Development and Standardization of Dental Plant-Based Preparation in The Republic of Uzbekistan

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Abstract

Nowadays herbal medicines are widely used in official medical practice in Uzbekistan. At the same time, the most common among both imported and domestic plant-based preparations are liquid dosage forms. The modern stage of application of these medicines is based on the rational use not only of the accumulated experience, but also on the achievements of scientific medicine in the study of the composition and features of biological activity of medicinal plants and isolated from them active compounds. Studies have shown that these drugs contain unique in composition combinations of biologically active substances, providing a wide range of therapeutic and preventive action. To solve the problem developed the composition and obtained on its basis a liquid extract for the treatment of diseases of the oral cavity of the mixture of plants such as chamomile drug, sage drug, St. John's wort, calendula drug in the ratio of raw materials - extraction fluid 1:1, established the most optimal extraction fluid - 70% ethyl alcohol and obtained alcohol extraction.

The resulting liquid extract was conditionally called "Parodonfit" and analyzed according to the requirements of regulatory documentation. Each of the plants has its own quality standard. Qualitative and quantitative analyses of biologically active substances of liquid extract "Parodonfit" with the use of physical and chemical method, such as thin layer chromatography, chromatography spectrophotometry, gas-liquid chromatography was carried out. Biologically active substances in the liquid extract - flavonoids and tannins - were determined during the research. The sum of flavonoids was determined by TLC, where compounds of routine, quercetin, cinarozid were isolated. Qualitative analysis of tannins and determination of heavy metals were conducted by pharmacopoeia method. Quantitative determination of active substances of cinarozid by chromatospectrophotometry was carried out. The tannins in recalculation for tannin - 3,2 % and in recalculation for proanthocyanides - 6,38 % were found out by chemical reactions according to the GD (governing documents). Quality indicators for liquid extract "Parodonfit" have been developed according to the requirements of the normative document: physical and chemical indicators, ethyl alcohol concentration, heavy metals, microbiological purity. The received results have shown perspective and expediency of development of highly effective multicomponent medical products on the basis of medicinal vegetative raw materials.

Keywords: *Plant-based preparation, dentistry, thin layer chromatography, chromatospectrophotometry, wild chamomile, St. John's Wort, calendula and garden sage.*

Introduction

Periodontal disease is one of the most difficult problems of modern dentistry. According to WHO, its level varies from 55% to 98%. In 15-19 years, it's about 55-89 %, in 35-44 years - 65-98 % [1,2]. Wide prevalence and significant «rejuvenation» of this pathology, adverse effect of periodontal infection foci on the body, as well as high level of complications up to complete loss of teeth - all this determines both medical and social significance of this problem. In addition to performing therapeutic and prophylactic dental manipulations, the protocols for the treatment of dental diseases include the prescription of various pharmaceutical preparations, such as antibacterial preparations, non-steroidal anti-

inflammatory preparations, calcium preparations, and preparations for the local therapy of periodontal diseases. Taking into consideration the continuous development of the pharmaceutical industry, the introduction of new drugs, the identification of new aspects of contraindications, side effects of certain drugs, all this requires a dentist to constantly update his knowledge in the field of pharmacotherapy [3].

In connection with this, recently in medicine herbal medicines (plant-based preparation - PBP) have been used more often for treatment of this disease[4], which is primarily due to good tolerability and absence of side effects in the vast majority of cases, as well as the presence of various biologically active substances and trace elements in their composition[5].

Many medications are used to treat diseases of the oral mucous membrane. Their choice depends on the nature of the course of inflammatory process (acute, exacerbation, chronic), the presence of pain, the type of elements of lesion, including soft tissue defect, oral hygiene. In diseases of the mucous membrane of the mouth used various dosage forms: decoction, infusion, infusion, ointment, plant-based paste, plant-based cocktail and their combinations.[6]

The results of the study showed that the range of Drug Products for the prevention and treatment of diseases of the oral cavity there are several types of DF (Dosage Forms) (solid, liquid, soft and gaseous), but the majority of drug products are produced in the form of solid DF. A relatively even distribution of liquid medications by quantity in Uzbekistan and abroad has been revealed. Soft dosage forms are mainly represented among drug products produced abroad.

At the analysis of Dosage Forms in solid aggregate state it has been revealed that among foreign there are three drug products produced in the form of tablets for resorption, while domestic medicinal products are represented by medicinal herbal substances (MHS) and produced in packets and filter - packets.

The analysis of the assortment revealed the presence of only two foreign drug products in the form of sprays.

At the next stage the analysis was carried out with a breakdown of the total assortment of dosage forms of sprays, the results are shown in Fig. 1.

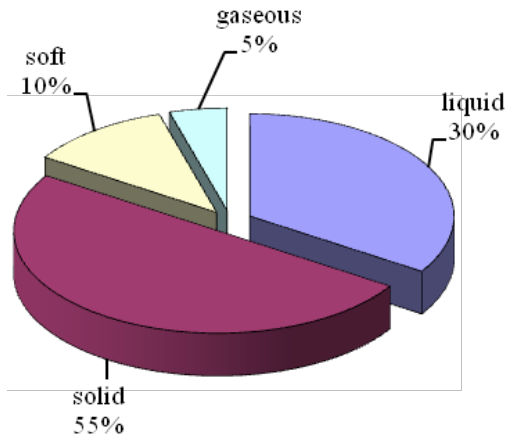

Figure 1: Specific weight of Dosage Forms in the range of drug products registered in the Republic of Uzbekistan, used for the treatment of stomatosis

Carried out marketing analysis of herbal drug products used for prevention and treatment of stomatosis in the pharmaceutical market [7]of the Republic of Uzbekistan, showed the prospects and feasibility of developing highly effective drugs based on local medicinal herbal substances (MHS).[8]

Standardization of multi-component preparations of plant origin is one of the most urgent tasks in modern pharmacy[9]. Determining the authenticity and analysis of the quality of these drugs is a complex procedure that must meet all modern requirements for the standardization[10] of multicomponent drugs. In addition, the growing demand for multicomponent preparations of plant origin used for prevention and treatment of stomatosis makes them potential targets for falsification [12].

Research Objective: Nowadays, studies on the conversion of multi-component mixtures of medicinal plants, which are used in the form of infusions and broths, in the total drugs - extracts are relevant. In obtaining the extracts provides maximum yield of biologically active substances, which increases the pharmacotherapeutic effect. The advantages of liquid extracts and dosage forms on its basis include convenience for use and resistance to storage. In addition, the standardization of active substances allows for control at the stage of production and the accuracy of dosing the finished product, so standardization of liquid extracts and dosage form is no less important.

In order to create medicinal products based on plant raw materials with standard active ingredients and

predictable therapeutic effects, it is necessary to study the relationship between the chemical composition and biological activity. A wide range of biological activity - anti-inflammatory, astringent - has flavonoids and tannins of medicinal plants. The qualitative and quantitative composition of flavonoids and tannins depends on many factors, such as differences in chemotypes and growth conditions of medicinal plants, production and storage technology, etc. In this regard, the need to develop rapid and effective method to determine the qualitative and quantitative composition of flavonoids and tannins, both in plant raw materials, and in phytotherapy based on it is an urgent task.

The article is based on the task of creating a composition for oral cavity treatment by means of such a selection of components that would provide a complex effect of the composition on the affected tissues of the oral cavity, resulting in a high therapeutic effect with simultaneous reduction of negative side effects.

Moreover, the composition of the components is selected in such a way that it represents different groups of biologically active compounds, which, on the one hand, provide a wide range of biological activity (antimicrobial, anti-inflammatory, astringent). Development of new approaches for the standardization of herbal medicines using thin layer chromatography, GLC chromatograph and chromatography spectrophotometry method.

Materials and Method

It is offered a dental product, which includes – wild camomile flowers of the pharmacy, sage leaves, calendula flowers, herb St. John's wort pierced in a ratio of 1:1:1:1.

To develop the technology of obtaining a phytopreparation, initially conducted pharmacognostic studies of raw materials. Wild camomile flowers of the pharmacy, sage leaves, calendula flowers of the medicinal product and the herb of St. John's wort pierced for research were prepared in autumn within the natural range.

We selected the optimal extraction fluid in the following way. We studied 4 extraction fluids: ethyl alcohol with concentrations of 40, 50, 60 and 70%.

Alcohol extraction from plants - liquid extract "Parodonfit" was obtained by repercalation in a battery consisting of three percolators. Repercolation, i.e. repeated (multiple) percolation, allowing the maximum use of the solvent capacity of the extraction fluid, to obtain concentrated extractions with complete exhaustion of the raw materials. The amount of raw material to be loaded into each of the three percolators is calculated. The same amount of raw material is loaded into each percolator. The method of Repercolation is considered to be economically justified for long-term production.

Medicinal plant raw materials are shredded to particles not exceeding 3 mm in size. The shredded raw materials are separated from the dust through a sieve with a hole diameter of 0.25 mm and 30 g are removed. Place the raw material in 3 percolators of 10 g. The first percolator is filled with extraction fluid (70% ethyl alcohol) with an open crane percolator to form a "mirror". The faucet is closed after the extractant displaces the air and left for 2 hours. The displaced liquid is poured into a second percolator. The first percolator is filled with a clean extraction fluid until a "mirror" is formed. After air displacement the first and second percolators are left for 2 hours. From the second percolator poured the amount of extraction in the third percolator, the extraction of the first percolator poured into the second percolator, the first percolator filled with pure extractant until the formation of the "mirror". Three percolators are left for 3 days. From the third percolator is pushed out extraction with a volume of 10 ml. From the second percolator extraction is poured into the third percolator, from the first to the second percolator. The second and third percolators are left for 2 hours. Extraction with the volume of 10 ml is displaced from the third percolator. Pour the extraction from the second percolator into the third percolator and leave for 2 hours. From the third percolator the last extraction of 10 ml is obtained. All portions of the finished product obtained from each percolator are combined, stored and filtered.

The resulting complex product refers to liquid alcohol-containing plant-based preparation. The current regulatory documentation contains general articles on such pharmaceutical forms as "Tincture" and "Extracts", for which the following indicators are mandatory[13]:

- Description
- Authenticity
- Heavy metals
- Alcohol concentration
- Quantitative determination

- Microbiological purity.

Authentication was determined by the following method of analysis: *for qualitative analysis of tannins* we add 1 g of sodium acetate crystals and 10 drops of 1% iron ammonium alum solution to 5 ml of filtrate.

Thequalitative composition of the extract for flavonoids was studied by the TLC test method on "Merck" plates with silica gel COF_{254} for aluminum with the size of 15x10 cm in the system of solvents n-buthanol: acetic acid: water (in a hundred 4:1:2). To detect phenolic compounds of the resulting extract chromatographed ascending method. To identify adsorption zones, 0.05 ml of water-alcohol extract and 0.05 ml of state standard reference sample alcohol solutions of rutin, quercetin and cinaroside were applied to the starting line. Detection of adsorption zones was performed in UV-light at 254 nm length. Three adsorption zones were detected: quercetin (Rf = 0.85); rutin (Rf = 0.55); and cinaroside (Rf = 0.75).

Determining heavy metals to 1 ml of liquid extract add 1 ml of concentrated sulfuric acid, gently burn and calcinate. The resulting residue is processed by heating 5 ml of saturated ammonium acetate solution. Filter through an ashfree filter, wash 5 ml of water and bring the filtrate volume to 200 ml. 10 ml of the obtained solution should pass the test for heavy metals (no more than 0.01% in the preparation). To 10 ml of the resulting solution add 1 ml of diluted acetic acid, 2 drops of sodium sulfide solution and after 1-minute compare with 1 ml of reference solution B, and the same amount of reagents as added to the test solution.

The ethyl alcohol concentration was determined by the developed method with the use of Agilent Technologies "GC 6850 Network GC System" gas chromatograph. Ethyl alcohol concentration in the obtained extract was determined by the developed method using the method of GLC on Agilent Technologies "GC 6850 Network GC System" gas chromatograph.

Chromatography was performed under the following conditions: DB-624 Capillary 30.0m x 250µm x 1.40µm; furnace temperature from 40˚C - 120˚C, duration of analysis-8.0 min, injection 1µl, split 50: 1, injector temperature 120°C; detector temperature 260°C; mobile phase 0.8 ml/min helium (He); flame-ionization detector (FID); air and hydrogen speeds 450 ml/min and 40.0 ml/min.

Preparation of the test solution: Place 10 ml of the test extract with a pipette in a measuring flask with a capacity of 100 ml and bring the volume to the mark with distilled water and stir. The solution is filtered through a 0.45 µm membrane filter.

Preparation of 70.0% RDF ethanol work standard sample solution. To do this, take 66.5 g (precision hanging) of 99% ethyl alcohol and placed in a measuring flask with a capacity of 100 ml, bring the volume to the mark with distilled water and stir. (Solution 1).

10 ml Solution 1 is placed in a measuring flask with a capacity of 100 ml, bring the volume to the mark with distilled water and stir.

Identification of ethyl alcohol on chromatograms of the tested sample is carried out by comparison of retention times of the standard sample. Chromatographs of 1.0 µl of the test solution and ethanol PSO solution shall be alternately taken, obtaining at least 3 chromatograms for each solution.

The reliability of the results of the analysis was checked by determining the suitability of the chromatographic system by: -resolution (R) of ethyl alcohol peaks (not less than 2.0); -association coefficient (T) of ethyl alcohol peaks (not exceeding 2.0); -relative standard deviation (RSD) (not exceeding 2.0%).

The main groups of active substances of the medicinal product "Periodonfit" were quantified. The quantitative content of the sum of flavonoids in terms of cinaroside was determined by chromatospectrophotometry. For this purpose, chromatographic plate "Merck" UF 254 (10 x 15) was divided into 3 strips. On the starting line was applied as a 3 cm strip of 0.05 ml of the extract, on the second strip 0.05 ml of work standardcinaroside solution, the third strip was left free - control. The plate with the applied samples is air-dried for 1 hour and chromatographed upward in a chamber with a pre-saturated mixture of solvents chloroform-methanol-acetic acid (4:2:2) for 30 minutes. When the solvent front passes 7 cm, the plate is removed from the chamber, dried in the air, marked in the UV light (254 nm) zones containing cinaroside at the level of the witness spot. The zone of sorbent containing cinaroside and the zone of control experience, transferred into flasks with lapped corks with a capacity of 50 ml, eat 10 ml of 80% ethyl alcohol and heated in a water bath (60°C) for 30 minutes. With constant stirring the eluate is filtered through the filter. paper and determine the optical density on the SF at a wavelength of 352 nm in the cuvette with a layer thickness of 10 mm.

Preparation of work standartCinarozid: 0.2 g (so-called) dissolve 80% alcohol in a measuring flask with a capacity of 100 ml (when heated).

Determination of the quantitative content of tannins in the drug: take a pipette 25 ml of the resulting extraction in a conical flask with a capacity of 750 ml, add 500 ml of water, 25 ml of solution of indigosulphonic acid and titrate when continuously stirred with a solution of potassium permanganate (0.02 mol/l) to golden yellow color.

In parallel, a control experiment is conducted.

1 ml of potassium permanganate solution (0.02 mol/l) corresponds to 0.004157 g of tannins in terms of tannins.

Microbiological purity. For the manufacture of high quality and effective plant-based preparation (EPBP) is a prerequisite for quality control (QC) procedure at various stages of the technological process. A special place is occupied by the control of microbiological purity (MBC) of the resulting EPBP.

In accordance with the requirements of the regulatory document definition was carried out in Petri dishes with sterile nutrients: meat - peptone agar - for the quantitative determination of growing colonies, blood agar - to determine hemolytic bacterial strains, yolk and salt agar - to detect pathogenic staphylococcus, Endo - to detect intestinal bacterial groups, Saburo - to excrete fungi. In nutrient media were brought the test drug in different concentrations (1: 2: 4), at least three cups of Petri. All cups with crops were incubated in a thermostat at 37°C. Initial recording of the results was made in 24 hours and 48 hours, the final one - in 5 days. After incubation, the growing colonies were counted and identified.

Results and Discussion

The quality and efficiency of therapeutic action of plant-based preparation directly depends on the method of isolation and receipt of biologically active substances from medicinal plants in the form of extracts. For example, it is known that such traditional method of extraction as decoction, infusion and tincture can allocate about 15-25% of biologically active substances in the medicinal plant. In this case, labile (unstable) biologically active substances are destroyed and the extract significantly loses its medicinal properties. At the same time with these method of allocation of biologically active substances in extracts together with them gets a large enough amount of ballast substances (resinous substances, hydrocarbons, fiber, hairs of plants). These substances, on the one hand, may have a negative effect on the body itself. And it should also be noted that the more used plants in the collection of the extraction, the more pronounced these negative effects, the harder to obtain a quality extract.

Camomile flowers, sage leaves, calendula flowers and St. John's wort herb are pharmacopoeia plants and are of great interest in dentistry as a source of BAS (biologically active substance). Preparations based on these plants are widely used in medicine.

The technical result obtained in the implementation of the invention is expressed in the increase of therapeutic effect with the simultaneous elimination of negative side effects, which ultimately leads to a reduction in the recovery time of patients. Moreover, the composition of the components was chosen in such a way that it represented different groups of biologically active compounds, which, on the one hand, provide a wide range of biological activity (antimicrobial, anti-inflammatory, regenerating, astringent, styptic, capillary strengthening, adaptogenic, immunocorrective action). In this regard, 4 extractants were studied - ethanol with concentrations of 40, 50, 60 and 70% for the liquid extract which includes - chamomile flowers, sage leaves, calendula flowers, St. John's wort herb pierced in the ratio 1:1:1.

The sum of flavonoids was quantified by spectrophotometry. Extraction properties of each object were studied three times; the results were statistically processed and are presented in Table 1.

Table 1. Quantitative determination of the sum of flavonoids in each extract by spectrophotometry.

Extraction fluid	Flavonoid content of extraction, in %
Ethyl alcohol 40%	0.37±0.05
Ethyl alcohol 50%	0.93±0.05
Ethyl alcohol 60%	1.22±0.05
Ethyl alcohol 70%	1.55±0.05

So, as a result of the conducted studies, the most optimal extraction fluid was found - 70% ethyl alcohol and alcohol extraction from a mixture of plants such as chamomile medicinal, sage medicinal, St. John's

wort pierced, calendula medicinal in the ratio of raw materials - extraction fluid 1:1.

The usage of the method of recolonisation instead of maceration allows for more complete extraction of the whole complex of BAS due to a higher concentration gradient, which is created by passing a pure extraction fluid through the raw material. This composition is a biologically active complex compound obtained by mixing components with known therapeutic properties, taken in the ratio providing a given therapeutic effect without negative side effects.

Further research was conducted on qualitative and quantitative indicators of liquid extract in accordance with the requirements of the regulatory document.

The resulting liquid extract is a transparent liquid of brown color, with a pleasant aromatic smell of medicinal herbs and a bitter spicy taste.

At the qualitative analysis of tannins in the liquid extract on the surface of the crystals in the neutral zone of the filtrate black-green coloring appears, which meets the requirements of the ND.

The qualitative composition of the extract for flavonoids was studied by the TLC (thin layer chromatography) method in the system of solvents n-buthanol: acetic acid: water (in a hundred 4:1:2). As a result of the conducted studies 3 adsorption zones were found: quercetin (Rf = 0.85); rutin (Rf = 0.55); cinaroside (Rf = 0.75). The results of TLC analysis of the flavonoid sum are shown in Fig. 2.

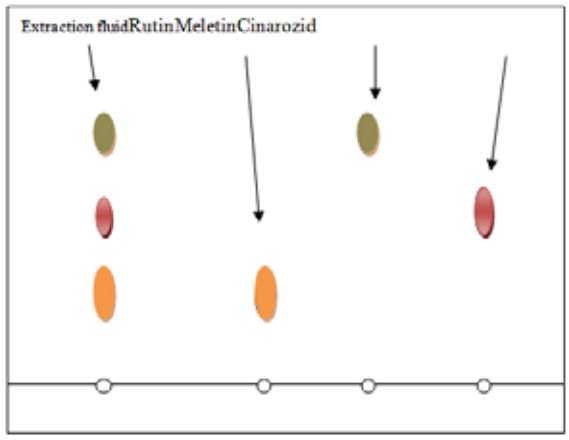

Fig. 2. Chromatogram of liquid extract and alcohol solutions of rutin, meletin and cinarozid as witnesses

When determining the qualitative indices, it was found out that there are no heavy metals in the extract (the color of the test solution is less than that of solution B). Low opalescence is observed.

Ethyl alcohol concentration was determined by the developed method with the use of GC (gas chromatography) method. Identification of ethyl alcohol on chromatograms of the investigated samples was carried out by comparison of retention times of a standard sample. The results are presented in Fig.3-4.

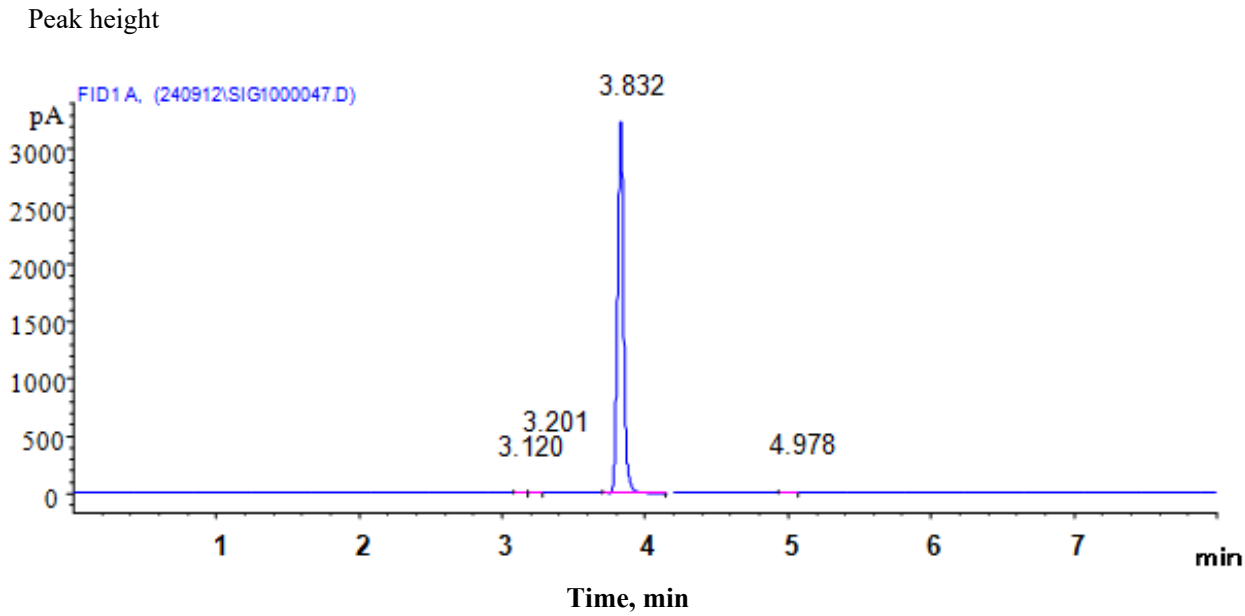

Fig. 3. Chromatogram of standard sample of ethyl alcohol

Peak height

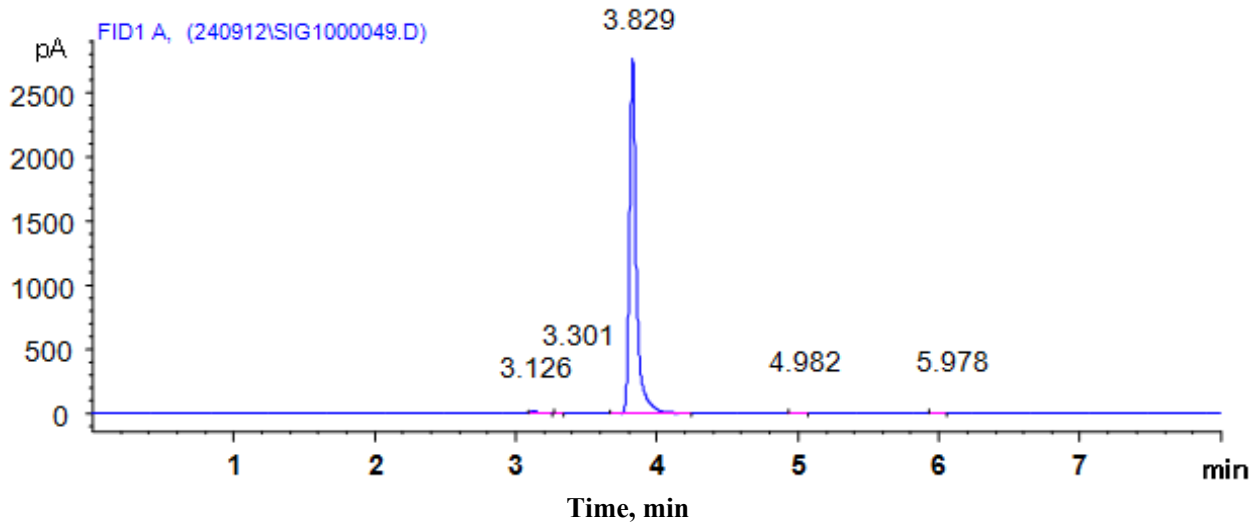

Fig. 4. Chromatogram of "Parodonfit" extract

On the chromatogram the peak with retention time of 3,829 min corresponds to work standard of ethyl alcohol with retention time of 3,832. The concentration of ethyl alcohol in "Parodonfit" extract was calculated by formula

$$X = \frac{H_1 \bullet m_0 \bullet 0 \bullet 100 \bullet P}{H_0 \bullet 100 \bullet 100 \bullet V}$$

H_1-peak height of ethyl alcohol from chromatograms of tested solution; H_0-peak height of ethyl alcohol from chromatograms of ethanol work standard solution; m0 - mass of 96% of ethyl alcohol in grams; V - volume of tested solution used for analyses, in milliliters; P - volume of 70% of ethyl alcohol, for preparation of ethanol work standard solution, in percent.

As a result of the tests carried out it was found out that the quantitative concentration of ethyl alcohol in "Parodonfit" extract was not less than 62.0%.

The quantitative determination of the main groups of active substances in the Parodonfit drug was performed. The quantitative content of the sum of flavonoids in terms of cinarozid was determined by chromatography spectrophotometry to determine the optical density in NF at 352 nm wavelength. The cinarozid concentration of the extract was 0.12%.

Determination of the quantitative content of tannins in the drug was conducted by chemical reactions given in the regulatory document. The concentration of tannins (X) in percent converted to absolute dry raw materials is calculated by the formula:

$$x = \frac{(V - V1) * 0{,}004157 * 250 * 100 * 100}{m * 25 * (100 - W)}$$

Where V - the volume of potassium permanganate solution (0.02 mol/l) spent on titration extraction, in ml;

V1- Volume of potassium permanganate solution (0.02 mol/l) used for titration in control trial, in ml;

0.004157 - amount of tannins corresponding to 1 ml of potassium permanganate solution (0.02 mol/l) (in terms of tannin), in grams;

m - Mass of raw materials in grams;

W - Loss in mass when drying the raw material in percent;

250 - Total extraction volume in ml;

25 - Extraction volume taken for titration, in ml.

The amount of tannins was 3.2 % in terms of tannins and 6.38 % in terms of proanthocyanides.

The research of microbiological purity was conducted in accordance with the requirements of the regulatory document «Method of microbiological control of drugs». A methodology for determining microbial quality was developed.

The results obtained are presented in Table 2.

Table 2: Microbiological control results

Target drug	Selected microorganisms				
	Aerobic Gram-positive bacilli	Staphylococcus	Micrococcus	Escherichia bacteria	Mushrooms
Liquid extract	0	0	0	0	0

Under studying "Microbiological purity" of liquid extract "Periodonfit" the obtained liquid drug corresponds to this index, according to which such drugs should contain no more than $5x10^3$ aerobic bacteria, 10^2 yeast mold fungi in the absence of *Pseudomonas aureginosa, Staphylococcus aureus, Escherichia coli, Salmonella* and no more than 102 other intestinal bacteria. The assessment of quantitative and qualitative composition of the contamination flora did not reveal any deviations from the requirements of SP (State Pharmacopoeia) XI. Quality indicators have been established and will be included in the draft regulatory documents.

Conclusions

1. Based on the data on application of medicinal plants in folk, traditional and scientific medicine, the review of the literature on chemical structure, taking into account a raw-material base it is proved and the optimum composition from four kinds of medicinal vegetative raw materials which is a basis for reception of anti-inflammatory stomatological means is developed.
2. The resource-saving technology was developed by the method of recolation, which consists in the extraction of phytocomposition with alcohol - 70%.
3. Method of standardization of phytocomposition - liquid extract - with the use of chromatographic and spectrophotometric method - TLC and chemical reactions have been developed, the presence of: flavonoids, tannins in liquid extract "Parodonfit" has been established. The phenolic composition of the liquid extract was studied and the following were identified: rutin, quercetin, cinaroside.
4. Tanning materials were studied by quantitative method of analysis calculated on tannin and proanthocyanides.
5. Quality indexes for liquid extract "Parodonfit" according to requirements of normative document are developed: qualitative and physical indexes, ethyl alcohol concentration, heavy metals, microbiological purity.
6. The results obtained can be further used to obtain, standardize and develop a regulatory document for a new combined herbal remedy "Periodonfit" used in dental practice.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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