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Preliminary Results of Complex Technological, Analytical and Pharmacological Research on the Development of New Medicinal Forms for the Treatment of Inflammatory Diseases.

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ABSTRACT

The paper presents some results of complex researches on the development of new targets drugs for the treatment of inflammatory periodontal diseases. Biologically active substances of the Juniperus plants are proposed to be used as the active ingredient. The work presents the results of the selection and justification of dental dosage form for the prevention and treatment of periodontal diseases based on the essential oil galberries of common juniper. In addition, the paper provides experimental materials of parallel study on fruit polyphenols of Juniperus oblonga Bieb for the purpose of its introduction into the category of officinal herbal products.

Keywords: periodontal disease, inflammatory diseases, therapy, natureceuticals, juniper.

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INTRODUCTION

Talking about the treatment of inflammatory periodontal disease, it is difficult to identify the aspects on which the positions periodontists different countries and schools would be fully harmonized. There are generally accepted views on the need of a comprehensive treatment, but different authors understand the essence of complex therapy differently [1]. However, the existence of medicinal agent presents in any scheme of therapeutic effects on the parodont.

Scientific basis for qualified and informed of complex therapy of inflammatory periodontal disease has been established by the end of the previous century. Periodontists have received a clear picture of the prevalence of this type of pathology, predisposing factors, species composition of the microflora of the oral cavity and periodontal pockets. The national classification of periodontal disease has been proposed for practical periodontics [1]. In this case, the existing range of target drugs does not permit to decide the problem of medications treatment of these nosological groups completely. In particular, this includes the therapy of accompanying side effects of bioactive compounds.

In this connection, the empowerment of the use of officinal species of medicinal plants, the study of new species of plants and their introduction in the official medicine in the form of medicines is urgent and reasonable. In this case, it is impossible not to rely on the huge reservoir of empirical knowledge, collected by traditional medicine.

Herbs and herbal teas are used rather often in different diseases in dentistry. The scientific community has accumulated extensive material on the effects of herbal medicines on the function of the periodontal tissues [1]. We have studied the medicinal plants that contain the essential oils. People have used these plants to treat diseases of the teeth and gums historically. Essential oils of some plants are used in the treatment of different dental diseases, e.g. gingivitis is treated with fir, sweet flag, wild marjoram, cedar, etc. .; stomatitis is treated with roses, sage, chamomile, eucalyptus and others [2].

Extemporaneous formulations such as a decoction of the galberries of common juniper are used for inflammation of the gums [3, 4].

The purpose of this first fragment of perspective complex researches plants of the genus Juniperus was a series of technical tests to establish the original dental dosage form with common juniper for the treatment of inflammatory periodontal disease, and a parallel study of fruits polyphenols Juniperus oblonga Bieb was for its introduction into the category of officinal plant raw material.

METHODS

Microbiological studies were performed according to the requirements Russian Pharmacopoeia XII.
Chromatographic studies were performed by reversed-phase high-performance liquid chromatography (RP-HPLC) on a chromatographic device company «Agilent Technologies 1200 Infinity» from USA with autosampler Agilent 1200, a vacuum micro degasser, a gradient pump and a heat bath.

Electronic absorption spectra were recorded using a spectrophotometric diode-array detector Agilent series 1200 (a wavelength range of 190 to 950 nm in a cuvette with a path length of 10 mm, volume 13 mcl), scanning step - 2 nm.

«Agilent Chem Station» software was used for registration and processing spectral data and chromatograms.

Column efficiency was determined by calculating the theoretical plate number N. When the column efficiency is higher, this value becomes greater and an extension of the original narrow band peak becomes smaller as advancing the strip through the column, the peak becomes narrower at the column outlet [5]. The value of the optimal test efficiency of the column provides not less than 5000 theoretical plates as an indicator of the efficiency of the column. The main evaluation criterion of adequate separate neighboring peaks was a separation factor Rs, which should not be less than 1.5 according to the European Pharmacopoeia. Optimum value of the asymmetry coefficient Tf was accepted figure - less than 2 [6].

Amounts polyphenolic complexes chromatographed under the following conditions: mobile phase: (A) - 0.5% aqueous formic acid (B) - ethyl alcohol in a gradient elution mode; column - Ascentis express C18 2,7µm × 100 mm × 4,6 mm; the speed of the mobile phase - 0.5 mL / min; column temperature - + 35 ° C; the injection volume - 5 µl. Detection was carried out: for flavones and flavonols at 360 nm, hydroxycinnamic acids - 325 nm [7].

Component identification was performed by matching the retention times of analytes with retention times of standard samples, which have been registered in similar experimental conditions and the results of diode array detection.

The relative content of individual flavonoids was determined as the ratio of the area of the chromatographic peak and the amount of peak areas all authenticated flavonoids according to the formula:

$$Xi = \frac{Si \times 100}{\sum S}$$

where Si - average value of the peak area on the chromatograms component amounts, ΣS - average value of the sum of all the peak areas at the chromatograms.

Registration of mass spectra were obtained by a mass spectrometer «Autoflex II» (modification Microflex) «MALDI TOF / TOF» (device error - no more than 0,5 Da) firm Bruker Daltonics GmbH from Germany. The detection of the resulting ions occurs in time of flight mass analyzer and reflected in the linear modes.

Previously analyzed sample solutions in an amount of 0,5 µl was applied using a dispenser to the target «MTP 384 target plate matt steel TF», dried and applied on top of the drop of the matrix. Used as a matrix α-cyanocinnamic acid (HCCA), the spectra were registered with the program «Flex Control», data processing was carried out in the program «Flex Analis», in reflection mode with positive polarity (Reflex Positive).

Choice and justification of dental dosage form for the prevention and treatment of periodontal diseases are based on essential oil of galberries of common juniper.

There was the analysis of dosage forms of drugs used for the treatment and prevention of periodontal disease with local action.

The range of dosage forms submitted solutions and gels, pills, sprays and with a certain amount of priority on the market of drugs. But, in our view, gels allow to achieve prolonged pharmacological effect.

Dominant synthetic compounds were found in the study of the nature of the active substances of targeted drugs. However, medicines from plants have certain advantages. They are complex chemical composition, and hence, a variety of biological activities, which applies to the normalization of functional disorders of the organs and metabolic processes. Therefore, the action of herbal remedies has a persistent and prolonged pharmacological effect, which determines their specificity and value. Herbal preparations have less pronounced allergenic action. The use of herbal medicines do not associate statistically with the presence of negative side effects [8].

Galberries of common juniper (Juniperus communis L.) were distinguished among the medicinal plants, having long-term significance for dentistry. In the therapy of gum disease juniper oil and water extraction from galberries were used. [3, 4, 8]. However, the industrial production of medicines from juniper components does not exist.

Thus, further development has been chosen, the dosage form was based on common juniper in gel form. And the first step in the implementation of this development was the selection of the forming component.

MAIN PART

We have considered the alginates and pectins to be a formative component without breaking the accepted concept of creating natureceutical drugs that are traditionally used in the compositions of the gels

hatchery. However, alginates are more susceptible to microbial contamination. Therefore, pectin is preferably a secondary connection developed formulation. For further research we have taken apple pectin, as the most accessible raw materials (GOST 29186).

The pectin solution with a concentration of 1, 3, 5, 7, 10 or 15% was studied to determine the optimal concentration of pectin to form the dosage form developed ones. Gellable consistency was obtained in the case of a 10% solution of pectin. Complete dissolution of the pectin does not happen with increasing concentration.

In the next phase of the study microbiological stability of 10% pectin solution adopted as the working was under study.

The first growth of microorganisms was recorded on the 4th day in the sample without conservant (Figure 1). After following 15 days, four colonies of microorganisms with diameter of 15, 9, 6 and 5 mm were formed. In this experiment, the task is to determine the kind of microorganisms was not intended.

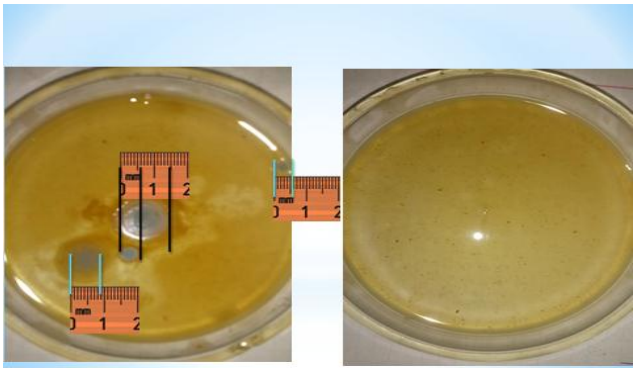

Figure 1: The microbial growth in the experiment

The results confirmed the necessity to introduce conservant in the structure under study. The latter was benzalkonium chloride in a concentration of 0.01%, and the concentration of benzalkonium chloride has bactericidal properties and does not show unpleasant taste sensations.

The chromatographic analysis of polyphenols Juniperus oblonga by RP-HPLC (Figure 2) showed the presence of flavonoids [7].

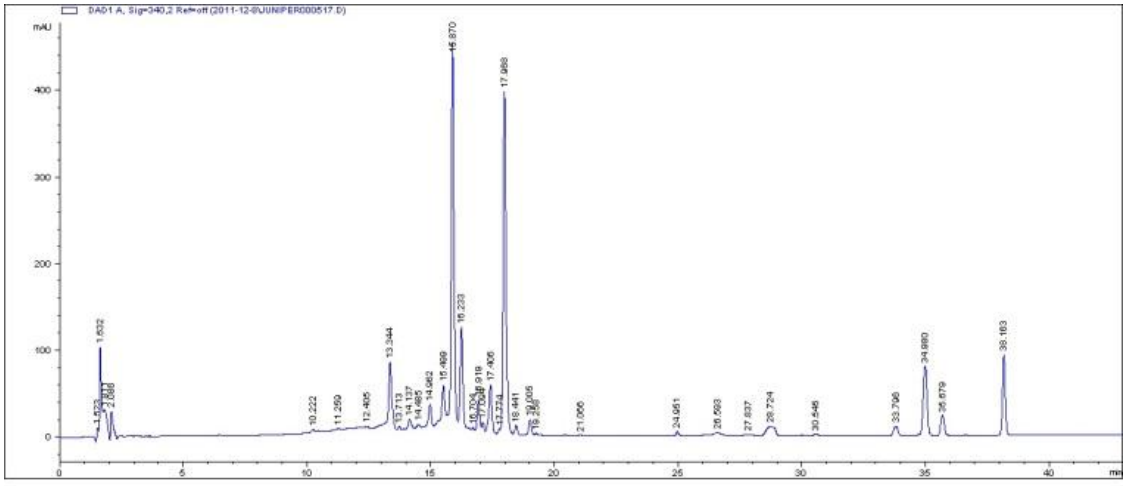

Figure 2: The chromatogram of the separation of flavonoids Juniperus oblonga (diode-array detection, λ = 360 nm)

The suitability criteria of the chromatographic system based on the results of chromatography and were presented in Table 2.

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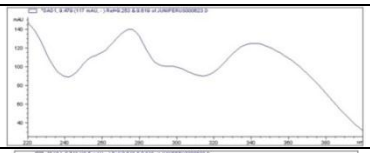
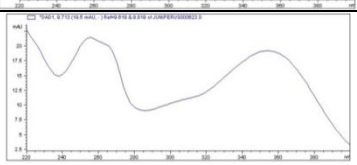
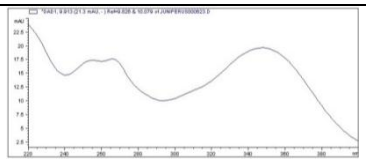
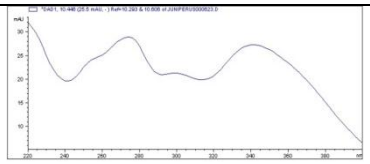
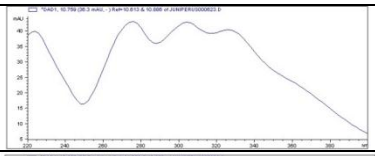
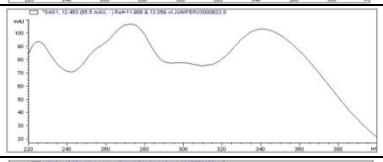
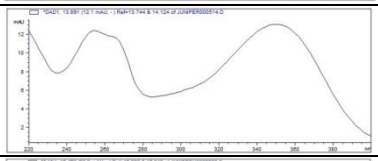
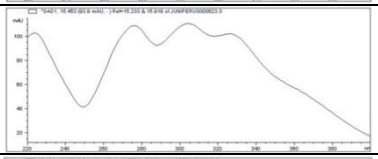
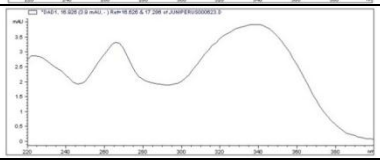
Table 2: Performance indicators of the system suitability

t_R	S	N	HETP	R_s	T_f	W_b
13,344	796	137776	1,08	5,6	1,128	0,1438
15,87	3095	339795	0,44	3,3	0,833	0,1089
16,233	803	323851	0,46	3,2	0,941	0,1141
17,968	3063	375425	0,4	2,04	0,727	0,1173

t_R - absolute retention time, S - peak area, N - number of theoretical plates, HETP - height equivalent to a theoretical plate, R_s - separation factor, T_f - asymmetry parameter, W_b - peak width

Table 3: Chemical composition of fruit polyphenols of Juniperus oblonga

Retention time, min	Ultraviolet spectrum	Content in an mount%	Identified component
9,479		15,8	Biflavonoid
9,71		2,4	Quercetin-3-glucoside
9,91		3,1	Luteolin-6-C-glucoside
10,45		4,6	Biflavonoid
10,76		3,0	Skutellyarein-6-O-glucoside
12,45		40,7	Biflavonoid
14,09		8,0	Luteolin-7-glucoside
15,45		21,4	Skutellyarein-8-O-glucoside
16,92		1,0	Apegenin-7-glucoside

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The findings of Table 2 indicate that the chromatographic system is suitable for the analysis because all eligibility criteria (N> 5000, Rs> 1,5, Tf <2) are in the range of valid values.

The component composition of the polyphenol complex of Juniperus oblonga was submitted on the results of diode array detection in Table 3.

The analysis results in Table 3 indicate that polyphenols Juniperus oblonga are presented exclusively by flavonoids.

The percent distribution diagram of flavonoids in galberries of Juniperus oblonga is presented in Figure 3.

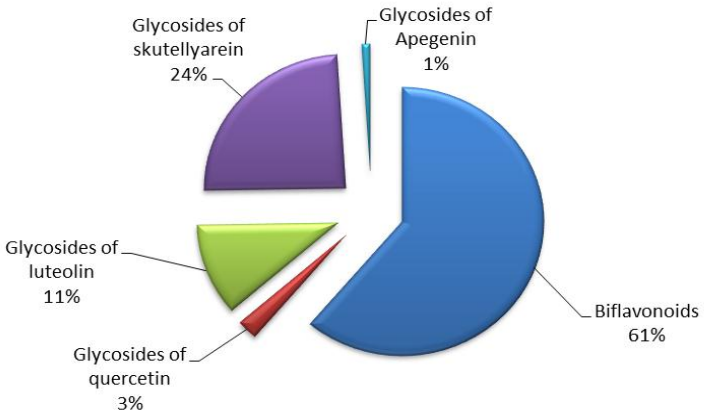

Figure 3: Percentage distribution of flavonoids J. oblonga Bieb.

The diagram shows that biflavonoids (61.0%) and glycosides skutellyareina (24.0%) are the dominant groups of flavonoids.

The strongest peaks of molecular ions flavonoid glycosides with m/z = 435, 449 and 465 are fragmented to form aglycones with peaks of molecular ions with m/z = 271 287 and 303 belong to apigenin, luteolin and skutellyareins are observed in the mass spectrum of polyphenolic complex J. oblonga Bieb. (Figure 4).

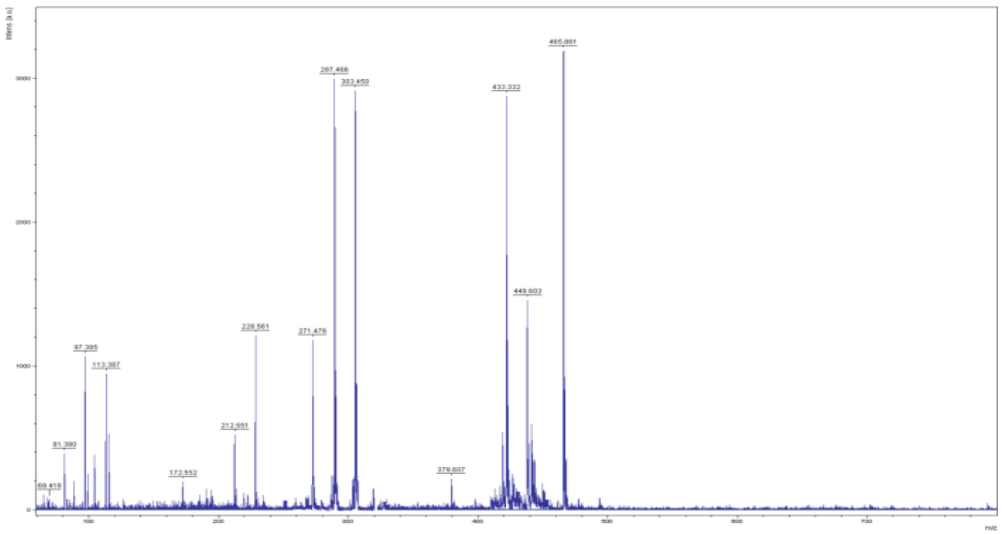

Figure 4: The mass spectrum amounts of polyphenols J. oblonga Bieb.

CONCLUSION

Thus, in accordance to the purpose of this study, the choice of basis of a new dental formulation in gel form for the prevention and treatment of inflammatory periodontal diseases based on juniper essential oil

galberries was implemented. In addition, the study of polyphenols galberries of Juniperus oblonga Bieb with a view to its elimination in the category of medicinal plants was held.

SUMMARY

Further experiments will be aimed at improving the proposed drug formulation, development of its industrial production technology, including the full range of traditional technology, analytical, pre-clinical and clinical works, and related research. To set up a specific target range of dosage forms, comparative study of their technological and therapeutic benefits is also planned.

Prerequisites for conducted comprehensive studies of the genus Juniperus were over-years profile studies of the present authors [9-10].

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