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Key Points in the Development of Medicinal Products for Electro- and Phonophoresis Based on a Phytocomplex in the Rehabilitation of Patients with Osteoarthritis

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Abstract

Creation of medicinal drugs for directed use with physical factors will significantly expand the range of pharmaco-physiotherapeutic methods of rehabilitation of patients with various diseases, increase their effectiveness, and shorten the time between the promotion of the idea of a new method and its introduction into medical practice. The scope is to develop the composition and optimal terms of obtaining multicomponent herbal medicinal product - a phytocomplex and to explore the possibilities of its use for electro-and phonophoresis in the rehabilitation of the patients with osteoarthritis. The phytocomplex was obtained from the crude marsh cinquefoil, creeping alfalfa and common hop. The optimum conditions of extraction were determined according to the multifactor experiment rules. The predominant flavonoids were isolated by thin-layer chromatography. The flavonoids were quantified by spectrophotometric method. Stability of the phytocomplex flavonoids to the action of pulse currents and ultrasound were evaluated in Franz-type diffusion cells. the composition of the phytocomplex – dry extract from crude marsh cinquefoil, alfalfa and common hop - has been developed (40:40:20), which can be used in the rehabilitation of the patients with osteoarthritis. The main biologically active substances of the phytocomplex were flavonoids, among which quercetin and rutin dominated. Flavonoids were resistant to the action of sinusoidal modulated and diadynamic currents and ultrasound. Optimum conditions for obtaining a phytocomplex have been established, and its main quality indicators have been determined. The key points in the development of medicinal products for electro- and phonophoresis have been shown based on the composition development, the determination of optimum conditions for obtaining, assessing the phytocomplex resistance to the action of physical factors in the rehabilitation of patients with osteoarthritis.

Keywords: electrophoresis, flavonoids, osteoarthritis, phonophoresis, plant extract, quercetin, rutin.

INTRODUCTION

In recent years, in modern physiotherapy interest in the development of pharmaco-physiotherapeutic methods has increased [1-5]. However, the process of creating new techniques is limited to a small number of drugs that can be used directionally with physical factors. The development of such drugs, for example, for electrophoresis, is not profitable for manufacturers due to the high cost of creating a drug and small sales volumes. In case of the use of this drug with several physical factors, for example, for electro- and phonophoresis, this complicates the search for drugs resistant to these factors, preserving pharmacological activity when used in conjunction with physical factors that have the necessary unified quality indicators and balanced dosage form. Thus, it is preferable to carry out electrophoresis using an aqueous solution, and phonophoresis – using the ointment or gel base.

The solution of the key issues for creating drugs for the use in conjunction with physical factors would significantly expand the range of pharmaco-physiotherapeutic methods of treatment of patients with various diseases, as well as reduce the time between the promotion of the idea of a new method and its introduction into medical practice.

This is especially true in case of inflammatory degenerative diseases of the musculoskeletal system, including osteoarthritis. The disease has significant and widespread prevalence, results from the interaction of multiple genetic and environmental factors, strikes mostly people of the older age group, is characterized by a long course with the tendency to recrudescence and progression, deterioration in the quality of life of patients, which defines the social significance of the disease [6-10].

Traditionally, treatment of osteoarthritis is complex and includes medical and nonmedical methods, including the physiotherapeutic ones [11-13]. Specificity of the disease makes it necessary to give preference to herbal drugs when using pharmaco-physiotherapeutic methods [14, 15]. However, so far, their range has been too small and included only monocomponent drugs of plant origin, and their application is not always substantiated from the scientific and experimental point of view.

Creating complex phytocomplexes with the multi-component structure for joint use with physical factors could make a significant contribution to the resolution of important medical and social issues of efficient rehabilitation of patients with osteoarthritis.

The scope of the work is to develop the composition and optimal terms of obtaining multicomponent herbal medicinal product - a phytocomplex, and to explore the possibilities of its use for electro-and phonophoresis in the rehabilitation of the patients with osteoarthritis.

MATERIALS AND METHODS

The crude marsh cinquefoil, creeping alfalfa and common hop have been used to obtain a multicomponent herbal medicinal product - phytocomplex. The isolation of flavonoids was performed by treating crude materials with 70% ethanol three times at a ratio of 1:1 with heating, the combined extracts were evaporated, the aqueous residue was purified with chloroform, then the phenol compounds were removed from the aqueous solution using ethyl acetate (5 times at a ratio of 2:3), and the latter was distilled. Flavonoids were separated by chromatography in a thin layer of sorbent on Sorbfil plates (Sorbopolymer, Russia) with a polar stationary phase on an aluminum and polymer matrix. For comparison, the plates on a low-polarity Polyamide 6 (Sigma-Aldrich) were used. A solvent system - ethyl acetate-acetic acid-water (70:10:20) - was used as the mobile phase. In chromatography on Polyamide 6, the acetonitrile-water (70:30) system was also used. The prevailing flavonoids were also identified using standard quercetin (Q 0125, Sigma) and rutin (R 5143, Sigma) samples. The quantitative content of flavonoids was determined by spectrophotometry using a Titrtek MCC 1340 spectrophotometer (Finland); and that of the extractive substances - using the gravimetric method. The experiment on the search for optimal conditions for the production of a phytocomplex - a dry extract - was mathematically planned in accordance with the rules for constructing a multifactor experiment and using the path-of-steepest-ascent method by Box-Wilson. The stability of flavonoids to the action of electric current and their polarity was determined in the modified Franz-type diffusion cells (for 3-chamber with electrodes) (SES GmbH-Analysesysteme,

Germany). To assess the stability of flavonoids under the action of ultrasound, Franz-type diffusion cells No. 4G-01-00-09-05 (SES GmbH-Analysesysteme, Germany) in the V6-SFCS system were used.

RESULTS AND DISCUSSION

The various kinds of drug plants were searched for based on the content of biologically active substances (BAS), which should combine the analgesic, anti-inflammatory and other effects, and could be used in osteoarthritis. The composition of a new phytocomplex has been developed - dry extract from crude materials of grass and roots of marsh cinquefoil *(Herba et radices Comari palustri)*, the alfalfa grass *(Herba Medicaginis sativae)*, and stems or strobili of common hop *(Strobili Humuli lupuli)* (40:40:20). All drug plants used are registered in Russia, have relevant regulatory documentation and sufficient crude material base. Active substances of drug plants being analyzed were represented by flavonoids, polysaccharides, coumestans, tannins, phenolcarboxylic acids, micro- and macro elements, essential amino acids and vitamins, due to which they could be used in the rehabilitation of patients with osteoarthritis.

A preliminary study of the new phytocomplex showed that the main BAS were flavonoids dominated by quercetin and rutin.

There was no systematic work in the literature on the effects of electric current and ultrasound on flavonoids. Therefore, the stability of flavonoids isolated from the analyzed crude material, and standard quercetin and rutin samples to the action of pulse currents (sinusoidal modulated and diadynamic) used in rehabilitation patients with osteoarthritis, as well as the ultrasound, was studied. Absorption spectra of flavonoids were recorded before and after the action of a physical factor.

It was found that after the action of sinusoidal modulated currents (for I, II, III, IV and V types of work lasting 5 and 10 minutes, the modulation frequency of 100 Hz, the modulation depth of 50% and 75%, the current strength of 5 mA on the "Amplipulse-6" device, Russia) and diadynamic currents (half-wave continuous and wave current with the frequency of 50 Hz, full-wave continuous with the wave frequency of 100 Hz, one-period rhythmic and short- and long-period modulated, with current intensity of 5-20 mA, total duration of 10 -30 minutes on the Tonus DTG device, Russia), the maximum of optic density of flavonoids and standard samples of quercetin and rutin did not shift and their quantitative content was preserved. The maximum electrophoretic mobility of the phytocomplex flavonoids was achieved when both the cathode and anode were used as working ones. Stability of flavonoids isolated from the analyzed crude material and standard quercetin and rutin samples to the action of ultrasound (the intensity from 0.1 to 1.0 W/cm2 on the UZT-1,07F device, Russia) has been shown.

In order to establish the optimum conditions for obtaining a phytocomplex - dry extract, the influence of various factors on the yield of BAS from the crude materials of the analyzed plants has been studied. Two parameters have been chosen as output optimization parameters: the yield of extractives and the yield of flavonoids. The influence of 5 factors has been investigated, namely: the ratio of crude materials and extractant (x_1), the duration of the extraction process (x_2), crude drug granulation (x_3), extraction temperature conditions (x_4), and the concentration of the extractant (ethanol) (x_5) (Table 1).

The analysis of the experimental data resulted in obtaining two regression equations: a regression equation with an output optimization parameter - the content of extractives:

$$y = 29.45 + 0.86x_1 + 0.31x_2 + (-0.15)x_3 + 0.04x_4 + (-1.57)x_1;$$ and

the regression equation with an output optimization parameter - the content of flavonoids:

$$y = 1.04 + 0.06x_1 + 0.05x_2 + (-0.03)x_3 + (-0.01)x_4 + 0.10x_5.$$

Checking the errors of parallel experiments and uniformity of variances by the Cochran criterion, the hypothesis of the adequacy of the model by the Fisher criterion, the significance of the regression coefficients by the confidence interval and the Student's t-test with significance level 0.05 have shown that the provided equations adequately and reliably describe the extraction process under study.

It has been found that the yield of extractives and flavonoids from the crude material under analysis increases with the increase in the crude material - extractant ratio, duration of extraction and the crude drug granulation. An increase in the extractant concentration contributed to the yield of flavonoids. The increase in the extraction temperature up to 60°C was directly proportional to the yield of extractives and flavonoids. A further increase in temperature caused a decrease in the yield of flavonoids.

The "extractant concentration" indicator had the greatest influence on the extraction process (the coefficients of the regression equations for extractives and flavonoids corresponded to 1.57 and 0.10). However, a significant increase in the ethanol concentration led to a decrease in the content of hydrophilic flavonoid glycosides, a decrease in the solubility of the dry extract, even in hot water, which ultimately could affect the possibility of using a phytocomplex for electrophoresis. Therefore, further optimization of the extraction process was carried out using the path-of-steepest-ascent method by the most significant factor - the extractant concentration, under conditions of the different multiplicity of the extraction process (Figure 1).

It has been found that the most rational way is to extract using 70% ethanol, in the ratio of crude material and extractant - 1:30, at a temperature of +60 °C, crude drug granulation - up to 1-2 mm, for 1.5 hours, then drain the extract, pour the rest with 70% ethanol at the above ratio of crude material and alcohol, extract for 1 hour and perform a third similar operation for 30 minutes, to combine, clean with double separation, and evaporate the extract in vacuum.

Two types of drying - sublimation and spraying - were studied to obtain a dry extract. Sublimation drying eliminated the thermal effect on the active substances. However, the resulting dry extract was more hygroscopic than the substance produced by spray drying. Dry extract after spray drying had more pronounced anti-inflammatory activity (inflammation markers) [16] than after sublimation, which was very important in the rehabilitation of patients with osteoarthritis. Therefore, for the production of a phytocomplex, it is recommended to use the spray drying.

The resulting phytocomplex - dry extract, represented an amorphous powder of a brownish-yellow color, with a fragrant smell, and a specific bitterish-brackish taste. Its main quality indicators were determined: the bulk weight at free fall - 0.38 + 0.01 g/cm^3, the bulk density at compaction - 0.69 + 0.01 g/cm^3, the flowability - 2.06 + 0.02 g/s, the angle of natural repose - 36.0 °, and the loss in the mass when dried - 3.84 + 0.03%. The phytocomplex was soluble in hot water (50-60 °C). After bringing to the required temperature, such a working solution can be used for electrophoresis. The phytocomplex was slowly soluble in the "Mediagel-T" special gel for ultrasound therapy (Geltek-Medica, Russia). Such a working composition can be used for phonophoresis.

The quantitative content of flavonoids in the phytocomplex was determined by the spectrophotometric method in terms of quercetin (method 1) and rutin (method 2). The selection of two predominant flavonoids for the estimation of the quantitative content of active substances is due to the use of a phytocomplex for electrophoresis (most important are hydrophilic glycosides, for example, rutin) and phonophoresis (lipophilic aglycons, for example, quercetin). To obtain more reproducible results (method 2), the reaction of rutin complex formation with aluminium

chloride was carried out in the presence of acetic acid. Previously, it was found that the phytocomplex did not shift the maximum of the optical density of quercetin (370 nm) and rutin (415 nm), the intensity of which would be used in photometry. The relative errors of the methods did not exceed +2.5% (Table 2).

When conducting experiments with additions of quercetin and rutin, it was found that the relative errors of the determination with a confidence probability of 95% were no more than +2.0%.

Table 1 – The factors influencing the extraction process and the levels of their variation

Plan characteristics	Factor				
	x_1	x_2, minute	x_3, mm	x_4, °C	x_5, %
Variation step	1:10	30	1	20	20
Upper level (+)	1:30 AM	90	3	80	90
Basic level (0)	1:20 AM	60	2	60	70
Lower level (−)	1:10	30	1	40	50

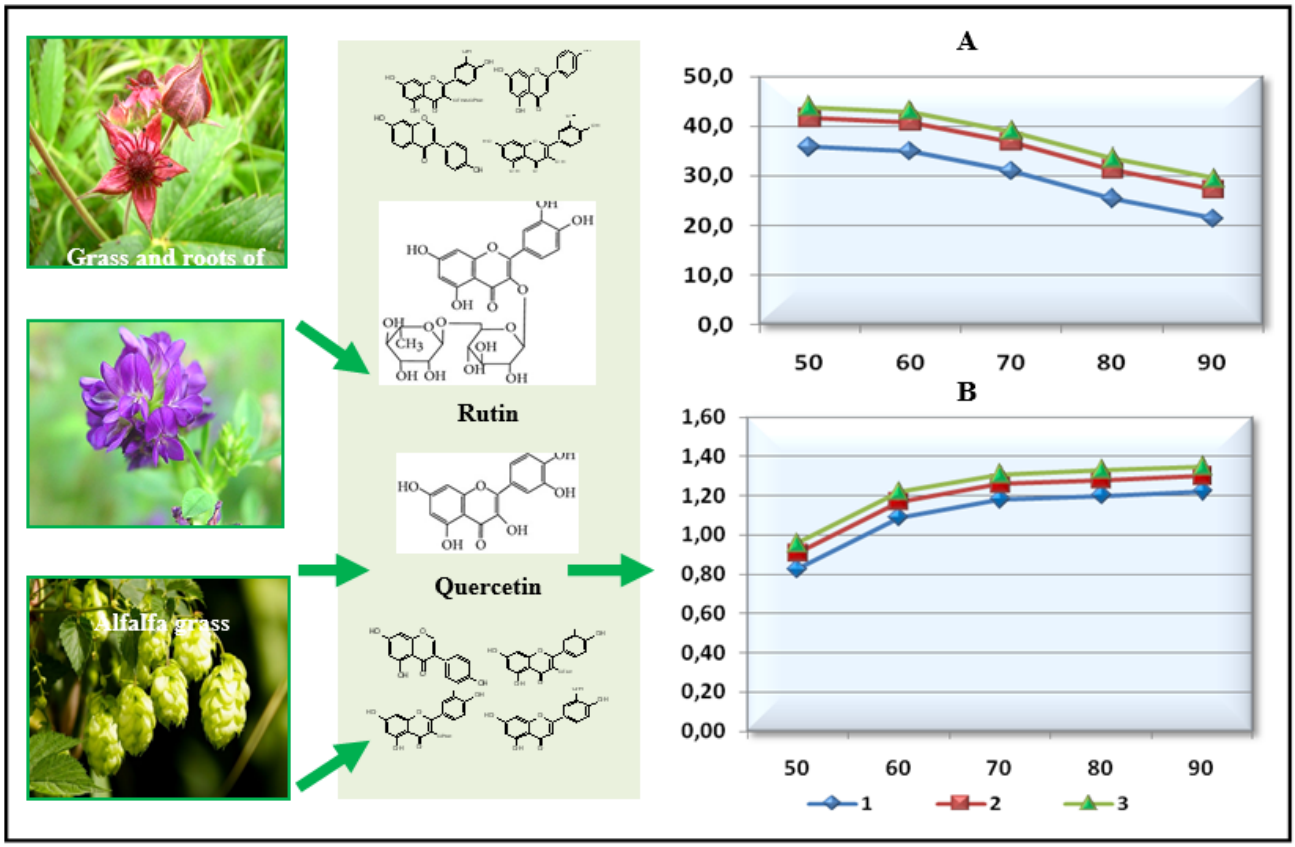

Figure 1. The influence of extraction conditions on the yield of extractives (A) and flavonoids (B) from the crude marsh cinquefoil, alfalfa and hop (40:40:20) (in % on absolutely dry crude material - along the ordinate axis) (scheme of the steepest ascent method by factor x_5 - the concentration of the extractant). Along the abscissa axis - the concentration of ethanol, %.

a) 1 - Single extraction;
b) 2 - double extraction;
c) 3 - triple extraction

Table 2 – Metrological characteristics of methods for the quantitative determination of flavonoids in a phytocomplex

Number of degrees of freedom (f)	Average content of flavonoids %	Variance	Standard deviation	Confidence coefficient (p), %	The boundary values of the confidence interval of the average result	Relative error of the average, %
Method I						
9	7,16	0,02416	0,15543	95	0.11	1,54
Method 2						
9	6,72	0,04855	0,22034	95	0.16	2,38

CONCLUSION

1. The composition of the new phytocomplex - dry extract from crude materials of grass and roots of marsh cinquefoil, the alfalfa grass and stems or strobili of common hop - has been developed (40:40:20), which can be used in the rehabilitation of the patients with osteoarthritis. The main BAS of the phytocomplex were flavonoids, among which quercetin and rutin dominated.
2. The possibility of using a phytocomplex for electro- and phonophoresis has been shown: flavonoids of the

phytocomplex were resistant to the action of various types of sinusoidal modulated currents, diadynamic currents, and ultrasound. The maximum electrophoretic mobility of the phytocomplex flavonoids was achieved when both the working cathode and anode were used.

3. The optimum conditions for obtaining the phytocomplex have been established: extraction with 70% ethanol, in the ratio of crude material and extractant - 1:30, at a temperature of + 60 °C, crude drug granulation - up to 1-2 mm, 3 times (1.5 hours, 1 hour and 30 minutes), cleaning with a double evaporation, evaporation in vacuum, and spray drying. The main quality indicators of the phytocomplex have been determined: the bulk weight at free fall - 0.38 + 0.01 g/cm^3, the bulk density at compaction - 0.69 + 0.01 g/cm^3, the flowability - 2.06 + 0.02 g/s, the angle of natural repose - 36.0 °, and the loss in the mass when dried - 3.84 + 0.03%. The spectrophotometric method for the quantification of flavonoids in a phytocomplex in terms of quercetin and rutin has been recommended.

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