature sources. It contains 20 tables, 14 drawings and 20 pictures.

**Brief content of work.**

The thesis work includes introduction, 5 chapters, the main conclusions and list of references.

In the introduction, attention is given to topicality, goal and objectives of research, as well as to research nоelty and practical bearing.

**The first chapter – Literature Survey,** describes the current state of research regarding the creation of soft dosage forms containing the extractive plant components with wound-healing activity. There are presented data on the phytocomponents, which are more frequently used for the creation of



dosage forms. There are also described the modern approaches to the external use of the dosage forms for pharmacocorrection of the wound process. The chapter also dwells on the analysis of a contemporary understanding of tea leaf lipid complex and its component parts, as well as of pharmacotherapeutic and wound-healing activity of lipid complex. There is formulated precondition for the selection and justification of ingredients containing biologically active substances of the wound-healing preparations. The chapter considers theoretical preconditions for the extraction of drug raw materials, and shows that the destruction of a certain number of cells has a great influence on the course of the extraction process that contributes to rapid solution of biologically active substances by the extracting agent, as well as to their outwashing from the intracellular structures.

The chapter describes the prospects for using the tea leaf extracting oil, stinging nettle and grape-stone sweet (unvinified) extracts, and plantain juice with anti-inflammatory, wound-healing, antibacterial and antiseptic action, for the creation of the external-action soft dosage forms.

**The second chapter** dwells on the objects and methods of research.

The object of the research is a little coarse and coarse tea leaves taken from the plantations Tskhaltubo district in 2011-2013; plants collected in Georgia's mountainous, environmentally pure regions (Guria, Adjara); stinging nettle and plantain – during the flowering stage, when the mentioned plants are characterized by higher content of biologically active substances; sweet grape-stone during the grape pressing process.

When performing this work, we used the standard, generally adopted and modified organoleptic and physico-chemical methods of research, high-effective liquid chromatography, spectrophotometry, and fluorometry. Research was conducted in accordance with international standards (ISO-International Organization for Standardization).

**Quantification of flavonoids** was carried out by using the method of high- [pressure liquid chromatography.

**Quantification of total phenols** – by spectrophotometric method, by using Folin-Ciocalteu reagent. Detection of the action of the reagent on phenolic compounds was carried out at 660 nm.

**Antioxidant activity** was determined by DPPH method.

**The qualitative composition of lipids** was determined by method of thin-layer chromatography on the "Silufol" plates.

For the experiment, we used modern methods of mathematical planning, particularly, the central composite rotatable design matrix, which is well suited for the extraction process. To determine the optimal regimes, we used the classical method of Lagrange infinite multipliers of the solution to compromise problems.

Pharmacological assessment of dosage forms and identification of physico-chemical characteristics were carried out by using the well-known standard methods.

**To study the elastic-viscose-plastic properties** in an experimental sample, there was used the rotation viscometer PB -8.

**Microbiological purity was determined** in accordance with the State Pharmacopeia XII, 1 edition, paragraph 32.

**Chapter 3 – Technological study of tea leaf extractive oil.** Based on the assigned tasks, the attention was given to obtaining extractive oil from tea leaf.

For the purpose of maximizing the yield capacity, there were carried out studies for determining the optimal parameters of the extraction, for which the extracting tea leaf was treated before being delivered to the extraction.

For the purpose of studying the influence of **the quality of dispersion,** the air-dried tea was dispersed to particles with sizes of 3 mm, 5 mm and 10 mm. The yield capacity and quality parameters of tea leaf extractive oil with the dependence on the quality of dispersion are presented in Table 1.

Table 1

**The dependence of the yield capacity and visual characteristics of tea leaf extractive oil on the quality of dispersion of raw materials (at 10-12% moisture content of raw materials)**

| The quality of tea leaf dispersion,mm | The yield of oil, % from the overall content | Color of extractive oil and its transparency |
|---|---|---|
| 10 | 80-83 | Oil-bearing, mobile liquid, green color. |
| 5 | 90-92 | Oil-bearing liquid, green color |
| 3 | 75-82 | Viscose, dark green color. |

From the data presented, we recommended the fraction with the quality of dispersion at 5 mm.



Acting with solvent of particles on the quality of wetting, as well as on the diffusion from fat particles, the **moisture content** of extractive oil has a significant influence on the extraction process. Since the moisture content of tea extraction raw material is within standards and does not exceed 10-12%, ot turned out that there is no need for undertaking additional measures to conduct an extraction.

Of the major factors influencing on the extraction process, the emphasis should be placed on a **temperature**, which is determined by temperatures of extracting raw materials and the extracting agent, and the ratio of their values.

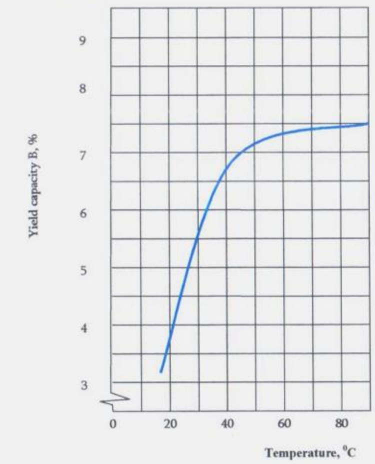



**Fig. 1. The temperature dependence of the yield capacity of tea leaf extractive oil**

Taking into account thermal instability of the main active substance in tea leaf extractive oil – carotenoids, fatty acids and tocopherols, when obtaining extractive oil from tea leaf, as an extracting agent there was selected chloroform with a relatively low boiling temperature (50-55°C).

As is seen from investigations, under the influence of the extraction temperature, the yield capacity of tea leaf extractive oil is increased significantly (Fig.1). A temperature between $52^0$ and 55°C was taken as an optimal temperature of the extraction.

Depending on yield capacity of oil., an important factor influencing on the extraction process is the **extraction duration**. Studies have revealed that extraction of product is reasonable close to the boiling temperature of extracting agent. The relationship between the yield capacity of oil and the extraction duration is illustrated graphically in Fig. 2, from which, it can be seen that by reducing (Curve 1), and by increasing (Curve 2) the share of a tender fraction in extracting raw material (Curve 1), the yield capacity goes down. The maximum yield capacity of product is achieved, when the content of a tender fraction in extracting raw material is 8-12% (Curve 3). The optimal content is 10%. In all cases, the stationary state is stabilizing 120-125 minutes after extraction.

The right choice of **the ratio of the extracting agent and extracting raw material**, i.e. the hydro-module, is the first prerequisite for increasing the yield capacity of product. The increase in the mentioned ratio leads to the increase in the yield capacity of product. The 75%-yield of product is achieved by the ratio of the extracting agent and tea leaves in a range of 5-7 l/kg Fig. 3).

For extraction of tea leaf, we used the matrix of the experiment's central composite rotatable design.

Geometric interpretation of the obtained regression equations is illustrated in figures 4 and 5.

Optimal conditions of the extraction of teaf leaf oil are as follows: extraction temperature t=52-55$^0$C; extraction duration - m=120-125 min; ratio extracting agent/tea leaf - n=5 l/kg; vibration frequency – 5 sec$^{-1}$; pulsation amplitude – 3 mm. In the case of extraction with chloroform, from each kg of raw material, it is possible to obtain practically at low unit cost on average not less than 75 kg of precious oil.

A technological scheme for extraction of tea leaf with organic solvent and



producing of oil is shown in Fig. 6.

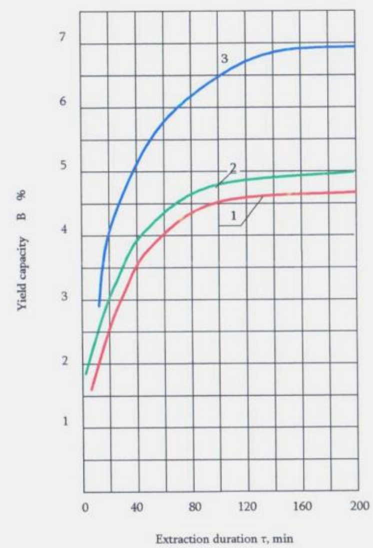



Fig. 2. The dependence of the yield capacity of tea leaf extractive oil on the extraction duration, in the case of shares of a tender fraction as follows: ,%: 1-2; 2-20; 3-10;

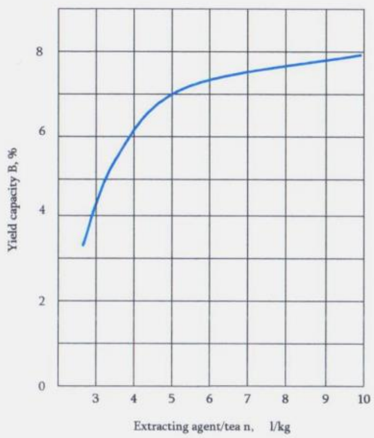
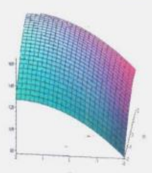
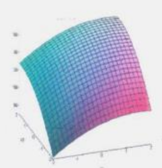



**Fig. 3. The dependence of the yield capacity of tea leaf extractive oil on the value hydro-module.**



$Y_1 = 72,5 + 9X_1 + 12X_2 - 3X_1^2 - 3X_2^2$

**Fig.4. The diagram of the dependence of the extraction temperature ($X_1$) and extraction duration ($X_2$) on the yield capacity of oil ($B_o$, kg/t)**



$Y_1 = 72,5 + 9X_1 + 14X_3 - 3X_1^2 - 4X_3^2$

**Fig. 5. The diagram of the dependence of of the extraction temperature ($X_1$) and "solvent-raw material" ($X_3$) on the yield capacity of oil ($B_o$, kg/t)**



Extracting raw material containing the dried out, a little coarse and coarse fractions with mosture content until 10-12%, is supplied to fixation, where fixation of tea leaf is carried out by means of vapor at temperature of 105$^0$C, within 20-25 minutes. Then, the fixated tea leaf is delivered to the rolling press grinder-crusher, from which, the crushed tea leaf, by means of dispenser, is supplied to the innovative batch extraction device with a pulsator. Here, organic solvent – chloroform, is added to the extracting tea leaf a ratio of 5 l/kg. The sequence of loading is conditioned by the difference of the densities of tea particles and chloroform (900 kg/m$^3$ and 1,483 kg/m$^3$, respectively), as a result of which tea leaf is completely washed with solvent; tea particles are floating to the surface, where the space, during the extraction process, is saturated with the vapor of solvent, and the environment is anoxic. This excludes the possibility of oxidation of extracting substances during the extraction process.

Extraction is lasting for 120-125 minutes, at temperature of 52$^0$-55$^0$C, vibration frequency of 5 sec$^{-1}$ and under conditions of pulsation at an amplitude of 3 mm.

After completion of the extraction process, the extracts are unloaded from the extractor, which is filtered, and in order to remove organic solvent chloroform, they are distillated in a R 1020-type batch rotary vacuum-evaporator under vacuum conditions at 7,90 – 92 kPa.

After completion of distillation, tea leaf extractive oil undergoes filtration, and it is collected in the tanks, but the vapor of solvent, together with water vapor, is condensed and flowing down into the Florentine vessel, where, under the influence of strictly different densities, it is separated into the water and solvent.

By chloroform extraction under optimal conditions, physico-chemical characteristics of oil obtained from the air-dried tea leaf are presented in Table 2, and chromatographic description of the fatty acid composition is shown in Pic. 1.

As the result of the analysis, we have identified palmic acid, oleic acid, linoleic acid and linolenoic acid, and their percentages have been determined.

It turned out that linolenoic acid prevails in tea leaf extractive oil - 43-45%, and accordingly, palmic acid - 23-24%, linoleic acid - 15,5-16%.

Thus and so, tea leaf extractive oil rich in biologically active substances is a very promising component for producing medical ointments and suppositories for healing wounds of different etiology.

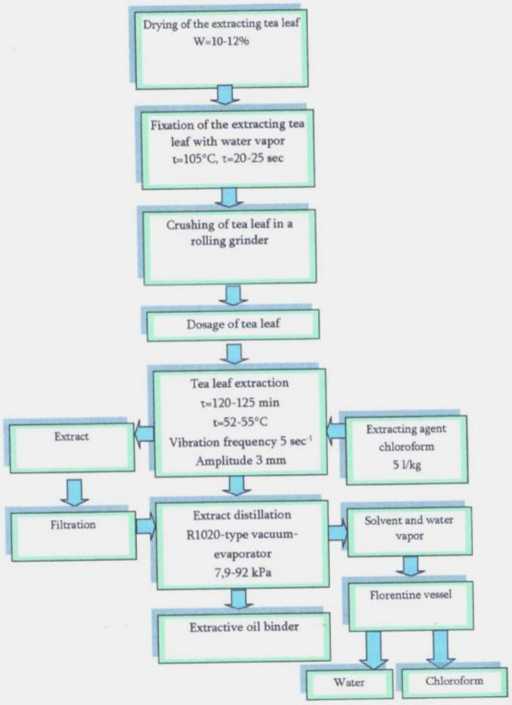


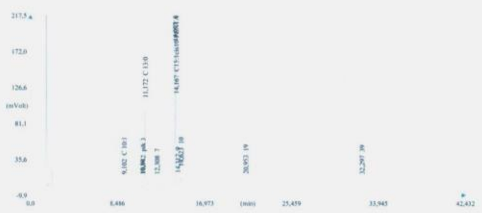

**Fig. 6. Technological scheme for producing tea leaf extractive oil**



Table 2

Physico-chemical characteristics of tea leaf extractive oil with chloroform

| Item name | Characteristics |
|---|---|
| Appaearance | Brown-green, quick-drying, viscose oil-bearing liquid, with a characteristic smell |
| Yield capacity, % | 6,0-7,5 |
| Density, ρ g/cm$^3$ | 0,920-0,925 |
| Acidity index, mg KOH/g | 0,40-0,45 |
| Iodine index, gJ$_2$/100g | 80-95 |
| Change index, $n^{20}_{s}$ | 1,470-1,475 |
| Total carotenoids, mg/g<br>β -carotine | 16,5-17,5<br>1,80-1,85 |
| Total tocopheols, mg/g<br>including:<br>α- tocopherol<br>β+γ – tocopheols<br>δ – tocopherol | 1,70-1,80<br><br>1,30-1,40<br>0,06-0,07<br>0,30-0,35 |
| Chlorophylls, mg/g | 17,0-18,0 |
| Pheophytins, mg/g | 25-26 |
| The content of basic fatty acids, % from total amount:<br>- palmic acid<br>- oleic acid<br>- linoleic acid<br>- linolenoic acid | <br>23-24<br>6,0-6,5<br>15,5-16,0<br>43-45 |
| Group composition of lipid complex, on average, % :<br>- polar lipids<br>- sterols<br>- higher alcohols<br>- free fatty acids<br>- triglycerides<br>- waxes<br>- ether sterols<br>- carbohydrates | <br>19,4<br>4,5<br>0,7<br>2,5<br>35,8<br>3,0<br>33,5<br>0,6 |
| Antioxidant activity AA,% measer..1:25 | 0,3 |



**Pic. 1. High-pressure chromatogram of tea leaf higher extractive oil**

**Chapter 4. Development of technology of phytocomponents and study of biologically active substances.**

To obtain hydrophilic stinging nettle extract we used the air-dried nettle leaves collected during the growing period the highlands region of Adjara, which comparative to the other periods, is characterized by higher content of biologically active and hydrophilic compounds.

To obtain thick hydrophilic stinging nettle extract, there was used a single retention of raw material -bismaceration. There were selected optimal conditions of conducting the extraction: the extracting agent-70%-alcohol-water solution; the quality of crushing the nettle leaves – 3 mm; hydro-module – 1:10; extraction frequency – 2; extraction time – 120 min; for the extraction, there were used the innovative batch extractor with a pulsator we developed (vibration frequency – 2-3 $sec^{-1}$ and an amplitude – 1-2 mm).

The calculated quantity of the extracting agent was divided into two rations, one ration was added to crushed raw materials loaded previously into the extractor and retained there for the extraction at 50-55$^0$C. During the extraction process, every 15 minutes, within 1 minute, there was carried out pulsation with frequency of 2-3 $sec^{-1}$ and an amplitude 1-2 mm.

After pouring off the primary extracted mass, raw materials were pressed out and then we added the second portion, and retained it again. At the end, we bridged both alcohol-containing extracts of nettle, and then placed them were bridged in a cool spot (in a cold-storage chamber at 4-8$^0$C for 12 hours. After the precipitation of ballast (resinous) substances, the



extracted mass was filtered through the paper ash-free filter, that resulted in obtaining transparent dark green alcohol liquid.

The alcohol-containing extracted mass filtered for obtaining thick extract (with mositure content not highre than 25%) was condensed by distillation of ethyl alcohol at 60$^0$C, in a R 1020-type rotary vacuum-evaporator.

The obtained extract is a dark-green thick, viscose mass with a pleasant specific smell and bitter taste. The thick extracting agent well soluble in water and alcohol. It is kept in glassware.

Chemical composition of extract was studied by method of high-pressure liquid chromatography. The results of this study are presented in Tables 3 and 4.

Table 3

The content of chlorophylls and carotenoids in stinging nettle extract mg/g calculated on a dry weight basis

| Name of sample | Chlorophylls | | Total chlorophylls | Carotenoids |
|---|---|---|---|---|
| | a | b | | |
| Nettle extract | 2,465 | 1,73 | 4,83 | 0,86 |

Table 4

The content and antioxidant activity of biologically active compounds in stinging nettle extract

| Name of sample | Total phenols mg/100 g calculated on a dry weight basis | Total flavonoids mg/100 g calculated on a dry weight basis | Antioxidant activity | |
|---|---|---|---|---|
| | | | Dissolution factor | AA,% |
| Nettle extract | 30,84 | 11,04 | 25 | 56,45 |

The results of the study reveal that stinging nettle extract is characterized by high content of biologically active substances, and has high antioxidant activity. The content of the mentioned substances is what conditions the wound-healing effect of nettle extract, as well as stimulation of regeneration of lesional tissues and mucous membrane. Thus, nettle extract is a significant component of the wound-healing soft drug phytopreparations.

In order to develop technology for obtaining extract of biologically active grape-stone, there was made preliminary determination of the extraction optimal parameters: hydro-module – 1:2; extraction duration – 2 hours; extraction temperature - $60^0C$; extracting agent water 2% with citric acid. Studies have shown that the most complete extraction of biologically active substances took place during preliminary drying of washed grape-stone, in a drying box at a temperature of $60^0C$. The dried out, crushed grape stone of 0,5 mm was placed in the innovative batch extractor with a pulsator (vibration frequency – 2-3 $sec^{-1}$ and amplitude – 1-2 mm).

After the completion of the extraction process, the extract 1 was poured out of the extractor, and raw materials remained in the extractor were subjected to the re-extraction by using the same method. After completion of the extraction process, there was obtained the extract 2, which was added to the extract 1, and then it was dried out in a drying box at $60^0C$ until the 50% of dry matter content.

The results of the organoleptic and biochemical analysis of obtained extract are presented in Tables 5 and 6, and chromatographic description of flavonoids is illustrated in Pictures 2 , 3.

Table 5

Organoleptic parameters of unvified grape-stone extract

| Name of sample | Parameters | | | |
|---|---|---|---|---|
| | Aggregate state | Color | Taste | Odor |
| Grape-stone extract | A little cloudy liquid | Cream-colored | Bitterish | Odor-free |

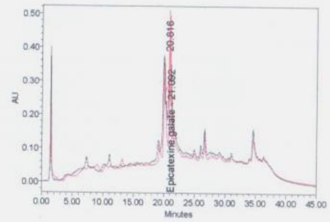
Table 6

Biologically active parameters of unvified grape-stone extract

| Name of sample | Parameters | | | | |
|---|---|---|---|---|---|
| | Total phenols, mg/ g calculated on a dry weight basis | Total flavonoids, mg/ g calculated on a dry weight basis | Flavonols, mg/g calculated on a dry weight basis | Leukoanthocyanins, calculated on a dry weight basis | Antioxidant activity, mg/g dissol. 1:20 |
| Grape-stone extract | 39,1 | 17,24 | 11,58 | 4,62 | 61,8 |

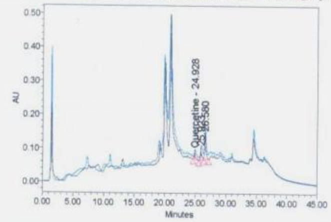
It has been established that the obtained sweet grape-stone extract is rich in biologically active substances, characterized by sufficiently high antioxidant activity. Extract is stable during the heating to $100^0C$. Accordingly, it would be feasable and practical to use this extract as a component of therapeutic phytopreparations.






| | SampleName | Acq Method Set | Injection Volume | Channel Description | ColumnType |
|---|---|---|---|---|---|
| 1 | wibwis eqstraqti 1 | flavonoidi 280360 06 40 | 10.00 | W2489 ChA 280nm | C 18 |

**Pic. 2. High-pressure chromatogram of flavonoids in sweet (unvinified) grape-stone extract**




| | SampleName | Acq Method Set | Injection Volume | Channel Description | ColumnType |
|---|---|---|---|---|---|
| 1 | wibwis eqstraqti 1 | flavonoidi 280360 06 40 | 10.00 | W2489 ChA 280nm | C 18 |

**Pic.3. High-pressure chromatogram of flavonoids in sweet (unvinified) grape-stone extract**

Plantain, as a herbaceous plant with a tanning effect, has a recognized position among medications using in practical medicine. Juice obtained from new herb is distinguished by wound-healing and antimicrobial action pathogenic microbes.

A significator factor of obtaining increased quantity of juice from the plantain leaves is the quality of dispersion of plantain leaves. We have established a method for crushing of plantain leaves and the optimal quality, which ensures more fast and complete drying off of leaves that results in increasing the total yield capacity of extracting substances. In this regard, we developed technology for producing natural plantain juice by using fermentation – previous biostimulation of leaves, for which, the newly-selected full plantain leaves were kept in the refrigerator at 5-8$^0$C for 10 days, that resulted in disintegration of leaves into particles of 3-5 mm, and then they were placed in thermostat at 37$^0$C for 24 hours.

Then, we stopped fermentation and re-disintegrated the blend, until obtaining a single coherent mass. This mass was pressed out in a press machine under pressure of 250-300 atmospheres. For the purpose of preservation, 25 parts of 90%-ethyl alcohol and 0,15% of sodium bisulphite were added to the obtained juice, under conditions of continuous stirring. The juice was retained for 7 days, and then it was filtered.

The results of the analysis of biologically active substances existing in the obtained plantain juice are presented in Tables 7 and 8.

Table 7

The content of chloropylls and carotenoids in plantain juice, mg/kg calculated on a dry weight basis

| Name of sample | Chloropylls | | Total chloropylls | Carotenoids |
|---|---|---|---|---|
| | a | b | | |
| Plantain juice | 55,76 | 86,73 | 148,33 | 69,29 |

Table 8

The content of biologically active compounds in plantain juice, mg/kg calculated on a dry weight basis

| Name of sample | Total phenols, mg/100 g calculated on a dry weight basis | Total flavonoids, mg/100 g calculated on a dry weightbasis | Antioxidant activity | |
|---|---|---|---|---|
| | | | Dilution factor | AA,% |
| Plantain juice | 648,65 | 217,44 | 25 | 51,75 |



The results of research demonstrate that plantain juice is distinguished by high content of biologically active substances, and high antioxidant activity. and it is a significant component of wound-healing sift phytopreparation.

**Chapter 5. Prospects for using tea leaf extractive oil for preparing soft dosage forms**

Preliminary studies of anti-inflammatory action of tea leaf extractive oil enabled us to develop rectal **suppositories** on its basis, one of the development stages of which is the selction of suppository bases, which is aimed at ensuring biopermeability, stability of the mentioned dosage form and evenness of distribution.

To produce suppositories, there have been selected the hydrophobic bases as follows: solid confectionery fat, witepsol H15, and cacao oil, and the mixture of cacao oil and bee wax (4:1).

Basis selection was made by visual assessment of suppositories, and according to such a traditional parameter as structural-mechanical property and the release rate.

Initially, during visual assessment of suppositories obtained from tea leaf extractive oil, there was excluded the use of solid confectionery fat as a basis, since suppository form made on this basis was indistinct, and the process of removal from dosage forms was complicated. Also, there was excluded the cacao oil and bee wax mixture, since the structural-mechanical parameters of this mixture, such as melting temperature (39,6°C) and total deformation time (18,4 min) are far greater than required by State Pharmacopeia standards. The preference was given to witepsol and cacao oil bases.

To select the optimal basis from the above mentioned ones, by method of dialysis on release of carotenoids, the results of studies have shown a slight difference in the release rate of suppositories. Thus, as an alternative to witepsol, it is possible to use also cacao oil, however, due to its low emulsionizing capacity, along with them, it should be taken into consideration the use of surface-active substances.

Suppositories have been obtained in polystyrene forms by casting method, with the following composition: tea leaf extractive oil - 0,5 g, witepsol – 2,0 g calculated on 2,5 g mass per suppository. Suppositories have been produced without emulsifier.

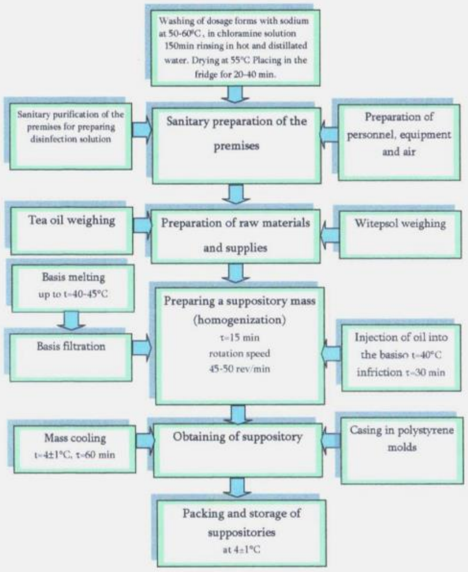



**Fig. 7. Technological scheme for producing suppositories**

Technological scheme of preparing suppositories from tea leaf extractive oil is shown in Fig. 7.

The quality of suppositories obtained from tea leaf extractive oil was assessed in accordance with requirements of the edition "Suppositories" (Edition 2, p. 151).



The quality characteristics and standards of suppositories obtained from tea leaf extractive oil are presented in Table 9.

**Then we carried out studies for developing wound-healing ointment.** Since the ointment base constitutes the required mass, soft constitution, proper concentration of therapeutic substances, and have an impact on the ointment stability, and the right choice of the ointment basis is of highly importance.

Table 9

Quality charactertistics and standards of suppositories obtained from tea leaf extractive oil

| Name of the item | Quality characteristics | |
|---|---|---|
| | Standardized requirement and determination methodology | Results of resaerch |
| Appearance and homogeneity of suppositories | Suppositories have a similar torpedo-like (rectal) form, with greenish coloration (visually) | Heterogeneous, green color, without impurities |
| Hardening temperature | From 20,0°C to 23,0°C (SP XI, edit.1, p.20) | 22,15±4,11 |
| Total deformation time | Not more than 15 min (SP XI, edit.2, p. 153) | 12,6±4,23 |
| Melting temperature | Not higher than 37°C (SP XI, edit.1,p.18,method2band edit.2, p.151) | 35,8±2,22 |
| Microbiological purity | Aerobic bacterium and fungus, totally up to $10^2$ in 1g, nonexistence of intestinal bacteria E. coli (SP XI, edit.2 $p_3$ .193, change 28.12.95) | Nonexistence of aerobic and other bacteria 15 |
| Carotenoids content calculated on a β-carotene basis, mg | Not less than 0,40 in each suppository (from 430 to500 nm, absorption maximum is observed at the wave lengths of 446±2 nm and 473±3 nm (UV – spectrophotometrically) | 0,46±2,54 |
| Acid index | Up to 5,0 (SP XI, Edit.1 p.191) | 4,45±0,46 |
| Storage terms | 24 months, temp. 3-5°C (storage method) | 24 months, 4±1°C |

Selection of bases was carried out with account for physico-chemical properties of ingredients selected for ointment, since the ointment composition envisages both lipophilic and hydrophilic extracts. In order to obtain homogeneous systems, it is necessary to solve the problem of their injection into the bases.

During the preparation of multi-component ointment, when selecting the optimal basis, there had been used the basis from German Pharmacopeia, which contains bee wax, cetylpalmitate and arachis oil. This basis is characterized by anti-inflammatory, wound-healing, strong bactericidal and regeneration stimulating action. In addition to this, there have been also used different polyethylene-oxide (PEO) smelt and arespol. Studies of aggregation stability of ointments obtained from them during the storage process, have shown that ointment based on polyethylene-oxide and arespol having different molecular masses is aggregately unstable at both room and 3-5$^0$C temperatures (dissection was evident three hours later), but by using bee wax and cetyl palmitate, there have been obtained the nonsegregating, viscose, stable mass in the form of ointment. The advantage of this basis was also evident in accordance with the carotenoids release rate.

The ointment composition we proposed is as follows: tea leaf extractive oil-20,0; sweet (invinified) grape-stone extract-12,0; stinging nettle hydrophilic extracts-14,0; plantai juice-12,0; garlic extract-16,0; arachis oil-16,0; bee wax-5,0; cetylpalmitate-5,0.

On the basis of carried out studies, we have developed technological scheme for producing environmentally safe ointment based on bee wax, cetyl palmitate and arachis oil, by using tea leaf extractive oil and phytocomponents, which is shown in Fig. 8.

In order to obtain ointment, by using the stand mixer, we place in a mixing device the formula components in kind of a lipophilic fraction, tea leaf extractive oil and arachis oil, to which, under conditions of continuous stirring, at a temperature of 60-70$^0$ C, we add previously in a steam boiler smelted bee wax and cetyl palmitate, and simultaneously, we weigh stinging nettle and grape-stone hydrophilic extracts and plantain juice in a measuring instrument, as a result of which, under conditions of continuous stirring in a mixing device, we add to a hydrophilic fraction lipophilic components. The obtained mixture is subjected to emulsionizing. Then we provide packing and storage of ointment in the fridge at a temperature of 4±1$^0$C.

It has been established that viscosity of ointment is ή=91,2 Pa/sec and the critical shearing stress (yield stress) is Q=56,4 Pa/cm$^2$. Characteristics of the proposed ointment is within the limits of the rheological optimum with its consumer properties, meets generally accepted requirements.

Quality assessment of ointment based on tea leaf extractive oil was made in accordance with requirements of State Pharmacopeia It has been established that ointment is heterogeneous, green colored, with characteristic



smell of ingredients, acidic index pH = 5,5±1,46, high osmotic activity, double water absorption, and slight change in the consistency.

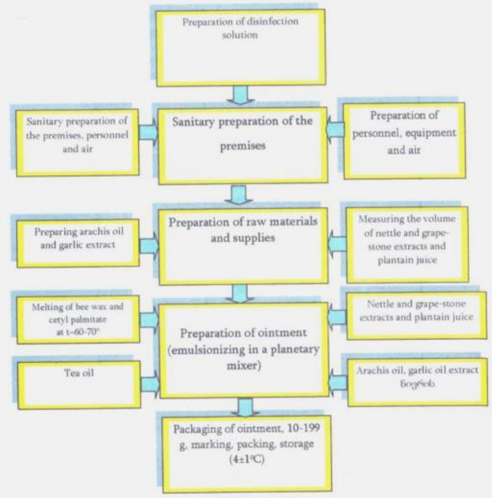



**Fig 8. Technological scheme for producing ointment by using tea leaf extractive oil**

Microbiological purity of ointment meets requirements of State Pharmacopeia, XI edition, and studies of stability and shelf-life of ointment, have revealed that samples of ointment produced from tea leaf extracting oil exhibit the correspondence of the quality parameters to standard requirements during the period of observations (24 months). Consequently, the storage time was defined as 24 months at a temperature of 4±1$^0$C.

The quality parameters and standards of ointment based on tea leaf extractive oil are presented in Table 10.

There has been studied that the ointment can be considered to be a relatively safe (6th category of toxicity).

Ointment has capacity to reduce accumulation of exudate in an area of inflamation and prevent creation of fibrose tissue. During traumatic injury, in both phases of wound, is distinguished by wound-healing action.

Table 10

Quality charactertistics and standards of ointment obtained from tea leaf extractive oil

| Name of the item | Quality characteristics | |
|---|---|---|
| | Standardized requirement and determination methodology | Results of research |
| Description | Thick, viscose, with smell and coloration charactiristic of its ingredients (visually) | Heterogeneous, thick, viscose green, char. of ingr. smell |
| Microbiological purity | Aerobic bacterium and fungus, totally up to $10^2$ in 1g, nonexistence of intestinal bacteria E. coli (SP XI, edit.2 $p_3$ .193, change 28.12.95) | Nonexistence |
| Carotenoids content calculated on a β-carotene basis, mg | Not less than 16 ( the maximums are observed at the wave lengths of 422-425 nm, 450-452 nm, and 470-475 nm) (UV – spectrophotometrically) | 16,5±3,52 |
| Acid index | 5,4-6,0 (SP XI, edit.1 $p_3$ .191) | 5,5±1,46 |
| Shelf-life | 24months,temp. 3-5°C°(storage method) | 24 months, 4±1°C |

**Basic conclusions**

1. Based on the analysis of literature sources, it has been established that successful muse of plant-origin therapeutic remedies is primarily due to their high biological activity, as well as due to the fact that natural compounds have less harmful effect on the human body than their synthetically produced analogues and substances having the artificially created structures.
2. In comparison with the synthetic-origin mono-preparations, of more relevance is the creation of wound-healing soft dosage forms with the composition consisting of tea leaf extractive oil, garlic-oil extract, sweet (unvinified) grape-stone extract, stinging nettle thick hydrophilic extract and plantain juice, in kind of the combined preparation, to allow for simultaneous realizing anti-inflammatory, antiseptic, antimicrobial, regenerating, bleed-stopping, pain-relieving and antioxidant actions at different stages of wound.
3. There has been developed technology for producing tea leaf extractive oil, with account for optimal conditions (raw material disintegration, moisture content, temperature, hydro-module, extraction duration, vibration frequency for pulsation, vibration amplitude), and phyto-chemical composition and quality characteristics of tea leaf extractive oil have been determined. This method for obtaining tea leaf extractive oil allows for making more complete extract with high content of integral components.
4. There has been developed technology for producing sweet (unvinified) grape-stone extract, stinging nettle thick hydrophilic extract and plantain juice, and biologically active substances in them have been studied.
5. Technological, bio-pharmaceutical and biochemical studies had determined an optimal ratio of therapeutic plant raw materials in the phyto-composition, and the composition of auxiliary substances with wound-healing action has been developed, in kind of bee wax, cetyl palmitate and arachis oil, and in the composition of suppositories – in the form of witepsol H15, due to their anti-inflammatory, wound-healing, strong bactericidal and regeneration stimulating action.
6. By using tea leaf extractive oil, there have been developed the compositions and technology for producing ointment and suppositories under conditions of modern pharmaceutical production, and quality and assessment and standards of therapeutic preparations have been determined.
7. It has been established that the developed ointment and suppositories represent the structured systems with thixotropic properties that is in full compliance with State Pharmacopeia requirements (according to XI Structural-Mechanical Properties).
8. There has been studied shelf-life of the developed soft dosage forms - suppositories and ointment at a temperature of 4±1°C, and it has been established that the correspondence of quality characteristics of experimental samples of ointment and suppositories obtained from tea leaf extractive oil with standard requirements was observed during the period of the observation (2 years), and consequently, the storage time was defined as 2 years at a temperature of $4\pm1^0$C.

9. Pharmacological studies have revealed that suppositories and ointment produced by using tea leaf extractive oil and phyto-components peratin to the sixth category of toxicity, and are distinguished by pronounced inti-inflammatory and wound-healing action.

**List of publications**

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6. Kopaleishvili T., Kipiani A., Chokhonelidze V. Clinical studies of wound-healing action of a new preparation based on tea leaf extractive oil. The center for scientific analysis "LOGOS". Proceedings of International Scientific-Practical Conference "Prospects for Integration of Science and Practice", Stavropol, 2014.

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