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Best regards,

Chief Editor, Professor

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MORPHOLOGICAL-ANATOMICAL STUDY OF *ASTRAGALUS VARIUS* S. G. GMEL HERB

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For the first time, a morphological and anatomical analysis of the astragalus varius herb (Astragalus varius S. G. Gmel.) was carried out and microdiagnostic features of raw materials were established, allowing the Astragalus varius to be distinguished from the Astragalus onobrychis. Such features include: weakly wavy walled cells of the upper and lower epidermis of the leaf, equal-arm or weakly– unequal-arm T-shaped hairs, the nature of the cuticle and the shape of which differ depending on their location on the plant (on the stem and leaves – equal-arm or weakly-, unequal-arm cuticles with pronounced rounded-warty outgrowths, on the calyx – equal-arm, weakly-unequal-arm or sharply – unequal-arm or almost single-armed cuticles with weakly expressed outgrowths).

Keywords: *Astragalus varius* S. G. Gmel., Fabaceae, morphological and anatomical study, diagnostic features

The *Astragalus* L. genus is the largest genus in the legume family (Fabaceae) and includes 3270 species grouped into 100 subgenera. Representatives of the genus are found on almost all continents; among them there are a large number of rare endemic species [1].

Plants of the astragalus genus, being promising sources of medicinal plant raw materials, have been actively studied during recent years. It was found that the chemical composition of astragalus is represented by various groups of biologically active substances such as flavonoids, triterpene compounds, polysaccharides, organic acids [2]. Currently, the only representative of the genus that is allowed for medical use in Russia is woolly-flowered astragalus (*Astragalus dasyanthus* Pall.), the herb of which is used in the form of an infusion as a diuretic, hypotensive and sedating medication [3]. However, the woolly-flowered astragalus has a limited geographical range of growth and, as a result, an insufficient raw material base.

In this regard, the task of studying unofficial species with pharmacological action becomes urgent

The range of *Astragalus varius* S. G. Gmel. covers the south of the European part of Russia, the North Caucasus, the south of Western Siberia, as well as the west of Kazakhstan. This species can be found in steppes, on sands, on outcrops of chalk and limestone, in forests [4]. It grows in sandy steppes, on fixed sands, deposits on sandy soils. It is distributed sporadically, but sometimes massively on the sands, in the steppe part of the Lower Volga region, rarely enters the Caspian lowland [5]. In natural habitats, the Astragalus varius is often found together with the *Astragalus onobrychis* L. and has some outward similarities with it. In the form of crushed raw materials, it is quite difficult to distinguish them.

A study of the chemical composition of the Astragalus varius herb showed that the plant contains flavonoids (kaempferol, quercetin, narcissin, populnin, isoquercitrin, astragalin, 3-O-β-D-glucopyranoside of isoramnetin) [6]. When studying the biological activity of extracts from the Astragalus varius herb, it was found that the amount of flavonoids causes a more pronounced decrease in systemic blood pressure than papaverine hydrochloride [6].

All of the above allows us to consider Astragalus varius as a promising source of medicinal plant raw materials. In this regard, there is a need to study the morphological and anatomical features of this species and to establish macro- and microdiagnostic features for the identification of crushed raw materials, its differences from similar externally species, for example, *Astragalus onobrychis* L. [7].

The purpose of this work was to conduct a morphological and anatomical analysis of the *Astragalus varius* S. G. Gmel herb to identify and establish the authenticity of raw materials.

MATERIALS AND METHODS

The object of the study was a whole plant raw material – the *Astragalus varius* S. G. Gmel herb, harvested in July 2020 in Saratov region, in Tatishchevsky district, in the vicinity of the Kurdyum station during the mass flowering period. The species was identified by the key specified in the monograph "Flora of the European part of the USSR". Vol. 6 [5].

During the macroscopic analysis, we were governed by the current regulatory documentation – SP XIV OFS.1.5.1.0002.15 "Herbs" [8]. Preparation of micro-preparations, microscopy and their analysis were carried out according to the generally accepted pharmacopoeia methodology set out in the SP XIV of OFC.1.5.3.0003.15 "Technique of microscopic and microchemical research of medicinal plant raw materials and herbal medicinal products" [8]. The prepared microscope slides were examined with a Carl Zeiss Primo Star microscope (Germany) with magnifications 10×4, 10×10, 10×40, 10×100. The photos were taken with a Levenhuk M1400 Plus digital camera. The resulting micrographs were edited in the Photoscape 3.7 program.

RESULTS AND DISCUSSION

Macroscopic description

The *Astragalus varius* S. G. Gmel is subshrub with height of 30–35 cm [9]. The studied raw material of the Astragalus varius consists of leafy stems with flowers, whole leaves and individual flowers getting lignified at a base. Stems are slightly ribbed, 1–2 mm in diameter, abundantly pubescent with pressed hairs. The unpaired pinnately-compound leaves are 4–8 cm long, consist of 6–8 pairs of leaflets, have free triangular stipules about 2 mm long. Individual leaflets of a compound leaf are located on short petioles, their shape is lanceolate, the tip

is blunted, the base is rounded and tapered, the length is 5–20 mm and the width is 1–3 mm. The leaves are pubescent on both sides with pressed hairs. The flowers are collected in racemes. The calyx is tubular-bell-shaped, with length of about 1.0 cm, slightly pubescent with hairs that are pressed to or slightly draw aside the calyx. The corolla is violet or purple, moth-like. The vane of the corolla is about 1.5 cm long, 0.4–1.0 cm wide. Wing petals of the corolla have the same size as the vane. The keel is slightly shorter than the wings. There are 10 stamens.

Microscopic description

On the cross-section of the stem of the Astragalus virgatus (Fig. 1a), it is noticeable that the stem has a fascicular type of structure, turning into a non- fascicular. Also, T-shaped hairs on the surface of the epidermis and the core, consisting of large thick-walled parenchymal cells, which are partially destroyed, that leads to the formation of cavities, are clearly distinguishable.

The entire stem is covered with a single layer of epithelial cells (Fig. 1b). Under the epidermis there is primary cortex, represented by the ectoderm, mesoderm and endoderm. Next there are phloem cells and xylem vessels. The sclerenchyme is located in conductive bundles between the layers of the endoderm and phloem and the cells of the collenchyme are located between the vessels of the xylem. The stomata of the epidermis of the stem are of the anomocytic type, surrounded by 3 or 4 cells of the epidermis and located mainly in the intercostal space.

The entire stem is covered with equal-arm or slightly unequal-arm T-shaped hairs. In those places where the hairs are torn off, the points of their attachment are clearly distinguishable (Fig. 2a). The conductive bundles on the stem surface form well-marked longitudinal ribs. The epidermis of the stem above these ribs

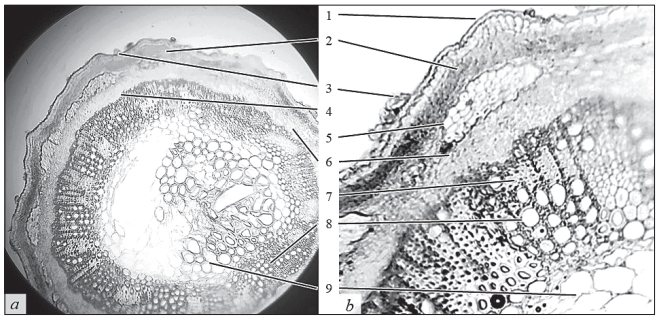

FIG. 1. *A cross section of the stem of Astragalus varius: a – the general plan of the structure of the stem (10×10); b – the conductive bundle (10×40). Designations: 1 – epidermis; 2 – primary cortex; 3 – T-shaped hairs; 4 – conductive bundle; 5 – collenchyma; 6 – phloem; 7 – sclerenchyma; 8 – xylem; 9 – parenchyma of the core*

is represented by strongly elongated cells of rectangular or almost rectangular shape with beveled walls arranged in even rows (Fig. 2a). Between the ribs, the epidermis consists of more rounded cells lying randomly (Fig. 2b).

The epidermis of the lower surface of the leaf (Fig. 3a) is formed by weakly wavy walled cells of various shapes and commonly occurring stomata, surrounded by 3 or 4 epidermis cells in the same way as on the stem. The cells of the upper epidermis of the leaf (Fig. 3b) are also weakly wavy.

T-shaped hairs are located on a 1–2-cell pedicle. The length of a single hair is 40–

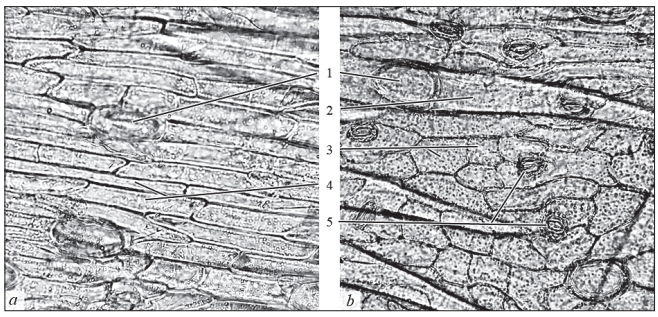

FIG. 2. *Epidermis of the stem of Astragalus varius: a – above the rib (10×40); b – between the ribs (10×40). Designations: 1 – the place of attachment of the hair; 2 – T-shaped hair; 3 – rounded epidermis cells; 4 – elongated epidermis cells; 5 – stomata*

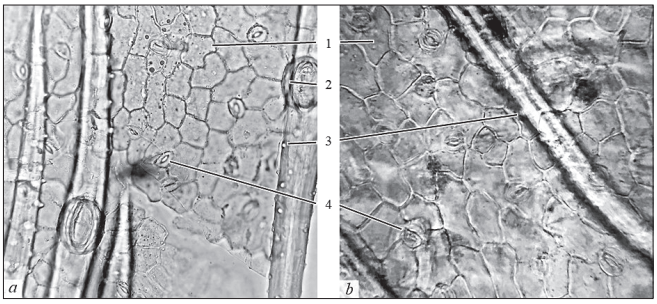

FIG. 3. *Epidermis of the leaf of Astragalus varius: a – lower (10x40); b – upper (10x40). Designations: 1 – weakly wavy cells; 2 – hair base; 3 – rounded-warty outgrowths of the hair cuticle; 4 – stomata*

50 microns. As well as on the stem, the shoulders of the hairs are equal or the difference in their length is small, and the cuticle has numerous pronounced rounded-warty outgrowths (Fig. 3).

From the outer and inner sides of the calyx, the epidermis is represented by straight-walled cells (Fig. 4a-4b). The T-shaped hairs are located only on the outside of the calyx. At the same time, at the base of the calyx (Fig. 4a) there are mainly short equal-arm hairs but as they move towards the tip, they stretch out and become unequal-armed or sharply- unequal-armed (Fig. 4b-4c). In the middle and upper parts of the calyx, there are also equal-arm hairs, but their number is small. On the teeth of the calyx (Fig. 4d) the hair metamorphosis can reach a stage when the short arm is almost completely or completely reduced and T-shaped hairs become similar to simple hairs. Rounded-warty outgrowths of the cuticle on the calyx hairs are much less pronounced than on the hairs located on the leaves and stem.

The shape of the epidermis cells of the petals on the outside and inside smoothly changes from strongly elongated rectangular with straight or beveled walls at the base to rounded or oval shape with slightly wavy walls at the tip (Fig. 5a-5c). The vessels are smooth, parallel, thin, branching twice or thrice at the tip (Fig. 5d).

When comparing the microscopic features of *Astragalus onobrychis* L., which are specified in accordance with the work of T.A. Pozdnyakova, E.S. Kuleshova and R.A. Bubenchikov [7], and Astragalus varius represented in this Article, the main distinguishing feature of these plants i.e. the nature of the lower and upper epidermis of the leaves, becomes obvious. Both the lower and upper epidermis of the leaves of Astragalus onobrychis are represented by strongly wavy cells. Both the lower and upper epidermis of the leaves of Astragalus varius are represented by weakly wavy cells.

The other characteristic features of Astragalus varius can include T-shaped hairs. On the stems and leaves, these hairs are characterized by equal-arm or slightly unequal-arm shape and have a cuticle with strongly pronounced

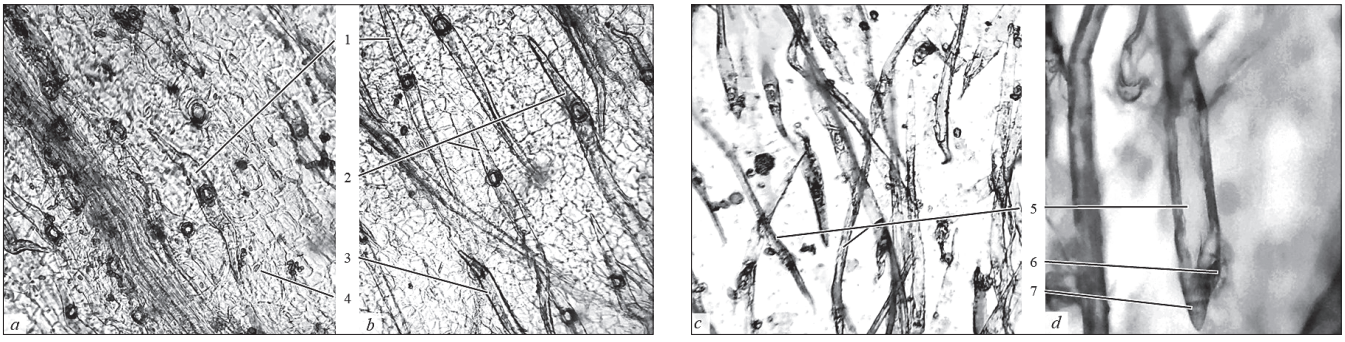

FIG. 4. *The epidermis and T-shaped hairs of the calyx of Astragalus varius: a – at the base (10×10); b – in the middle part (10×10); c – in the upper part (10×10); d – on the tooth (10×40). Designations: 1 – equal-arm hairs; 2 – unequal-arm hair; 3 – unequal-arm hair; 4 – stomata; 5 – long arm; 6 – base of the hair; 7 – reduced arm*

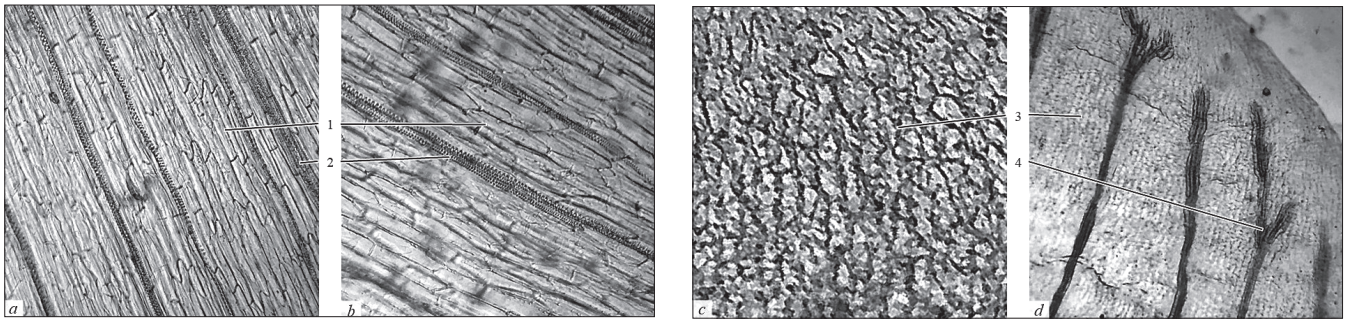

FIG. 5. *The epidermis of the corolla of Astragalus varius: a – at the base (10×10); b – in the middle part (10×10); c – in the upper part (10×10); d – in the upper part (10×4). Designations: 1 – elongated epidermal cells; 2 – vessels; 3 – wavy-walled cells; 4 – branching of vessels*

rounded-warty outgrowths. On the calyx, the shape of the hairs can vary greatly – from short equal-armed to long sharply unequal-armed. On the teeth of the calyx, the short arm of the hair can be completely reduced, and the hair becomes like a simple one. The cuticle on the T-shaped hairs in all parts of the calyx is noticeably less pronounced than on other parts of the plant.

CONCLUSIONS

As a result of the study, macro- and microscopic diagnostic features of the Astragalus variuis herb were identified, allowing us to identify raw materials and distinguish it from a similar species – Astragalus onobrychis. The most characteristic distinguishing features of the Astragalus variuis herb are weakly wavy-walled cells of the upper and lower epidermis of the leaf, equal-arm, weakly- or sharply- unequal-arm T-shaped hairs of the teeth of the calyx with weakly pronounced cuticle outgrowths, on the stem and leaves – unequal-arm or weakly-unequal-arm with pronounced rounded-warty outgrowths of cuticles. The cuticle of T-shaped hairs has rounded-warty outgrowths, which are most pronounced on the hairs of the leaves and stem and less pronounced on the hairs of the calyx.

The obtained data will allow us to develop the section "Microscopic characteristic features" of the pharmacopoeial monograph "Astragalus varius herb".

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VALIDATION OF THE PROCEDURE OF QUANTITATIVE ANALYSIS OF THE COMPONENTS OF A MIXTURE OF MEDICINAL PRODUCTS BY THE METHOD OF DERIVATIVES OF UV ABSORPTION SPECTRA USING CHEBYSHEV POLYNOMIALS

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The results of the development and validation of a procedure for the quantitative determination of substances in medicinal products using differentiation of UV absorption spectra by Chebyshev polynomials are presented in the article. The correctness of the procedure was controlled using the solutions of Citramon tablets from different manufacturers.

Keywords: spectrophotometry, validation, numerical differentiation, polynomial approximation

Spectrophotometry in the UV region of the spectrum is widely used in pharmaceutical analysis. Many methods have been developed to solve the problems of pharmaceutical analysis i.e. to determine the authenticity of the mixture, the quantitative composition of the mixture [1]. Identification and quantification of medicinal substances in a mixture by their absorption spectra is complicated by the fact that the absorption bands overlap. In these cases, the method of derivative spectroscopy is used. On the ordinate axis, not the optical density is recorded, but the derivative of the optical density along the wavelength [2,3]. This method allows you to clearly determine the position of the wavelength at maximum absorption, narrows down the absorption bands and allows you to determine substances at close wavelengths, excluding their influence on each other. This method is successfully used in the quantitative analysis of two- and – less often – three-component mixtures. The application of the method is based on the existence of a directly proportional dependence of the absorption value on the concentration of the substance in the analyzed solution:

$$D = \varepsilon \cdot c \cdot l.$$

At the same time, it is necessary to observe the principle of additivity of the optical densities of the mixture [4].

A variation of the method of derivative of spectrophotometry is the method of differentiation with smoothing. UV absorption spectra are described by polynomials of the nth degree [2]. The authors have developed a method for finding derivatives using tabular values of Chebyshev polynomials, which are related to derivatives by simple relations. The method is not widely used, because for its implementation it is necessary to carry out mathematical calculations, which requires automation of the calculation process.

In our work [5], a procedure was described for determining the concentration of a medical product in a two-component mixture by the method of derivative of UV spectrophotometry using Chebyshev polynomials. The UV absorption spectrum was approximated by an optimal n-th order polynomial. The coefficients of the polynomial corresponded to the values of the n-th order derivative. All calculations were carried out in a computer program [6].

The purpose of the study is to develop and validate a procedure for quantifying the composition of three-component mixtures of medical products using derivatives of UV absorption spectra with the use of Chebyshev polynomials.

MATERIALS AND METHODS

The objects of the study were medicinal products – Citramon-P tablets from different manufacturers and their models. The concentrations of the components of the mixtures for creating the tablet models corresponded to the pharmacopoeial monograph and were similar to their concentrations in tablets. Absorption spectra were taken with spectrophotometer SF-2000-02. Derivatives of absorption spectra were calculated in a computer program [6].

RESULTS AND DISCUSSION

The whole process of spectral analysis in order to determine the concentration of components in a mixture can be divided into several stages.

1. Preparation of solutions for the study

An important point at this stage is the choice of solvent. It is known that the profile of the UV spectra of medicinal substances, especially those associated with p-π, π-π* electronic transitions, depends on the pH of the medium. To study the nature of the spectra in the UV region of the molecular and ionic forms of the objects of study, 0.01 mol/l hydrochloric acid solution and 0.01 mol/l sodium hydroxide solution were used with the addition of ethanol in a ratio of 1:10. It was shown that the absorption band with a maximum of the ionic form in relation to the molecular form of acetylsalicylic acid was shifted hypsochromically by 18 nm (222±2 nm and 194±2 nm), the ionic form of paracetamol 242±2 nm in alkaline solution was shifted bathochromically by 10 nm (254± 2 nm). Caffeine as a compound that does not have pronounced acid-base properties, being in molecular form, both in acidic and alkaline media, has a constant maximum in the absorption band at 272±2 nm. Considering that both acidic and alkaline solvents in the range of 180–200 nm themselves strongly absorb light (Fig. 1), it is impractical to analyze substances in this range, for example, to determine acetylsalicylic acid by the maximum absorption of 194±2 nm in an alkaline medium. It is better to use an acidic medium in which the maximum absorption is 222±2 nm.

2. Working with the device СФ-2000

It follows from the characteristics of the device that when measuring the optical density up to 1, the error will be 0.01. This adds another condition for taking spectra – to dilute samples

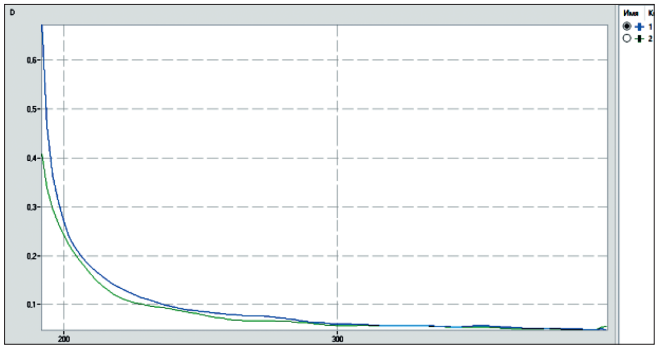

FIG. 1. *UV absorption spectra of compensation solutions relative to air:*
1 – a solution of 0.001 M sodium hydroxide with the addition of ethanol in a ratio of 1:10 (blue),
2 – 0.001 M solution of hydrochloric acid with the addition of ethanol in a ratio of 1:10 (green)

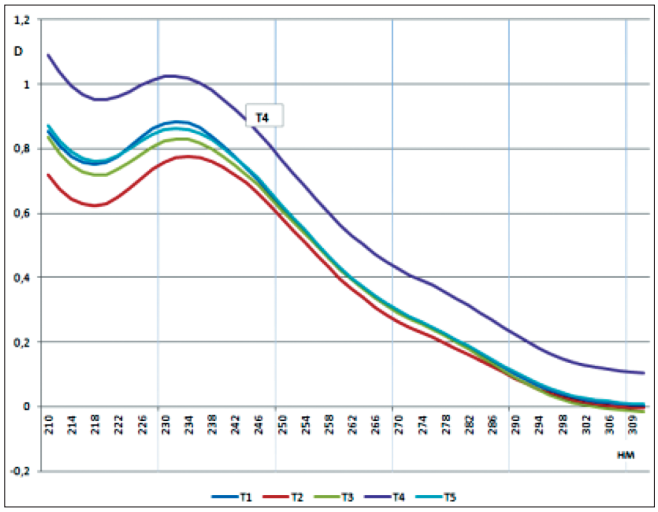

РИС. 2. *UV absorption spectra of the solution of Citramon-P tablets from five manufacturers (solvent – 98% ethyl alcohol and 0.01 mol/l hydrochloric acid in a ratio of 1:10). СФ-2000, step equal to 2 nm*

to concentrations at which the optical density does not exceed 1. Photometric reproducibility at an optical density of 1 does not exceed 0.005.

Figure 2 shows the UV absorption spectra of solutions of Citramon-P tablets from different manufacturers.

The spectra are identical and the difference is only in height, which is connected with the different weight of the tablet powder during the preparation of the mixture. The absorption spectrum of solution T No. 4 is higher than the others and has optical density values greater than 1. The calculation of the concentration of medicinal substances of this solution showed an unreliable result. Therefore, when preparing solutions to determine the concentration of medicinal substances in mixtures of the same composition, it is necessary to provide the equal sample weights.

In the spectral analysis of the mixture, some components are included in very small quantities, for example, caffeine in Citramon tablets. With a very strong dilution, the optical density becomes less than 0.01 in the areas of caffeine absorption. Therefore, for analysis such mixtures are excluded.

3. Choice of the scan step

The resolution and duration of collecting the spectral data depend on the scan step. The smallest resolved spectral range of the СФ-2000 spectrophotometer is 1±0.4 nm in the range of 190–390 nm (http://www.labteh.com/productID411/). We conducted a number of experiments to determine the optimal scanning step, for which the UV absorption spectral data for 0.002995% solution of a model mixture of Citramon tablets were collected in increments of 0.2 nm, 0.5 nm, 1 nm, 2 nm and 4 nm. The results are shown in Fig. 3. Analysis of the graphs shows that the scan step of 4 nm does not allow obtaining good resolution

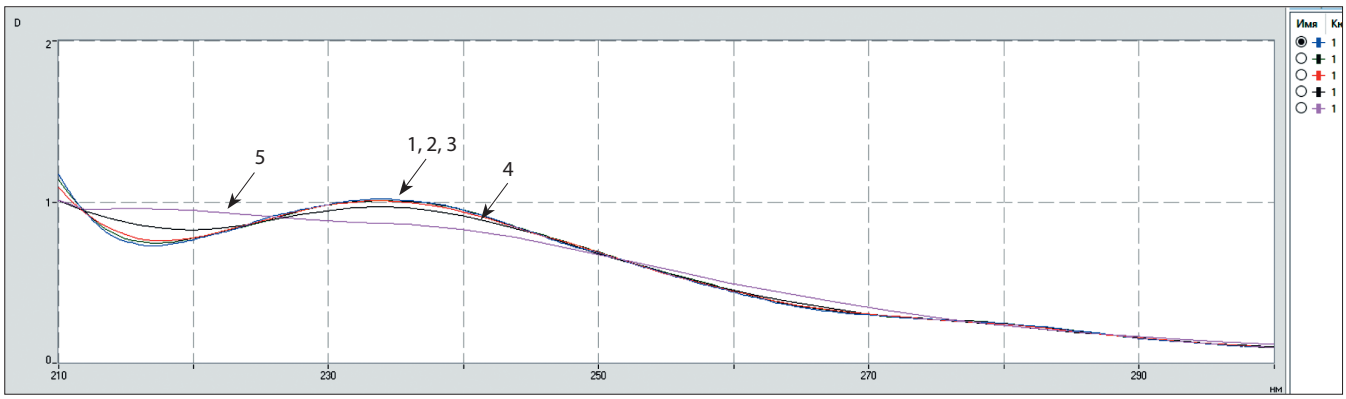

FIG. 3. *UV absorption spectrum of a solution of a model mixture of Citramon tablets in increments of 0.2 nm (1), 0.5 nm (2), 1 nm (3), 2 nm (4), 4 nm (5) (solvent – 98% ethyl alcohol and 0.01 mol/l hydrochloric acid in a ratio of 1:10)*

and the absorption spectrum has no maxima. At a step of 2 nm, the spectrum view is not distorted, it is the same as at a minimum step of 1 nm, therefore, the scan step of 2 nm can be considered as optimal.

4. Derivative of UV spectrophotometry and derivative of UV spectrophotometry with the use of Chebyshev polynomials

The СФ-2000 software allows calculating the first or second derivative of the selected spectrum. If we take the second-order derivative of the second-order derivative, we get the fourth derivative. Fig. 4a shows the UV absorption spectrum of Citramon tablets, Fig. 4b shows the second and fourth derivatives of the absorption spectrum along the wavelength.

Citramon contains acetylsalicylic acid with a maximum absorption of 222±2 nm, paracetamol – 240±2 nm, caffeine – 272±2 nm. The purpose of using the method of derivative of spectroscopy is to isolate individual bands of the UV spectrum. The spectrum is the sum of overlapping absorption bands or bands that do not have a clearly defined maximum (Fig. 4a). At each step of spectrum differentiation, the bands with pronounced maxima and minima appear. This allows identification of the composition of the mixture. In Fig. 4b, on the graph of the 2nd derivative, three main maxima and side ones, which are called satellites, are marked. The first maximum is in the region of 218 nm, the second is 258 nm and the third is in the region of 290 nm. However, it is inappropriate to continue differentiation for this mixture, since the number of maxima increases (Fig. 4b is the fourth derivative of the absorption spectrum). The positions of the maxima do not correspond to the wavelengths of the maximum absorption of the three components of the mixture such as 222 nm, 242 nm and 272 nm (aspirin, paracetamol, caffeine, respectively). Using the method of spectrum derivatives for the study of Citramon tablets will lead to great mistakes.

5. Differentiation of absorption spectra by the polynomial method

The main purpose of differentiating the absorption spectra by the polynomial method is to approximate the absorption spectrum of the mixture under study by the Chebyshev polynomial. The coefficients of the polynomial are related to the value of the derivatives by a simple relation. The derivatives at the points of the spectrum maxima are due to only one component of the mixture, which makes it possible to estimate the contribution of each, without dividing the absorption bands, using

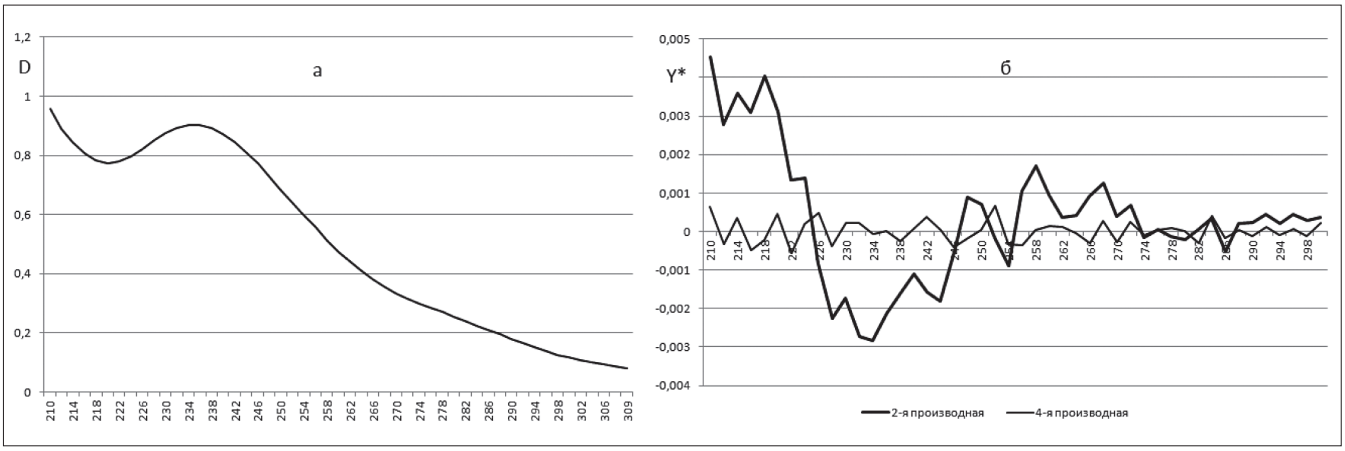

FIG. 4: *a) UV absorption spectrum of the solution of Citramon tablets. Step 2 nm (solvent – 98% ethyl alcohol and 0.01 mol/l hydrochloric acid in a ratio of 1:10); b) derivatives of the UV spectrum of 2nd order and 4th order. СФ-2000*

only the values of the coefficients of the optimal polynomial.

To calculate the coefficients, a computer program was compiled that allows approximating the absorption spectrum to any accuracy [7]. Optical densities are entered into the program as a file with the ANSI extension, into which the spectrum file is easily converted (a column of numbers into *спекmр.txt*), issued by the СФ-2000 device. The accuracy was determined by the minimum error, which was calculated by the formula:

$$\sigma = \sqrt{\frac{(D_i - y(\lambda_i))^2}{n-1}},$$

where D_i and $y(\lambda_i)$ – the values of the optical density of the absorption spectrum and the approximating polynomial, respectively.

Calculations of the optimal derivative using Chebyshev polynomials have shown that the smallest error corresponds to a polynomial of the 9th order (Fig. 5). In the first column there are calculated errors of polynomials from 1 to 11 orders. The second column in Fig. 5 shows the bit depth of the number of coefficients of the polynomials

```
CONCLUSION OF RESULTS
            1. VALUES OF DERIVATIVES
Choose the optimal derivative value,
corresponding to the minimum error
( N = derivative order)
-------------------------------------------------------
error                          max derivative value
-------------------------------------------------------
L 0 - .3019672                              25.83374
L 1 - 8.376991E-02                          217.7893
L 2 - 8.188932E-02                          174.3461
L 3 - 5.217582E-02                          8047.501
L 4 - 5.204165E-02                          6107.554
L 5 - 3.509493E-02                          800491.9
L 6 - 1.166569E-02                          8764276
L 7 - 1.139097E-02                          9162908
L 8 - 5.459004E-03                          4.883734E+08
L 9 - 3.638987E-03                          5.193789E+07
L 10 - 1.259523E-02                         1.525704E+11
L 11 - 1.304155E-02                         6.18418E+11
-------------------------------------------------------
OPPOLINOM=
?
```

FIG. 5. *Optimal polynomial of the 9th order (minimum error – 3.6.10^{-03}). (Computer program [6].)*

The errors of the coefficients of the polynomials of the 8th and 9th orders are approximately the same 5,4.10^{-03} (the 8th order polynomial) and 3,6.10–03 (for the 9th order). Both polynomials approximate the absorption spectrum well and can be chosen as the optimal polynomial. Figure 6 shows the absorption spectrum and the approximating absorption spectrum – a polynomial of the 9th order.

6. Calibration graphs

Finding the optimal derivative of the absorption spectra is necessary to find its values at the points of maximum absorption of the components of model mixtures used to construct the calibration graphs. A larger variation of concentrations of standard solutions is irrelevant due to the limited range of possible changes in the concentration of the test substance in tablets. The second reason that calibration graphs are constructed with a slight difference in concentrations is that the optimal derivatives should be of the same order. In our case, the range of concentrations of calibration mixtures was: for acetylsalicylic acid – 0.0011–0.0020%; for paracetamol – 0.0009–0.0015%, for caffeine – 0.00015–0.00030%

The following equations were obtained: for acetylsalicylic acid (222 nm) – Y* = 1716.25 +

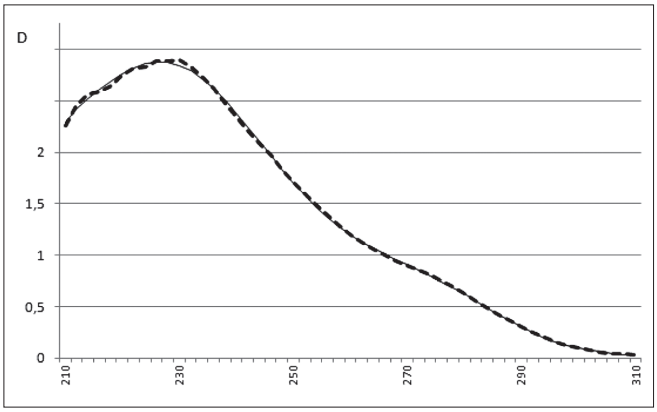

FIG. 6. *UV absorption spectrum of 0.00253% solution of the Citramon tablet model is a solid line and approximation by the 9th order polynomial is a dotted line*

Table 1

COMPARISON OF THE FOUND AND EXPECTED VALUES OF THE WEIGHTS OF MEDICINAL SUBSTANCES IN TABLETS FROM DIFFERENT MANUFACTURERS CONTAINED IN THE WEIGHED SAMPLES USED FOR THE STUDY

Citramon Tablets	Expected value, mg	Found value for Acetylsalicylic acid, mg	Expected value, mg	Found value for Paracetamol mg	Expected value, mg	Found value for Caffeine, mg
Tablet 1	242±12	246±4	181±12	191±0.3	33±2	30±0.5
Tablet 2	221±11	206±4	166±8	177± 0.3	28±1	27±0.5
Tablet 3	229±11	233±4	172±9	184±0.3	29±1	30±0.5
Tablet 4	248±12	304±4	186±9	227±0.3	31±2	45±0.5
Tablet 5	238±12	246±4	178+9	191±0.3	27±1	31±0.5

$5.9E^{+12} \cdot C$, $5 \cdot 10^{-5}$%; for paracetamol (242 nm) – $Y^* = 896.8186 + 1.98E^{+12} \cdot C$, $2 \cdot 10^{-6}$%; for caffeine (272 nm) – $Y_9 = 693.0353 + 1.09E^{+13} \cdot C$, $4 \cdot 10^{-6}$%. Y* is the value of the optimal coefficient of the 9th order polynomial. The correlation coefficient is 0.99 for all equations.

To control the correctness of the procedure used, the solutions of Citramon-P tablets from different manufacturers were used:

- TNo.1 – Tathimpharmparaty JSC, Kazan (batch 550920, valid until 10.24);
- TNo.2 – Medisorb JSC, Perm (batch 39042020, valid until 05.2024);
- TNo.3 – Dalkhimpharm JSC, Khabarovsk (batch 440920, valid until 09.24);
- TNo.4 – Pharmstandard – Leksredstva JSC, Kursk (batch 7941120, valid until 12.24);
- TNo.5 – Industrial Pharmaceutical Company "Obnovlenie" JSC, Novosibirsk (batch 11120, valid until 12.24).

Table 1 includes the expected values of the weight of medicinal substances contained in the sample weights of tablets, and the values found using the calibration graphs. By comparing the obtained weights with the expected ones (sample weight ±5%), it can be concluded that the found weights of acetylsalicylic acid, paracetamol, caffeine in tablets No. 1, No. 2, No. 3, No. 5 are included into the intervals (5% error) of the weights of medicinal substances in the weighed samples taken for the study The content of substances in mixture No. 4, as expected, does not meet the requirements of the State Pharmacopoeia of the Russian Federation of the XIV edition, since the sample weight of this medical product differed from others by 0.03 g and with appropriate dilution, the optical density of this medical product exceeded 1.

CONCLUSION

1. The analysis of the implementation of the algorithm of the method of derivatives of the UV absorption spectra of medical product mixtures by Chebyshev polynomials to determine the concentrations of their components is carried out.

2. At each stage, possible violations leading to errors in the use of the proposed method are identified. The ways of their elimination are presented.

3. Following the requirements of the method (solvent selection, range of spectral analysis of UV absorption spectra, scan step, calculation of the optimal derivative and construction

of calibration graphs) allows us to assert that the method is applicable for the analysis of a multicomponent system of substances that do not react chemically with each other. The method allows you to find concentrations of medical products in a mixture quickly and to good accuracy.

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FEATURES OF SORPTION OF LEAD (II) IONS BY PECTIN SUBSTANCES EXTRACTED FROM PLANT RAW MATERIALS

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The study of pectin substances (PS) isolated from the leaves of black currant, garden loosestrife herb, inflorescences of gaillardia pulchella, showed that for all samples of pectin substances the complex formation proceeds according to the reaction of the first order. The rate constants for pectin substances isolated from different plants were $8.9 \cdot 10^{-3}$ min^{-1} (PS from black currant leaves); $26.3 \cdot 10^{-3}$ min^{-1} (PS from garden loosestrife herb); $27.8 \cdot 10^{-3}$ min^{-1} (PS from gaillardia pulchella inflorescences). The study of the sorption capacity of polysaccharides showed the presence of a high ability to bind Pb^{2+} ions in 40 min.: PS of the gaillardia pulchella inflorescences – 95.1%; PS of the garden loosestrife herb – 91.9%; PS of the leaves of black currant – 72.3%. Calculations of the experimental adsorption value according to the Langmuir and Freundlich equations revealed the ratio of these values. The results indicate that the sorption process for PS from all samples of plant raw materials in a greater degree adheres to the Langmuir equation. The high sorption capacity of the studied PS in relation to lead ions makes it possible to consider them as effective natural detoxifiers. Further study of the physical and-chemical properties of PS – for example, the effect of amino acids on the binding properties of PS, the effect of PS on the therapeutic effect – in case of development of combination medications, etc. it will allow to evaluate the possibility of their medical and biological application.

Keywords: pectin substances (PS), the leaves of black currant, garden loosestrife herb, the inflorescences of *Gaillardia pulchella,* complex-forming ability

Currently, the research is being conducted for the physical and chemical study of the properties of polysaccharides in order to find new plants of this series, study their properties and possibilities for medical use. Pectins have complex-forming ability with respect to heavy metal ions: it is known, for example, that pectins adsorb Pb^{2+} ions more strongly than activated carbon [1,2].

The sorption properties of PS depend on the source of its isolation, on the content in the macromolecule of pectins of carboxyl and hydroxyl groups involved in the sorption process and contributing to the formation of strong complex compounds with divalent metal ions [2,3].

Due to the deterioration of the environmental situation associated with the emission of greenhouse gases and exhaust gases, a significant increase in chemical additives in food, beverages, clothing and shoes, the need to find cheap and affordable detoxifiers is becoming increasingly urgent. Therefore, it is necessary to comprehensively study the physical and chemical properties of natural sorbents, including pectin substances.

Pectin substances are widely used in the food industry (jelly-forming agents, stabilizers, improvers of rheological properties of the product, sources of dietary fiber in therapeutic and preventive nutrition); in geology (pectin glue during drilling); in printing (curing the printed materials); in medicine (immunomodulatory properties, anticarcinogenic, gastroprotective, detoxifying, enhancing the therapeutic effect of medicines); in pharmacy (stabilizers, emulsifiers, as a carrier matrix of biologically active components) [2–4].

The biological activity of pectins depends on their molecular weight and degree of esterification. Previously calculated by us, the average molecular weight of pectin substances isolated from the leaves of black currant, the inflorescences of *Gaillardia pulchella and garden loosestrife herb,* was 26153, 1823 and 830 g/mol, respectively [5].

All the studied pectin substances are characterized by a low degree of esterification, which probably may indicate their high complex-forming ability [6]. To confirm this assumption, it was necessary to determine the complex-forming activity of the obtained pectin substances in relation to lead ions.

The purpose of the work was to study the kinetics of complex-forming of PS and lead ions, to determine the sorption capacity of pectin substances isolated from the leaves of black currant, inflorescences of *Gaillardia pulchella and garden loosestrife herb.*

MATERIALS AND METHODS

PS isolation was carried out by the method of N.K. Kochetkov and M. Sinner [7]. Pectin substances are based on pectin acid, which is a high-molecular polygalacturonic acid [7,8]. The sorption properties of PS were studied on the basis of their ability to complex-forming with respect to lead ions. In the process of taking, the pectin, turning into pectin acid, combines with Pb^{2+} ions, forming insoluble salts to be excreted [1,7].

The Pb^{2+} ion content in the raffinate after sorption was determined by titrimetry using sodium ethylenediaminetetraacetate disubstituted in an acetate buffer medium (pH=5) in the presence of Xylenol orange [8]. The mechanism of complex-forming was evaluated by determining the order and rate constant of the reaction [9].

PS weighted samples were prepared and 0.035 M lead acetate solution was added. An indicator of complex-formation is the formation of loose sediment, to which 100 ml of distilled water was added with stirring.

Filtration was carried out at certain intervals and 10 ml of acetate buffer solution (pH = 5) was

added to 10 ml of filtrate, diluted with water in a measuring flask up to 100 ml. To determine the amount of bound lead ions, titration was carried out with a solution of sodium ethylenediaminetetraacetate, double-substituted in the presence of Xylenol orange before the transition of raspberry color to lemon one.

The weight of lead ions (mg/100 mg) was calculated according to equation 1.

$$m_{Pb^{2+}_{(mg)}} = \frac{E_{Pb^{2+}} \times N \times V}{m}, \quad (1)$$

where V – titrant volume (EDTA), ml; N – normal concentration (EDTA), ml; $E_{Pb^{2+}}$ – molar equivalent weight of lead ions.

The adsorption value (A_{exp}) in the experiment was calculated by equation 2:

$$A = \frac{(C_0 - C_{comp}) \times V}{m}, \quad (2)$$

where m – weight of sorbent, g; V –volume of the solution from which the adsorption occurs, l; C_0, C – initial and equilibrium concentrations of lead ions in solution, mmol/l.

The sorption capacity of PS was evaluated using the Langmuir and Freundlich equations.

Langmuir equation:

$$A = \frac{A_\infty \times c}{b + c}, \quad (3)$$

where A∞, b – constant; C – equilibrium concentration.

Freundlich equation:

$$A = KC^{\frac{1}{n}}, \quad (4)$$

but it is convenient to use it in logarithmic form:

$$\lg A = \lg K + \frac{1}{n} \lg C, \quad (5)$$

where K and $\frac{1}{n}$ – constants; C – equilibrium concentration; A – adsorption.

RESULTS AND DISCUSSION

The yield of pectin substances in the inflorescences of the *Gaillardia pulchella* was 5.1 ± 0.1%, in the *garden loosestrife herb* – 10.7 ± 0.2% and in the leaves of the black currant – 9.9 ± 0.2%. Quantitative determination of PS was carried out in 6 repetitions.

The sorption capacity of PS *in vitro* to lead ions was studied, since pectins are effective detoxifiers with different sorption capacity. Sorption capacity is expressed by the amount of metal ions bound to 1 g of polysaccharide.

The ability of PS to complex-formation is due to the presence of hydroxyl and carboxyl groups in the polymer molecule [8,10].

The insoluble salts formed are excreted from the body naturally. Another mechanism of excretion of heavy metal ions from the body is due to the ability of the low-molecular fraction of pectin substances to penetrate into the blood, forming complexes with subsequent natural extraction.

The sorption capacity of PS was studied using the Ostwald isolation method. Complex-forming is carried out with an excess of sorbent and a lack of reagent.

The results of determining the complex-forming ability ($K_{mg/g}$) are presented in Table. 1. Its value was estimated as the ratio of the change in the amount of lead ions (mg) during sorption to the weight of the sorbent (g).

We have experimentally found that within 40 minutes the highest percentage of binding of lead ions is 95.0% that is characteristic of pectin substances isolated from the inflorescences of *Gaillardia pulchella*. Slightly less sorption was observed in PS isolated from *garden loosestrife herb* – 92.5%, in PS isolated from black currant leaves – 72.5%. The sorption capacity of PS remains unchanged for 1 hour.

Table 1

BINDING ABILITY OF PS FROM *BLACK CURRANT LEAVES, GARDEN LOOSESTRIFE HERB, GAILLARDIA PULCHELLA* INFLORESCENCES TO LEAD (II) IONS

t, min	Number of ions Pb^{2+}, mg			Content of ions Pb^{2+}, mmol/L			% of binding ions Pb^{2+}			K_1C, mg/g		
	$PS_{bl.cur.}$	$PS_{g.l.}$	$PS_{G.p.}$	$PS_{bl.cur.}$	$PS_{g.l.}$	$PS_{G.p.}$	$PS_{bl.cur.}$	$PS_{g.l.}$	$PS_{G.p.}$	$PS_{bl.cur.}$	$PS_{g.l.}$	$PS_{G.p.}$
0	82.9	82.9	82.9	40.0	40.0	40.0	–	–	–	–	–	–
10	26.9	12.4	10.4	13.0	6.0	5.0	67.6	85.0	87.5	560.1	704.3	725.0
20	25.4	8.3	6.5	12.5	4.0	3.5	68.8	90.0	91.3	579.3	745.7	761.5
30	24.9	8.3	6.2	12.0	4.0	3.0	70.0	90.0	92.5	580.3	745.7	761.5
40	23.0	6.2	4.1	11.0	3.0	2.0	72.5	92.5	95.0	599.1	761.5	787.9
60	23.0	6.2	4.1	11.0	3.0	40.0	72.5	92.5	95.0	599.1	761.5	787.9

Note: $PS_{bl.cur.}$ – pectin substances isolated from the leaves of black currant; $PS_{g.l.}$ – pectin substances isolated from garden loosestrife herb; $PS_{G.p.}$ – pectin substances isolated from the inflorescences of gaillardia pulchella

The kinetic characteristics of complex-forming were evaluated by determining the order of the reaction and the values of the rate constants.

The reaction order was determined by the dependence of ln/C on time, where C is the content of Pb^{2+} ions, mol/L. The linearity of the obtained dependence (Fig. 1) allows us to assert that complex-formation proceeds by a reaction of the first order [8,9].

The reaction rate constants were determined by the tangent of the angle of inclination of the straight lines to the abscissa axis. The values found (min^{-1}) for PS from currant leaves were $8.9 \cdot 10^{-3}$, for PS from gaillardia inflorescences – $26.3 \cdot 10^{-3}$, for PS from garden loosestrife herb – $27.8 \cdot 10^{-3}$.

The sorption capacity of natural biopolymers was evaluated on the basis of experimental

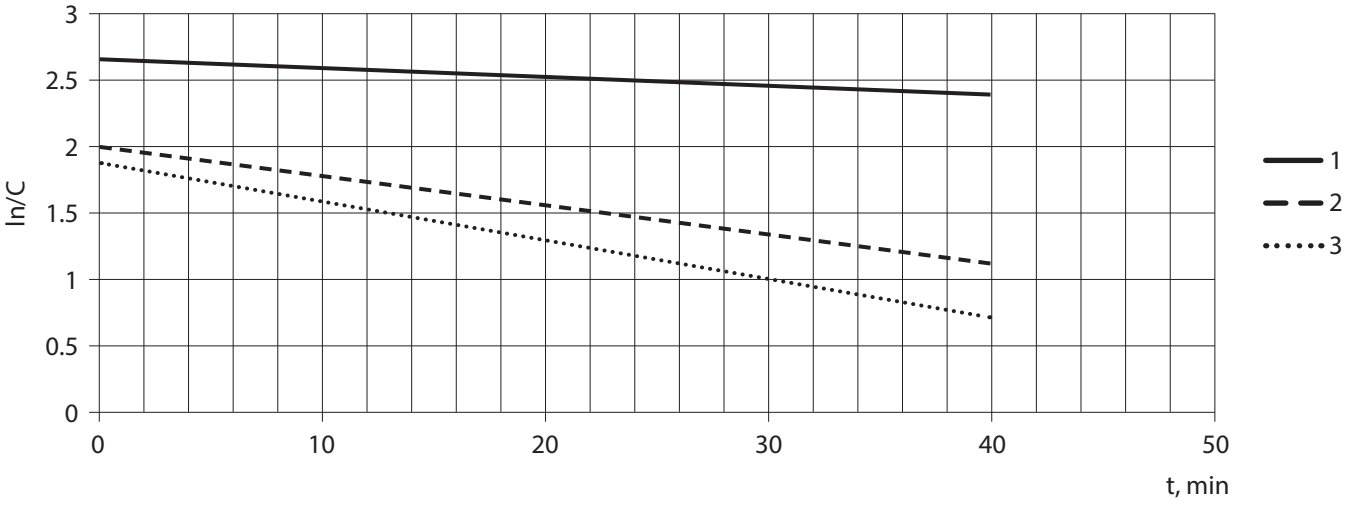

FIG. 1. *Dependence ln/C on t for the reaction of I order (1 – PS isolated from black currant leaves; 2 – PS isolated from the inflorescences of Gaillardia pulchella; 3 – PS isolated from garden loosestrife herb)*

data (Table. 1) and the calculated Freundlich and Langmuir equations. The applicability of the equations was determined by the ratio of the experimental and calculated values of adsorption. Depending on the nature of the sorbent, the adsorption process may obey one of the equations [10–12].

To find the constants of the Langmuir equation, a graphical dependence of 1/A on 1/ΔC was used (Fig. 2). The segment cut off by a straight line from the ordinate axis of 1/C corresponds to the value of 1/A. The coefficient b is numerically equal to the concentration at which the adsorption is half of the limit value. Its value was found by additional constructions. The values found were for the adsorption of Pb2+ ions on PS: from black currant leaves – A = 40.3; b = 32.3; from garden loosestrife herb – A = 55.8; b = 28.6; from inflorescences of gaillardia – A = 35.9; b = 27.8.

The adsorption equilibrium constant b depends on the affinity of the adsorbate to the adsorbent [10,12]. This affinity is expressed the more, the greater the value of the constant. For PS from the inflorescences of gaillardia, it was 40.3, while the percentage of binding capacity was 95%.

The values of the constants were found by the graphical dependence of lgA on lgΔC and extrapolation to the intersection with the ordinate axis. The segment cut off from the ordinate axis is lgK, and the tangent of the angle of inclination to the abscissa axis is 1/n (Fig. 2).

The coefficients found were for the adsorption of Pb^{2+} ions on PS: from garden loosestrife herb – K = 20.4; 1/n = 0.43; from the inflorescences of gaillardia pulchella – K = 23.98, 1/n = 0.90.

The constant K depends on the diffusion coefficient and the size of the adsorbing surface of the sorbent. According to the largest value K = 39.8 for PS from gaillardia inflorescences, we again observed a correlating dependence on its binding ability in relation to Pb^{2+} ions (95%).

The faster the saturation limit of the sorbent surface is reached, the greater the K value is. In our case, K increased from 29.38 to 39.8 in the studied samples. At the same time, the sorption capacity also increased – from 72.5% to 95%.

Comparative data of calculated and experimental values of adsorption are given in Table 2.

It follows from the data in Table 2 that the functional dependence of sorption capacity in all pectin substances in a greater degree adheres to the Langmuir equation. The applicability of the Langmuir model indicates the monomolecularity of PS adsorption on the active centers of

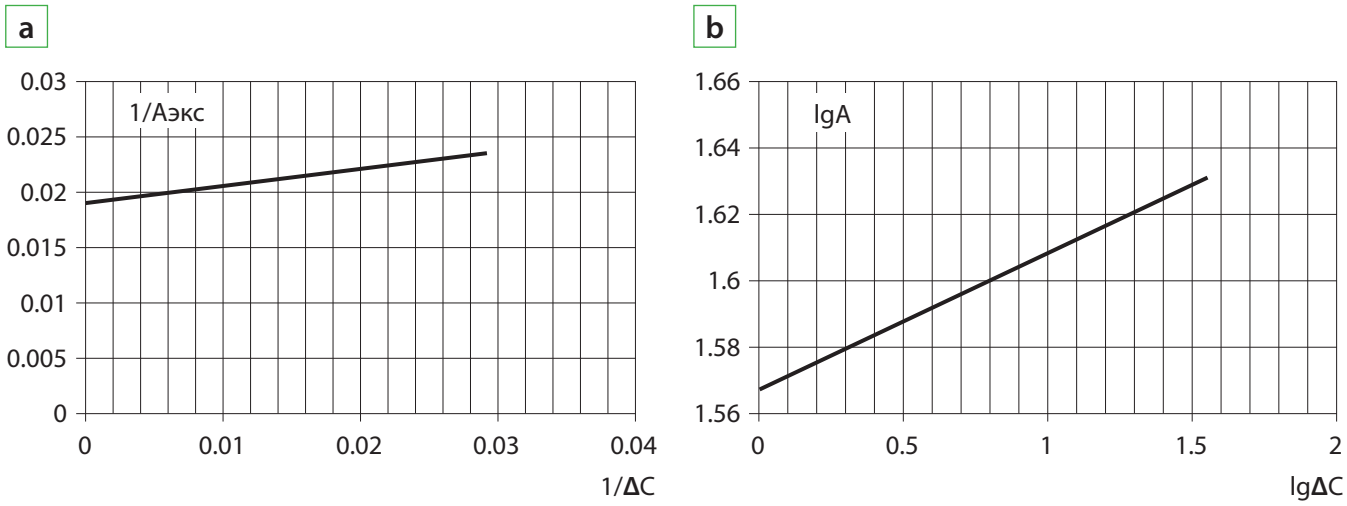

FIG. 2. *Isotherms of adsorption of lead ions on PS from garden loosestrife herb: a) in the coordinates of the linear form of the Langmuir equation; b) in the logarithmic coordinates of the Freundlich equation*

Table 2

THE RATIO OF THE EXPERIMENTAL VALUE OF ADSORPTION OF PB2^{+} IONS ON PS ISOLATED FROM PLANT RAW MATERIALS

t, min	ΔC, mol/L	A_{exp}, Mmol/g	A_l, Mmol/g	A_f, mol/g	A_{exp}/A_l	A_{exp}/A_f
PS of black currant leaves						
10	27,0	32,4	18,2	695,3	1,7	0,047
20	27,5	33,0	18,4	707,6	1,8	0,047
30	28,0	33,6	18,6	719,9	1,8	0,047
40	29,0	34,8	18,9	744,6	1,8	0,047
Average values					1,8	0,047
PS of garden loosestrife herb						
10	34,0	40,8	26,1	502,6	1,6	0,08
20	35,5	42,6	26,7	519,0	1,6	0,08
30	36,0	43,2	26,9	524,4	1,6	0,08
40	37,0	44,4	27,3	535,1	1,6	0,08
Average values					1,6	0,08
PS of inflorescences of gaillardia pulchella						
10	35,0	42,0	27,3	244,0	1,5	0,17
20	36,5	43,8	27,9	249,2	1,6	0,18
30	37,0	44,4	28,1	251,0	1,6	0,18
40	38,0	45,6	28,5	254,4	1,6	0,18
Average values					1,57	0,18

the sorbent surface and witnesses its possible homogeneity.

The adsorption mechanism is complex, which is confirmed by deviations of experimental data from theoretically calculated ones. It is possible this is explained by the surface of adsorbents, which is geometrically, chemically and energetically heterogeneous. Therefore, the rate of adsorption on different parts of the surface is not the same.

Analysis of the data obtained allows us to conclude that it is possible to use pectin substances isolated from plant raw materials for binding the lead (II) ions and as natural detoxifiers.

CONCLUSION

1. The sorption properties of PS isolated from the leaves of black currant, garden loosestrife herb, gaillardia pulchella inflorescences in relation to lead (II) ions were used.

2. It is shown that complex-formation proceeds according to the kinetic mechanism of the first order.

3. It is established that the degree of extraction of Pb^{2+} ions of PS from plant raw materials for PS of gaillardia pulchella inflorescences is 95.1%, for PS of garden loosestrife herb – 91.9%, for PS of black currant leaves – 72.3%.

4. The experimental data obtained allow the use of PS isolated from plant raw materials for the extraction of lead ions from aqueous solutions of various nature.

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APPLICATION OF FTIR SPECTROSCOPY FOR AUTHENTICATION OF NIGELLA SATIVA SEEDS

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The paper shows the possibility of using the method of FTIR spectroscopy for the purpose of establishing the authenticity of Nigella Sativa seeds and pharmaceutical products prepared from them. In this case, the best is to identify the Nigella Sativa plants by the presence of thymoquinone, which is one of the main active substances and to a significant extent determines its pharmacotherapeutic potential. The proposed FTIR spectroscopy method can be used to assess the quality of Nigella Sativa seeds, black seed oil and supercritical carbon dioxide extract.

Keywords: FTIR spectroscopy, thymoquinone, Nigella Sativa seeds, black seed oil, supercritical carbon dioxide extract

The FTIR spectroscopy method is one of the priority research methods that have been successfully used for decades to establish the authenticity of compounds of various nature [1,2]. A high degree of confidence in this method is connected to the fact that the spectral characteristics, namely, the positions of the absorption band maxima on the wavenumber scale, as well as their intensity, are individual for each chemical compound, i.e., essentially, its specific characteristic [2,3].

Currently, the implementation of this method is carried out with the predominant use of FTIR spectrophotometers, which are widely used to study the chemical composition and establish the authenticity of plant raw materials for the main classes of biologically active substances (BAS) [4–9].

The use of FTIR spectroscopy for the study of plant raw materials, which are of interest from the point of view of their implementation into medical practice, is particularly true. To solve this task, it is necessary not only to obtain the detailed information about the composition of BAS, but also to standardize raw materials according to the selected components [7].

Promising plant raw materials for use in medical practice are Nigella Sativa seeds, from which black seed oil is currently prepared, as well as supercritical carbon dioxide extract [10,11].

According to the scientific literature, thymoquinone is the main biologically active

compound of Nigella Sativa seeds, which determines their pharmacotherapeutic potential [10]. This compound is characterized by a diverse spectrum of pharmacological action. It has been established that thymoquinone has analgesic, choleretic and hepatoprotective activity [10,12]. Along with this, the protective effect of thymoquinone *in vitro* on the pulmonary and cardiovascular system, as well as its ability to inhibit the process of gluconeogenesis in the liver was revealed [10]. A number of authors have found that thymoquinone is able to reduce the frequency of asthma attacks, has broncholytic and antihistamine effects [10,12–14]. The latest data obtained by Algerian scientists by molecular docking in relation to the main BAS of Nigella Sativa, including thymoquinone, demonstrate their ability to inhibit the SARS-CoV-2 virus and have a preventive effect against a new coronavirus infection [15,16].

Currently, the identification and quantitative determination of thymoquinone in the Nigella Sativa seeds and pharmaceutical products based on them is carried out mainly by the method of high-performance liquid chromatography (HPLC) [17–19]. However, for the purposes of confirming the authenticity of plant raw materials, FTIR spectroscopy is a more convenient and faster method, as described above. Therefore, the use of this method in the pharmacognostic analysis of Nigella Sativa seeds is an extremely urgent task.

The purpose of the work was to study the possibility of using the method of FTIR spectroscopy to establish the authenticity of Nigella Sativa seeds and pharmaceutical products based on them.

MATERIALS AND METHODS

As objects of the study, Nigella Sativa seeds cultivated in Krasnodar Territory (Krasnodar, Vasyurinskaya station) were used, which were collected and harvested in August-September 2020 during their full maturation period; as well as a certified commercial sample of black seed oil (RUSOIL LLC, Russia) produced by cold pressing and supercritical carbon dioxide extract of Nigella Sativa seeds, the fluid technology of which was developed by the authors from KubSMU, were also applied [11].

Identification of Nigella Sativa seeds, black seed oil and carbon dioxide extract was carried out by the characteristic absorption bands in comparison with the IR spectrum of the certified reference standard of thymoquinone (99%, Sigma).

The test was carried out using FTIR spectrometer IRTracer – 100 (Shimadzu, Japan) provided with the LabSolutions IR software package, in the transmission mode within the range from 4000 to 400 cm^{-1} with 20-fold scanning with resolution of 4 cm^{-1}. To record the absorption spectra, the analyzed samples were ground with a minimum amount of nuyol oil in accordance with the requirements of the RF SP, Edition XIV.

The resulting suspension was compressed between two plates made of a special non-hygroscopic material (CaF_2).

RESULTS AND DISCUSSION

On the IR spectrum (in the region of characteristic frequencies) of the thymoquinone reference standard the following absorption bands are clearly visible: 1130 (very weak), 1238 (weak), 1377 (medium), band group 1464–1460 (medium), 1661 (medium), band group 2853–2953 (strong) cm^{-1} (Fig. 1)

Comparison of the IR spectra of the reference standard and the test samples (Fig. 2 and 3) in the region of characteristic frequencies allowed us to identify the absorption bands of thymoquinone, which were clearly

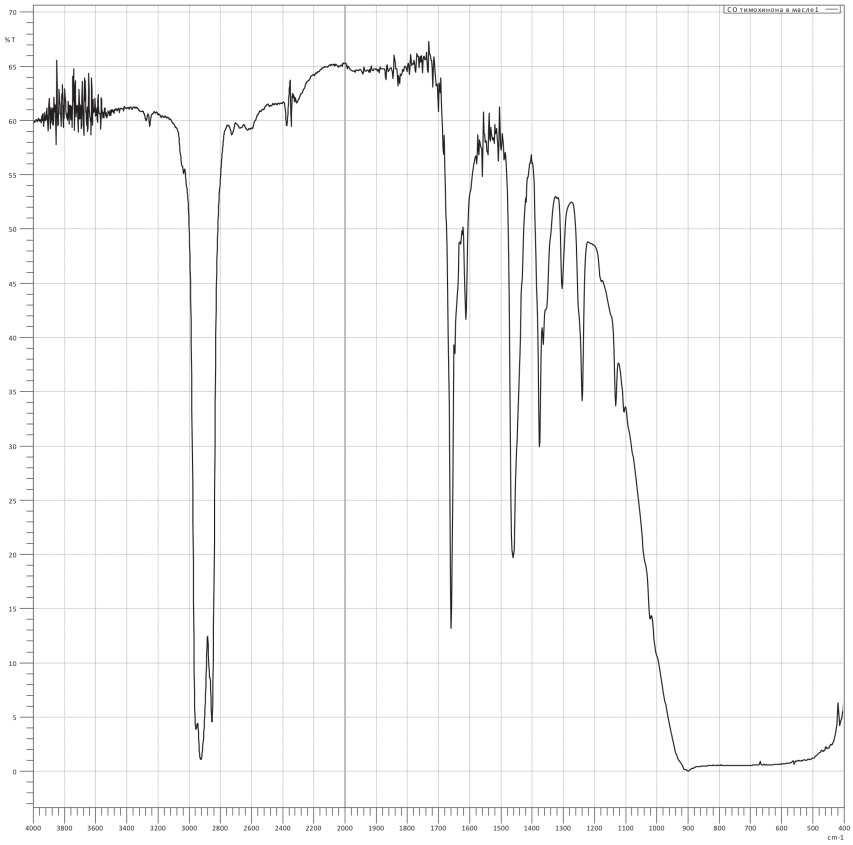

FIG. 1. *IR spectrum of the thymoquinone reference standard*

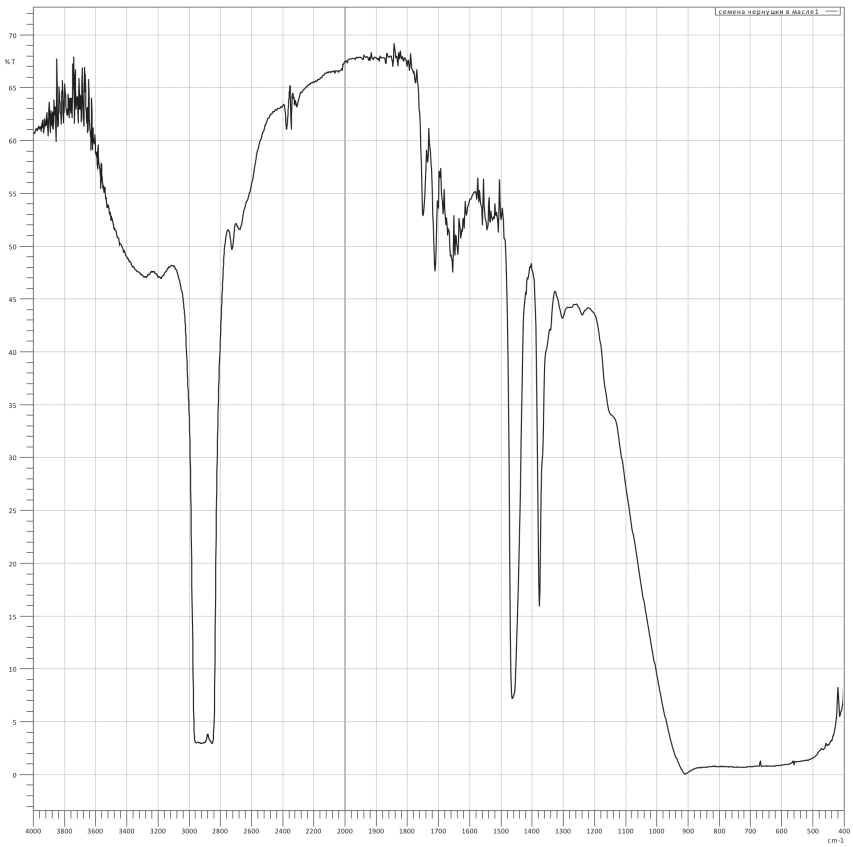

FIG. 2. *IR spectra of Nigella Sativa seeds*

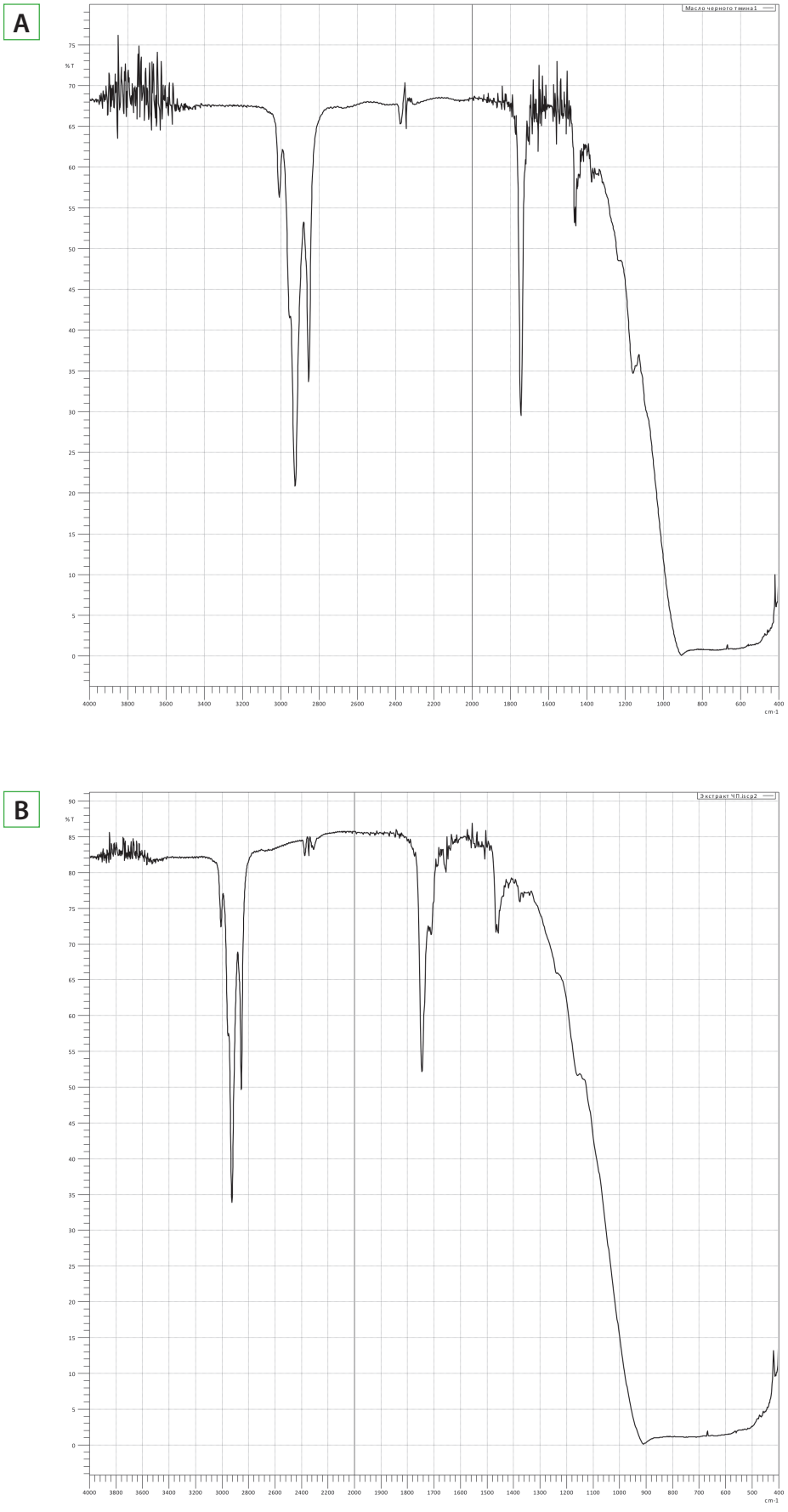

FIG. 3. *IR spectrum of black seed oil (A) and supercritical carbon dioxide extract of Nigella Sativa seeds (B)*

Table

THYMOQUINONE CHARACTERISTIC BANDS MANIFESTED IN THE SPECTRA OF THE TEST SAMPLES

Sample	Absorption bands in the characteristic region, cm^{-1}
Thymoquinone reference standard*	1130 (very weak), 1238 (weak), 1377 (medium), 1464–1460 (medium, set of bands), 1661 (medium), 2852–2953 (strong, set of bands)
Certified commercial sample of black seed oil	1460 (medium), 2900–3000 (strong, set of bands)
Supercritical carbon dioxide extract of Nigella Sativa (black cumin)	1240 (weak), 1377 (weak), 1466–1458 (medium, set of bands), 2855–2955 (strong, set of bands)
Nigella Sativa (black cumin) seeds (after grinding in nuyol)	1240 (weak), 1377 (cp.), 1462–1456 (strong, set of bands), 1661 (medium), 2800–3000 (strong, set of bands)

** Specified for comparison*

manifested in the spectra of the test samples (see Table).

From the data presented in the table, it can be seen that the largest number of thymoquinone bands manifested in the characteristic region of the IR spectra is observed for the supercritical carbon dioxide extract of Nigella Sativa (black cumin) and its seeds ground in nuyol. In this regard, it seems appropriate to propose two methods of sample preparation before registering the IR spectra of Nigella Sativa seeds: supercritical carbon dioxide extraction or grinding of seeds of raw materials with nuyol. The latter option of sample preparation is simpler, faster and cheaper, does not require significant labor and special equipment and can be recommended for routine quality control of raw materials. Moreover, this sample preparation option in a line with supercritical fluid extraction makes it possible to register the largest number of thymoquinone characteristic absorption bands, which guarantees its confident identification against the background of a complex matrix.

CONCLUSION

The possibility of using the method of FTIR spectroscopy in the analysis of Nigella Sativa seeds, black seed oil and supercritical carbon dioxide extract has been studied. The conducted studies have shown that the method of FTIR spectroscopy can be used and recommended to confirm the quality of Nigella Sativa seeds and pharmaceutical products based on them by the presence of thymoquinone.

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COMPARATIVE ANALYSIS OF THE MINERAL COMPOSITION OF DIFFERENT VARIETIES AND TYPES OF *APIUM GRAVEOLENS* L. RAW MATERIALS AS DIETIC NUTRITION

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By the method of inductively coupled plasma atomic emission spectrometry, a comparative analysis of the elemental composition of various botanical forms and varieties of wild celery (Apium graveolens L.), that is a food crop used in dietary and functional nutrition for the correction of excess body weight, was carried out. It is shown that different morphological parts of the plant accumulate different elements in the maximum amount. For the leaves, the calcium and rubidium content prevailing relative to other botanical forms of celery is noted, the accumulation of sodium and strontium is maximal in the petioles. The celery root crop has a high content of potassium and zinc. The celery leaves (particularly the "Nezhnyj" variety) are characterized by the largest number of macro- and microelements, which makes them the object of choice in dietary nutrition and as a source of biologically active additives for the correction of excess weight. The majority macro- and microelements are magnesium, iron, calcium and zinc, the metabolism and action of which, according to literature data, play an important role in the correction of obesity.

Keywords: wild celery (Apium graveolens), mineral compound, atomic emission spectrometry, obesity, functional nutrition

Currently, obesity and overweight are a progressive pathology, as well as a risk factor for the development of a number of chronic diseases. For example, insulin resistance resulting from obesity leads to type 2 diabetes mellitus, and elevated levels of lipids and glucose in blood plasma lead to cardiovascular diseases and an increased risk of cardiovascular events. According to the literature data [1,2], obesity is quite often characterized by a deficiency of some macro- and microelements, for example, magnesium and iron. First of all, this is explained by the presence of a chronic inflammatory process of adipose tissue. It has been established that this pathological process is accompanied by a violation of iron absorption [3], which can lead to a decrease in the level of hemoglobin in blood plasma. Magnesium deficiency is a risk factor for complications such as atherosclerosis, type 2 diabetes mellitus and osteoporosis [4]. Thus, additional intake of iron and magnesium may be recommended in the combination therapy of obesity

Literature data have shown that certain micronutrients, such as calcium and zinc, contribute to the reduction of excess body weight

and the treatment of obesity [5,6]. According to studies, calcium acting through calcium signaling pathways, increases energy consumption by enhancing metabolism and differentiation of adipocytes, as well as reducing appetite, thereby contributing to the reduction of excess body weight [5]. It has been found that zinc increases the biogenesis and energy consumption of brown fat, and also reduces insulin resistance [6]. In addition, zinc deficiency has been proven to be a risk factor for obesity and type 2 diabetes mellitus [7]. The ability to reduce insulin resistance has also been established for chromium [8] and is due to increased sensitivity of insulin receptors. In addition, a positive role in the prevention and treatment of overweight was noted for potassium [9] and selenium [10].

The study of the elemental profile of medicinal plants is a mandatory stage of phytochemical screening, because minerals in complexes with biologically active substances (BAS) can affect the manifestation and severity of the pharmacological effect of plant extracts. Taking into account the participation of macro- and microelements in the prevention of obesity, it is relevant to study the mineral composition of plants that are positioned as sources of substances for therapeutic and dietary nutrition.

Wild celery (*Apium graveolens* L.) is a food biennial herbaceous plant of the *Apiaceae* family, which exists in 3 botanical forms such as root, petiole and leaf and is characterized by a diversity of agricultural varieties. Thus, according to the state register of selective breeding results approved for use [11], 65 official varieties of celery were registered in Russia as of February 26, 2020, of which 31 varieties are root types, 34 are petiole and leaf types. The chemical composition of celery is very diverse and includes mono- and polysaccharides, amino acids, flavonoids, hydroxycinnamic acids, coumarin derivatives, essential oils, organic and fatty acids [12]. According to literature data [13], celery reduces the level of glucose and lipids (triglycerides, cholesterol, LDL, HDL) in the blood, lowers blood pressure and the likelihood of cardiovascular events. Thus, it can be assumed that celery is a potential source of various biologically active substances (BAS) that contribute to the prevention of obesity and reduction of excess body weight, and, as a result, the study of the mineral profile is an important stage of a comprehensive phytochemical study of this plant.

The purpose of the work was to study the macro- and microelement composition of various botanical forms of raw materials (root crop, petioles and leaves) of wild celery, including comparing the different varieties of leaf celery and determining the elements of maximum accumulation. To achieve this purpose it was necessary to solve the following tasks:

- Select sample preparation conditions for isolation of macro- and microelements from medicinal plant raw materials;
- Carry out the actual analysis by atomic emission spectrometry, process and interpret the results obtained;
- Carry out systematization and comparative analysis of the data obtained and select the elements of maximum accumulation in various botanical forms of celery;
- Compare the mineral composition of different varieties of celery leaves and choose a variety that is characterized by the maximum accumulation of target elements.

MATERIALS AND METHODS

Root crops (botanical variety "Olymp"), petioles (botanical variety "Malachite") and leaves (botanical varieties "Nezhnyi", "Zakhar", "Pascal" and "Bodryi") of wild celery were used as objects of study. The leaves were harvested on a household plot in the Leningrad region (Lembolovo village) in August-September 2020, root crops and stems were purchased in grocery supermarkets in St. Petersburg (according to

the information on the packaging, the country of origin is Russia, the harvesting period is September 2020). Fresh raw materials were dried by natural shadow drying in compliance with the rules of drying of essential oil raw materials (thick layer of raw materials, frequent flipping) to residual humidity of no more than 15%.

The mineral composition of the raw materials was determined by inductively coupled plasma atomic emission spectrometry using the Optima 8000 AES-ISP device (Perkin Elmer, USA) controlled by WinLab 32 software. The analysis was carried out on the basis of the CCU "Analytical Center" of the Saint Petersburg State University of Chemistry and Pharmacy of the Ministry of Health of Russia.

Sample preparation. About 0.4 g (exact weight) of dried crushed (3–5 mm) raw materials were placed in Teflon reactors, 5 ml of nitric acid (Nitric acid Puriss. p.a., 65%, Honeywell Fluka, Germany) and 3 ml of 30% hydrogen peroxide solution (batch 11/D 2, Neva Reactiv LLC) were added, gently mixed, left for 10 minutes to remove vapors. The samples were decomposed using the BERGHOF SpeedWave Entry Two microwave system. After cooling, the obtained solutions were quantitatively transferred to polymer measuring flasks with a capacity of 50 ml and brought to the mark with deionized water (with an electrical conductivity of less than 0.5 Cm · cm^{-1}). The obtained samples were used for qualitative and quantitative determination of elements.

As a reference standard (RS), Multi-Element Calibration Standard 3 (Perkin Elmer, USA) was used with concentration of all ions (Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, Zn, U, V) equal to 10 µg/ml (Perkin Elmer, USA), from which the calibration solutions (conc. = 0.1; 0.5; 1.0 mg/l) were prepared and calibration dependences were built based on these solutions.

As a solvent (blank), 5 ml of nitric acid and 3 ml of a 30% hydrogen peroxide solution were used, which were placed in a 50 ml volumetric flask and brought to the mark with deionized water (with an electrical conductivity of less than 0.5 Cm · cm^{-1}).

The device settings are shown in Table 1.

The solvent and the tested solutions (5 solutions) were analyzed sequentially. The content of elements in the sample (X, mg/kg) was calculated by the formula:

$$X = \frac{C_X \cdot V_K \cdot w}{a} \times 1000,$$

where C_X is the concentration of the analyte along the calibration line, mg/l; V_K is the volume of the measuring flask, ml; a is the weight of the sample, g; 1000 is the conversion of g into kg; W is the dilution coefficient for macronutrients (calcium, magnesium, sodium, potassium) = Vmk/Va.

The obtained data were processed by the method of mathematical statistics in accordance with the recommendations of the RF SP OFS.1.1.0013.15 "Statistical processing of the results of a chemical experiment" [14]. The reliability of the results was assessed by the RSD value (the RSD acceptance criterion is 2%, for microconcentrations of elements, the permissible RSD value is 30%).

Table 1

ANALYSIS CONDITIONS – OPERATING SETTINGS OF THE DEVICE

Integration time	1–2 sec
Number of integration repeats	3
Plasma-forming gas flow rate	10 l/min
Additional gas flow rate	0,2 l/min
Gas flow rate for sample spraying	0,7 l/min
Plasma power	1300 W
Position of the emission review	axial
Sample rate of feeding	1,5 ml/min

RESULTS AND DISCUSSION

According to the results of the study, 12 macro- and microelements were found in various types of raw materials of *Apium graveolens* L. The results are shown in Tables 2–3.

The obtained data on the study of the mineral profile in a comparative aspect for different botanical forms of celery are presented in the diagrams (Figs. 1 and 2). Heavy metals and arsenic were not identified in the analyzed plants (their content is lower than the QL for the method used).

Table 2

MACRO- AND MICROELEMENT COMPOSITION OF VARIOUS TYPES OF WILD CELERY RAW MATERIALS

Group of elements	Element	Content of elements, mg/kg (n=5)		
		Leaf celery ("Nezhnyi" variety) $x_{av} \pm \Delta x_i$	Petiolate celery $x_{av} \pm \Delta x_i$	Root celery $x_{av} \pm \Delta x_i$
Macro-elements	Ca	(27 ± 4) × 103	(11.2 ± 1.1) × 103	(8.3 ± 0.2) × 103
	K	(13.7 ± 0.5) × 103	(18.6 ± 0.6) × 103	(30.5 ± 1.1) × 103
	Mg	(2.8 ± 0.3) × 103	(2.9 ± 0.3) × 103	1381 ± 18
	Na	(6.9 ± 0.3) × 103	(51 ± 5) × 103	(3.1 ± 0.1) × 103
Micro-elements	Fe	160 ± 20	178 ± 16	101 ± 24
	Cd	–	–	–
	Co	0.045 ± 0.015	0.11 ± 0.03	0.199 ± 0.015
	Cr	–	–	–
	Cu	7.2 ± 1.3	6.9 ± 1.3	16.4 ± 1.7
	Al	225 ± 16	241 ± 21	62 ± 21
	As	–	–	–
	Ba	62 ± 4	3.7 ± 0.6	4.9 ± 1.5
	Mn	46 ± 6	28.2 ± 1.0	22.4 ± 1.2
	Bi	–	–	–
	Ni	–	–	–
	Pb	–	–	–
	Hg	–	–	–
	Rb	18.1 ± 0.3	26 ± 4	11.5 ± 0.4
	Se	–	–	–
	Sr	75 ± 4	43.2 ± 1.7	23.9 ± 0.4
	Zn	65 ± 4	26.4 ± 0.6	55 ± 2

Note: "–" – the content of the element is below its QL for this method; controlled elements are highlighted in italics in accordance with the RF SP OFS.1.5.3.0009.15

As can be seen from the data in Table 2 and Fig. 1–2, the different botanical forms of celery accumulate different elements. Thus, as of the macronutrients, the calcium content prevails in the leaves, which can be explained by its accumulation in the form of calcium oxalate, and the maximum concentration is observed in the "Nezhnyi" variety (about 27 g/kg). The accumulation of magnesium is maximum in the chlorophyll-bearing parts such as celery petioles and leaves (from 2.5 to 3 g/kg). The highest potassium content is characteristic of the leaves

Table 3

MACRO- AND MICROELEMENT COMPOSITION OF VARIOUS VARIETIES OF WILD LEAF CELERY

Group of elements	Element	Content of elements, mg/kg, (n=5)		
		"Zakhar" variety $x_{av} \pm \Delta x_i$	"Paskal" variety $x_{av} \pm \Delta x_i$	"Bodryi" variety $x_{av} \pm \Delta x_i$
Macro-elements	Ca	(15.5 ± 0.5) ×103	(15.8 ± 0.1) ×103	(15.5 ± 1.1) ×103
	K	(29 ± 3) ×103	(29.2 ± 0.3) ×103	(37 ± 8) ×103
	Mg	(27.1 ± 0.5) ×103	(2.6 ± 0.2) ×103	(2.5 ± 0.3) ×103
	Na	(5.2 ± 0.4) ×103	(6.1 ± 0.6) ×103	(5.4 ± 0.5) ×103
Micro-elements	Fe	127 ± 10	158 ± 12	112 ± 11
	Cd	–	–	–
	Co	0.055 ± 0.012	0.06 ± 0.03	0.15 ± 0.04
	Cr	–	–	–
	Cu	2.61 ± 0.01	2.35 ± 0.30	2.7 ± 0.3
	Al	205 ± 6	263 ± 14	154 ± 5
	As	–	–	–
	Ba	18.0 ± 0.7	14.1 ± 1.3	12.35 ± 0.15
	Mn	32.8 ± 0.5	28 ± 4	26.6 ± 1.4
	Bi	–	–	–
	Ni	–	–	–
	Pb	–	–	–
	Hg	–	–	–
	Rb	42.3 ± 1.6	54 ± 3	65 ± 12
	Se	–	–	–
	Sr	13 ± 2	13 ± 3	31.5 ± 0.2
	Zn	24.26 ± 0.10	23.0 ± 1.6	24.7 ± 1.1

Note: "–" – the content of the element is below its QL for this method; controlled elements are highlighted in italics in accordance with the RF SP OFS.1.5.3.0009.15

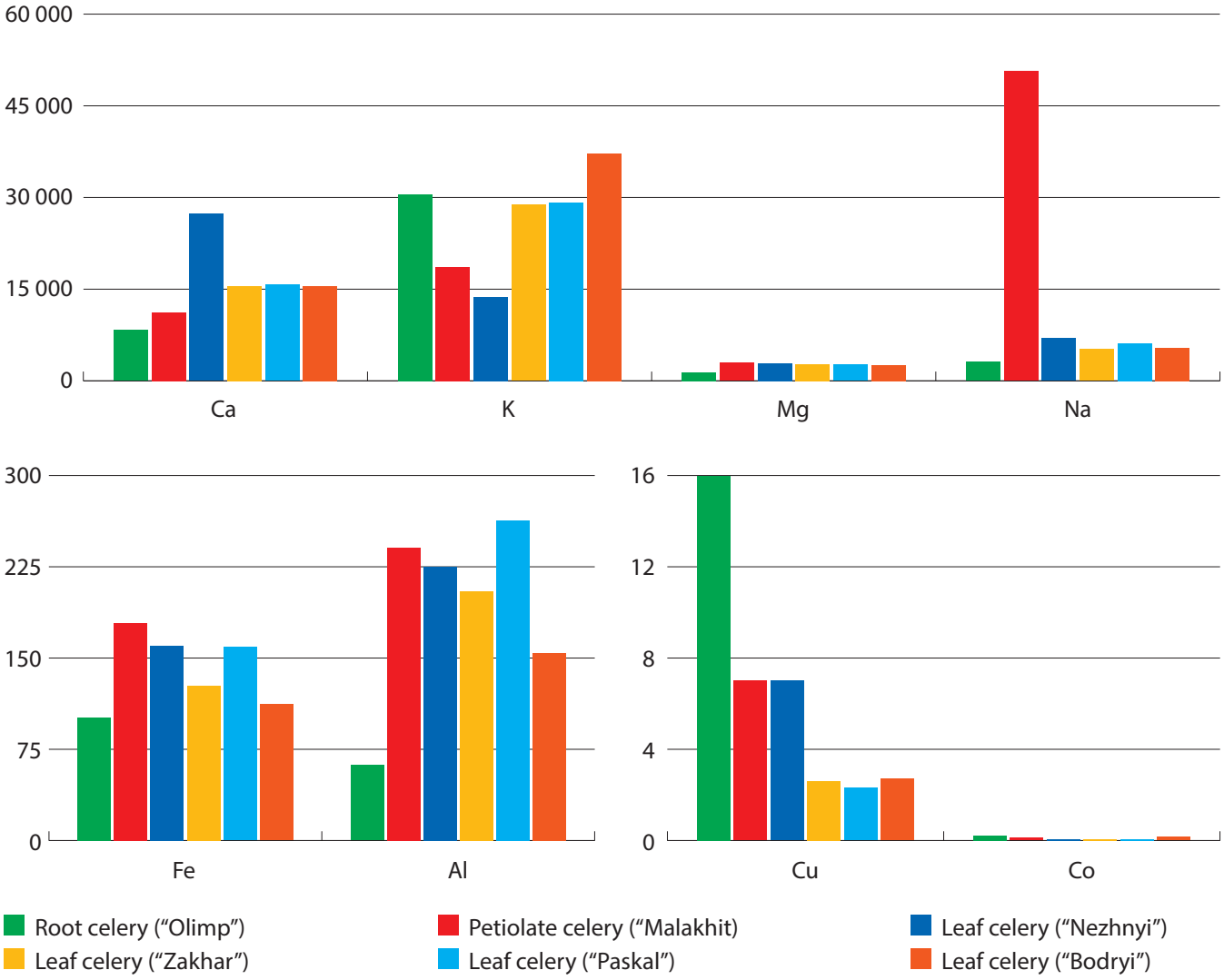

FIG. 1. *Macroelement profile of wild celery (mg/kg)*

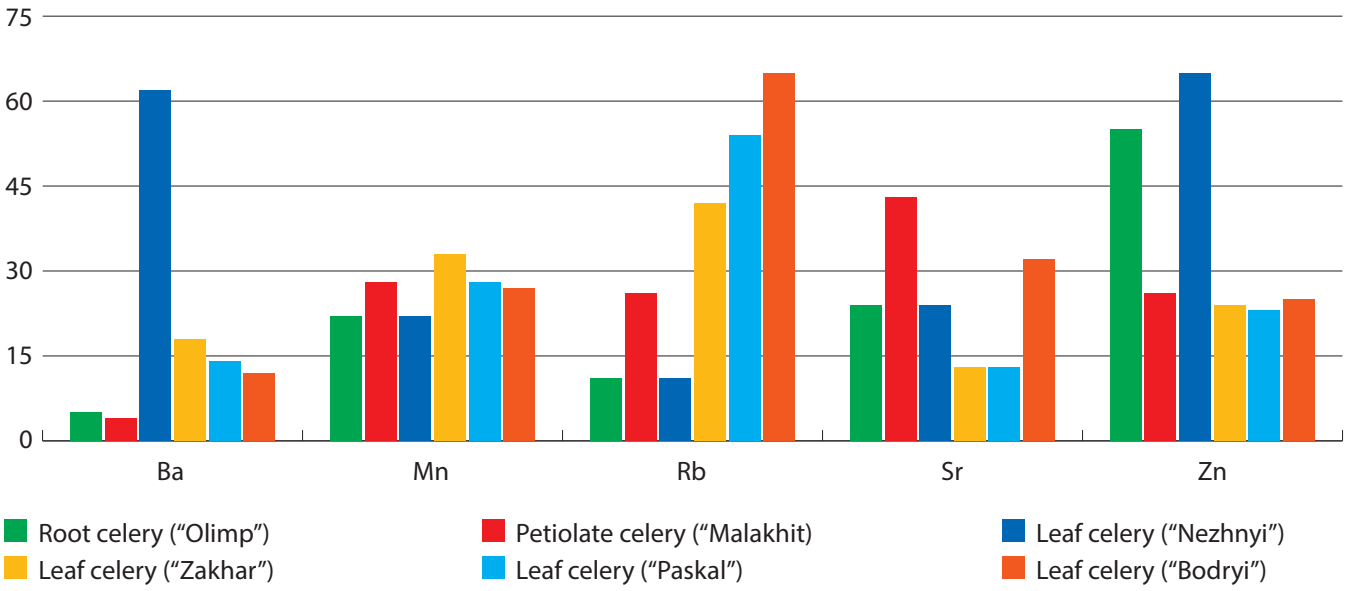

FIG. 2. *Microelement profile of wild celery (mg/kg)*

of the "Bodryi" variety and the root crops of the plant

The majority microelements of celery are iron, aluminum, zinc and strontium, the accumulation of which differs in different botanical forms and varieties of the plant. The iron content is maximum in petiolate celery (about 180 mg/kg), as well as in the leaves of the "Nezhnyi" and "Pascal" varieties (about 160 mg/kg). The greatest accumulation of zinc is observed in the leaves of the "Nezhnyi" variety (about 65 mg /kg) and the root crop (about 50 mg /kg) of celery. In principle, for leaves, it is possible to note a greater accumulation of all microelements (except copper, which significantly prevails in root crops) in comparison with other morphological parts. It is also necessary to note the accumulation of rubidium (65 mg/kg) in the leaves of celery of the "Bodryi" variety and to position this variety as a source of biologically active supplements of a neurological profile due to the combined action of rubidium and magnesium with increased nervous excitability.

Thus, the greatest accumulation of target elements such as calcium, zinc, magnesium, iron, which are involved in the mechanism of preventive action of plant BAS in case of excess weight, is characteristic of leaf celery of the "Nezhnyi" variety, which makes it an object of choice in dietary nutrition and as a source of biologically active supplements for the correction of excess weight. In addition, the significant calcium content in this plant variety (about 25 g/kg) allows it to be attributed to biological indicators of calcium-strontium soils.

CONCLUSION

1. The mineral composition of various types of raw materials (root crops, leaves and stems) of wild celery has been studied by inductively coupled plasma atomic emission spectrometry, and different varieties of leaf celery have been considered in a comparative aspect. It is shown that different morphological parts of the plant accumulate different elements in the maximum amount. The greatest number of microelement is contained in celery leaves.

2. It was found that the largest number of target (magnesium, iron, calcium, zinc) elements indicated for dietary nutrition in case of excess body weight is accumulated by celery leaves of the "Neznyi" variety.

3. Thus, based on the assessment of the elemental profile of wild celery, it is possible to assume a positive effect of the use of this plant in the framework of combined therapy of obesity. The development and standardization of medicines and biologically active supplements based on wild celery is a promising and relevant direction in the prevention and treatment of overweight.

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STUDYING THE BASE FOR PRODUCTION OF MEDICINAL CHEWING GUM HEALTH IN GUM® USING THE HECKEL AND KAWAKITA MATHEMATICAL MODELS

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Mathematical modeling of the tablet mixtures compression is a valuable tool for studying the processes occurring during this stage, and allows you to optimize the process, adjust the composition of excipients in the finished dosage form. Coprocessing excipients for direct compression of medicinal chewing gum are a relatively new, promising group of excipients, which makes it possible to simplify the procedure for production of this dosage form. The study of Health in Gum® is a necessary stage for the development of the formulation of the finished dosage form, since this excipient is the main component composition of medicinal chewing gums. The article describes the study of the processes occurring during the compression of the base for medicinal chewing gums Health in Gum® by mathematical modeling. During the study, the mathematical models of Heckel and Kawakita were used. The most optimal compression force and the point of transition to deformation of the tableting mass particles are determined. The compression pressure under which the main redistribution of particles occurs is studied and conclusions are drawn about the need for additional introduction of excipients and the use of other models that take into account the elastic deformation of Health in Gum®.

Keywords: medicinal chewing gums, compression, mathematical modeling, Heckel model, Kawakita equation, *Health in Gum® Cafosa®*

Medicinal chewing gum is a solid dosage form (DF) containing the base of chewing gum (gum base) with a pharmaceutical substance (PS) and intended for chewing for a certain period of time without subsequent ingestion in order to provide local action in the oral cavity and throat or systemic action [1,5]. The direct compression method is the most cost-effective method of preparation of medicinal chewing gum which is the most technologically acceptable for pharmaceutical use [2]. This process uses standard tableting equipment. For this process the specially developed bases for chewing gums in the form of loose powdered excipients are used. One of the most common brands of mixtures for direct compression is *Health*

in Gum®, which has the properties of flowability and compactness as well as contains elastomeric components and basic excipients for creating the medicinal chewing gums. This mixture, developed by *Cafosa Gum SAU*®, is a coprocessed (jointly processed) mixture that can be used as ready-made form by adding PS, or mixed with additional amount of excipients to provide the required characteristics to the dosage form [4]. When compressing *Health in Gum*® or mixtures with this base, the medicinal chewing gums are produced, which in appearance are similar to tablets for oral use. They have a higher hardness and friability than medicinal chewing gums, made in the traditional way by melting-extrusion. Gums *Health in Gum*® are available in three varieties: *HiG PWD-01, HiG PWD-03* and *HiG PWD-04,* which contain 25, 35 and 30% elastomeric base, respectively.

The literature describes several different concepts for describing the compression of powder materials. The most common concept is the representation of compression as a process proceeding for several successive and partially overlapping stages. Each stage describes the physical and chemical state of the powder material at a certain compression pressure and is associated with one or more dominant compression mechanisms. In addition, various interpretations of this concept are considered in the literature both in terms of the number of stages of the compression process step, and in terms of which physical processes dominate at each stage. The modeling of the compression stage used in this article is based on a four-stage model, including the initial rearrangement of particles, their fragmentation, plastic deformation and, finally, elastic deformation of a briquette or tablet. Initially, at low compression pressures, the particles move closer to each other, and the porosity and volume of the powder layer decrease. Under a certain applied pressure, the particles reach the most compact structure, and any further rearrangement becomes impossible. Therefore, the next decrease in volume is associated with re-sizing of the particles themselves. This re-sizing can be either temporary as a result of elastic deformation, or permanent as a result of plastic deformation. Re-sizing of the particles can also be connected with a result of their brittle destruction (fragmentation). Subsequently, the particles undergo a secondary rearrangement followed by plastic and/or elastic deformation.

Numerous attempts have been made to develop, based on a physical understanding of the powder compression process, a mathematical model of compression from which compression parameters reflecting the actual properties of the material can be obtained. The dominant approach is to take into account the entire layer of powdered material or tablet during simulation (so-called global models), linking either the porosity of the powder or the volume of the powder with the applied pressure. However, in the field of pharmaceutical technologies, the global models of Heckel and Kawakita are most often used. The reason for this may be their rather simple mathematical form, as well as the fact that a significant part of other models are based on the information received from them. In addition, the presented models are considered reliable from the point of view of the physical value of the compression parameters [3,6].

The purpose of this research is to study the features of the parameters of the compressibility of the gum base *Health in Gum*® (Cafosa®) and the physical processes occurring during tableting, using mathematical models of Kawakita and Heckel.

MATERIALS AND METHODS

Used materials: *Health in Gum*®, *Cafosa*® (Spain).

Used equipment: pycnometer, automatic tapper (ERWEKA SVM 221), manual hydraulic press

PRG-50, mechanical strength tester TBF 1000, Copley Scientific®.

Method: The true density is determined using a pycnometer, the bulk density before and after compaction is determined using an automatic tapper, according to OFS.1.4.2.0016.15 SP XIV.

Compression of the base for the production of medicinal chewing gums was carried out on a manual hydraulic press PRG-50. During compression, the standard conditions were observed i.e. the same speed, the standard retention time of the maximum compression force (20 s). The characteristics of the tablets were measured 15 minutes after compression. The measured characteristics of the resulting tablets include the height and weight of the tablet, tablet breaking force, briquette density, porosity (ε) and degree of volume reduction (C).

Based on the data obtained, dependence graphs were constructed in accordance with the Heckel and Kawakita equations.

Heckel equation is one of the most common equations describing the mechanism of volume reduction during compaction.

The mathematical model is based on the assumption that the compression of the powder follows the first-order kinetics and the pores between the particles act as a reagent, and the compaction of the powder acts as a product. In this case, the degree of compaction with an increase in compression pressure is proportional to porosity – and, accordingly, the degree of compaction with an increase in compression pressure is directly proportional to the porosity value:

$$\frac{dD}{dP} = k\varepsilon, \quad (1)$$

where D is the relative density under applied pressure P; ε is the porosity. Relative density is defined as the ratio between the compaction density under pressure P and the true density of solid particles.

Porosity is defined as:

$$\varepsilon = 1 - D, \quad (2)$$

after that, the equation can be transformed:

$$\frac{dD}{dP} = k(1 - D), \quad (3)$$

and after solving the differential equation:

$$\ln\left[\frac{1}{(1 - D)}\right] = kP + A. \quad (4)$$

Based on this equation, a graph of the dependence of $\ln(1/(1 - D))$ on the applied compression force is constructed with a linear section, slope k and intersection point A. The graph describes three stages of compaction occurring in the tablet mass during tableting (particle redistribution, plastic deformation, fragmentation). The inverse value of the parameter k expresses a material-dependent constant known as pressure fluidity D, which reflects the ability of the material to plastic deformation under pressure. Thus, low values of D indicate the beginning of plastic deformation under low pressures. The line segment A expresses a parameter that depends on the initial volume of compaction during the filling of the matrix and the initial redistribution of particles.

The relative density D_a is calculated by the equation:

$$D_a = 1 - e^{-a}, \quad (5)$$

where D_a – the relative density during the redistribution phase under low compression pressures, which represents the difference between D_A and D_0 (relative density of the powder in the absence of pressure) [6–8].

Kawakita equation [6,7,9,10] was developed to study powder compaction using the degree of volume reduction C, expressed as:

$$C = \frac{V_0 - V_p}{V_0} = \frac{abP}{(1 + bP)}. \quad (6)$$

Equation (6) can be transformed by the following way:

$$\frac{P}{C} = \frac{P}{a} + \frac{1}{ab}, \quad (7)$$

where V_0 – initial powder volume; V_p – powder volume under pressure P.

Constants *a* and *b* are obtained from the slope and intersection of the graph of the dependence of *P/C* on P. The constant *a* expresses the minimum porosity of the powder before compression, and the value *(1 – a)* indicates the initial relative density ρ_0. The constant *b*, also known as the compression ratio, is related to the properties of the plastic material. Its inverse value (*1/b* – cohesiveness) expresses the pressure parameter P_k, which is the pressure required to reduce the volume of the powder by 50%. For plastic materials, the P_k value is inversely proportional to the degree of plastic deformation during the compaction process, so lower P_k values indicate a higher degree of plastic deformation.

RESULTS AND DISCUSSION

The experiment was carried out according to the methods described in the section "Materials and methods". During the experiment, the value of the true density of the *Health in Gum*® base was established, which was 1.4086 g/cm^3, as well as the value of the bulk density equal to 0.235 g/cm^3 was determined. Table 1 has been compiled, reflecting changes in the mass, height and strength of tablets relative to the compression pressure. During the compression process, the pressures from 35.36 to 707.36 MPa were applied, which corresponds to 1–20 kN/m^2.

Based on the obtained data on the porosity of tablets, a graph of the dependence of the natural logarithm of the porosity ln (1/ε) on the compression pressure is constructed (Fig. 1).

A linear approximation was carried out using the least squares method, as a result of which the equation $y = bx + a$ was transformed as follows: $y = (0{,}00268 \pm 0{,}00069)\,x + 1{,}9296 \pm 0{,}048$.

With confidence probability values $p = 0{,}95$, the number of measurements – 7, Student coefficient – $t = 2{,}37$, the absolute errors are calculated: for *a* – $\Delta a = \pm 0{,}11397$, for *b* – $\Delta b = \pm 0{,}00163$.

Table 1

DEPENDENCE OF TABLET CHARACTERISTICS ON COMPRESSION PRESSURE

Compression pressure (P), MPa	Weight of a tablet, g	Height of a tablet, cm	Tablet breaking force, N	Briquet density, g/cm^3	Porosity (ε)	ln (1/ε)
6.50 ± 0.1	0.998 ± 0.010	0.578 ± 0.0058	44.5222 ± 0.2150	1.1216	0.1569	1.8521
16.24 ± 0.1	1.0105 ± 0.008	0.577 ± 0.0024	49.1313 ± 0.3739	1.1377	0.1449	1.9319
32.48 ± 0.1	1.0425 ± 0.007	0.5785 ± 0.0020	57.2218 ± 0.1541	1.1706	0.1201	2.1196
48.72 ± 0.1	1.004 ± 0.011	0.5575 ± 0.0024	56.8295 ± 0.0826	1.1699	0.1207	2.1148
64.96 ± 0.1	1.007 ± 0.010	0.557 ± 0.0024	54.0346 ± 0.1359	1.1744	0.1172	2.1436
97.44 ± 0.1	1.001 ± 0.015	0.552 ± 0.0040	53.4953 ± 0.1784	1.1780	0.1145	2.1668
129.92 ± 0.1	0.998 ± 0.007	0.5455 ± 0.0010	57.1728 ± 0.2604	1.1885	0.1067	2.2379

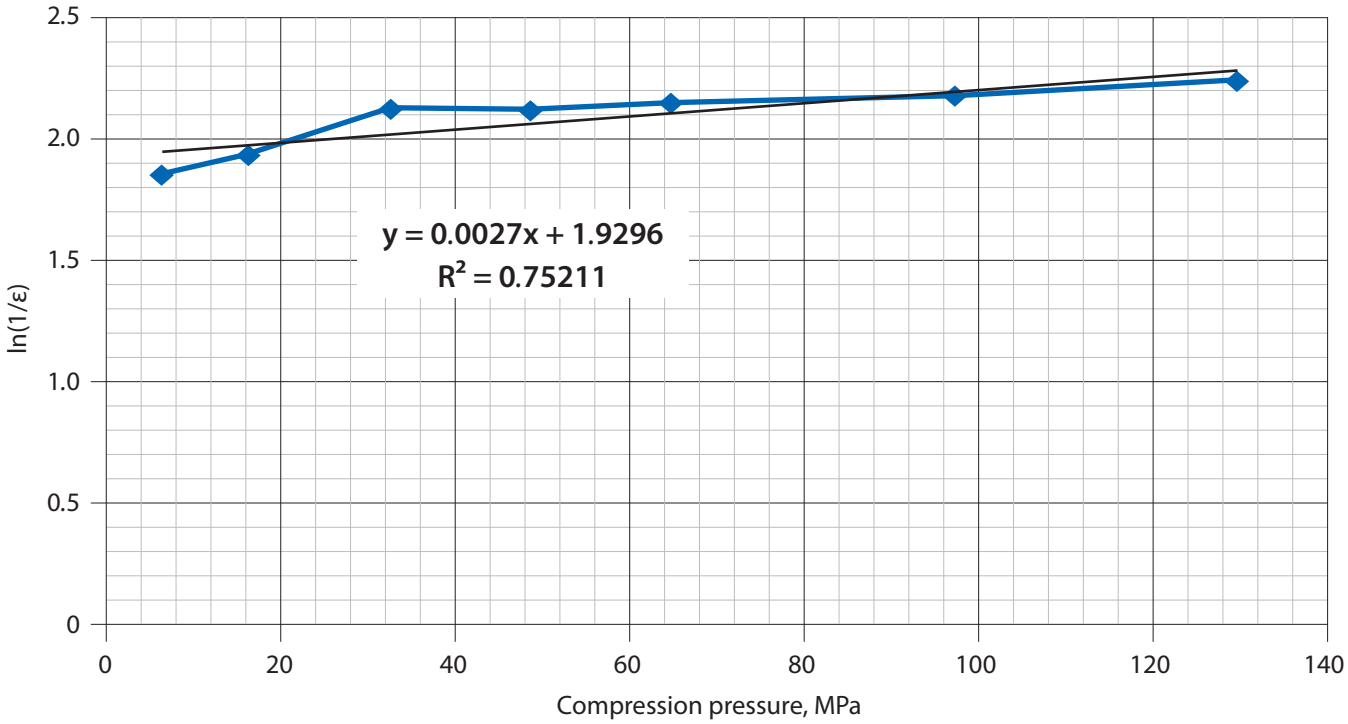

FIG. 1. *Dependence of the natural logarithm of the porosity of gum base tablets on the compression pressure*

According to the analysis of the obtained values, the fragmentation prevails during the compression process, as evidenced by a sufficiently high value of the coefficient *A*. The D_b value is less than the D_a value, which indicates the redistribution of particles during the compression process.

The straight section of the graph is located in the range of compression pressure values from 48 to 129 MPa (10–20 kN/m^2), and the highest predicted (extrapolation method) value of the compression pressure for production of medicinal chewing gums is 373.9 MPa and corresponds to 57.55 kN/m^2, which indicates the beginning of plastic deformation at very high values.

Further, based on the data obtained for the Kawakita equation, a graph of the dependence of P/C on the compression pressure is constructed (Fig. 2).

A linear approximation was carried out using the least squares method, as a result of which the equation $y = bx + a$ was transformed as follows: $y = (2{,}042 \pm 0{,}015)\,x + 1{,}5439 \pm 1{,}043$.

With confidence probability values $p = 0.95$, the number of measurements – 7, Student coefficient – $t = 2{,}37$, the absolute errors are calculated: for a – $\Delta a = \pm 0.441$, for b – $\Delta b = \pm 0.015$.

The Kawakita dependence graph characterizes the behavior of the powder as in bulk volume before compaction, in the briquette state and characterizes the particles by the frequency

Table 2

THE VALUE OF THE COEFFICIENTS OF THE HECKEL EQUATION

A (a)	*k* (b)	D_a	D_b	1/*k*, P_k, MPa
1.9296 ± 0.048	0.00268 ± 0.00069	0.8548	0.6198	373.87 ± 38.90

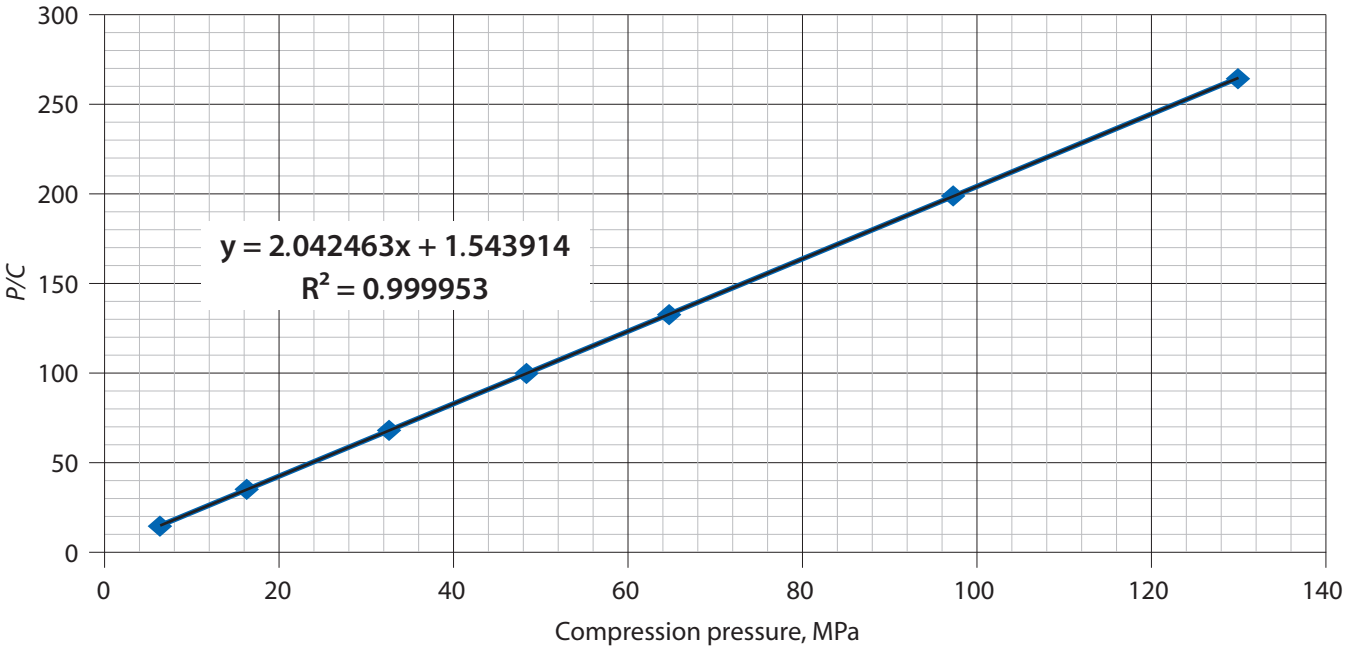

FIG. 2. *Dependence of P/C of gum base tablets on compression pressure*

of rearrangement. The value of the product *ab*>0.1 witnesses a good ability of the particles to redistribute during compression (a good degree of granulate flowability), at the same time a low value *(P_k)* shows a high degree of elasto-plastic strain. The pressure required to reduce the material in volume by 50% was 0.756 MPa, which corresponds to 0.116 kN/m^2.

Using the mathematical model of Heckel, the process of compression of the base for the medicinal chewing gum *Health in Gum*® is described. The optimal compression pressure for the production of the medicinal chewing gums containing the base was determined, which was 373.9 MPa. The Kawakita equation showed that the resulting tablet mass has a good ability of particles for redistribution during compression and a very high ability to elasto-plastic strain. The pressure required to reduce the material in volume by 50%, which was 0.756 MPa, was identified.

CONCLUSION

As a result of the analysis of the data obtained, a conclusion was made about an extremely high degree of elasto-plastic strain and elasticity of the material due to very low pressure to reduce the material in volume. Fig. 2 shows a low decrease in the volume of the tablet with an increase in the compression pressure, which additionally indicates elastic deformation of the particles, which negatively affects the strength of the medicinal chewing gums. In addition, a low increase in strength was noted with increase in compression pressure. Accordingly, the addition of fillers increases the mechanical strength,

Table 3

THE VALUE OF THE COEFFICIENTS OF THE KAWAKITA EQUATION

1/*ab* (a), MPa	1/*a* (b)	*a*	*b*	*ab*	1/*b*, P_k MPa
1.5439 ± 1.043	2.042 ± 0.015	0.4896	1.323	0.647	0.7559 ± 0.0391

compensates for the elasticity and elasto-plastic strain of the material. For further study of this excipient, it is necessary to select a mathematical model that more fully describes the compression process and takes into account the elastic deformation of the gum base.

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ASSESSMENT OF THE INFLUENCE OF THE PROCESSING CONDITIONS ON THE QUALITY OF WATER EXTRACTS FROM ALDER LEAVES OF THE SPECIES *ALNUS INCANA* (L.) MOENCH AND *A. GLUTINOSA* (L.) GAERTH

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The actual problem of modern pharmacy is the search for new types of medicinal plant raw materials and the development of quality criteria for medicines based on it. In the Russian Federation, the alder multiple fruits are official medicinal plant raw materials, however, the analysis of the literature data shows a significant interest of researchers in alder leaves of pharmacopoeial species. This raw material was also used in traditional medicine of different peoples in the form of an aqueous extract-infusion. The purpose of this work was to assess the effect of the processing conditions on the quality of water extracts from alder leaves of the species Alnus incana (L.) Moench and A. glutinosa (L.) Gaerth. The object of the study was water extracts prepared from raw materials of different degrees of grinding and in different technological modes. The following parameters were determined in the prepared aqueous extracts: description, dry residue, pH, tannin content, flavonoid content.

According to the description, all the studied aqueous extracts from alder leaves of the species Alnus incana (L.) Moench and A. glutinosa (L.) Gaerth., as well as mixtures of leaves of pharmacopoeia species were brown or greenish-brown liquids with a faint odor and astringent taste. The dry residue in the studied extracts ranged from 2.11 to 2.34%. The content of tannins was 0.801–0.831%, the total content of flavonoids was 0.064–0.079%. This study presents for the first time the results of a techno-analytical study of aqueous extracts from alder leaves of pharmacopoeia species.

Keywords: grey alder, black alder, alder leaves, water extracts, dry residue, tannins, flavonoids

In the Russian Federation, the pharmacopoeial raw materials are alder multiple fruits harvested from two species – Alnus incana (L.) Moench and A. glutinosa (L.) Gaerth., belonging to the birch family (Betulaceae). However, the chemical composition of the leaves and the spectrum of their pharmacological action are as good as the pharmacopoeia raw materials, which allows us to consider alder leaves as a promising source of new medicines [1,2]. It should also be noted that alder leaves are included in the State Pharmacopoeia of the Republic of Belarus, and Borshchagovsky Chemicals and Pharmaceuticals (Ukraine) produces the Altan medicine containing alder leaf extract, used as an anti-ulcer, reparative, anti-inflammatory agent [3]. In addition, the Altabor medicine is produced, containing alder extract enriched with ellagotanins [4–7], recommended for treatment of influenza and acute respiratory viral infections, the medicine is also characterized by antimicrobial action against microorganisms Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella. The main pharmacological effects

inherent in alder leaves due to the presence of ellagotanins [9–12] are shown in Fig. 1.

Taking into account the good solubility of tannins in water, one of the ways to use alder leaves may be preparing the aqueous extracts from this raw material.

The purpose of this work was to assess the influence of the processing conditions on the quality of water extracts from alder leaves of species Alnus incana (L.) Moench and A. glutinosa (L.) Gaerth.

MATERIALS AND METHODS

The object of the study was air-dry raw alder leaves harvested in the summer of 2020 from wild plants growing in ecologically favorable areas of the Moscow and Tver regions. Sampling for research was carried out in accordance with the requirements of article OFS 42-0013-03 "Rules for acceptance of medicinal plant raw materials and sampling methods".

Water extracts from raw materials were prepared in a ratio of 1:10 in accordance with the requirements of OFS.1.4.1.0018.15 "Infusions and decoctions". The quality of the prepared aqueous extracts from alder leaves of pharmacopoeia species was assessed according to the following parameters: description, pH, dry residue content, tannin content, flavonoid content. pH value was evaluated using the pH 211 Microprocessor pH Meter. The content of tannins was determined in accordance with the requirements of the OFS.1.5.3.0008.15 "Determination of the content of tannins in medicinal plant raw materials

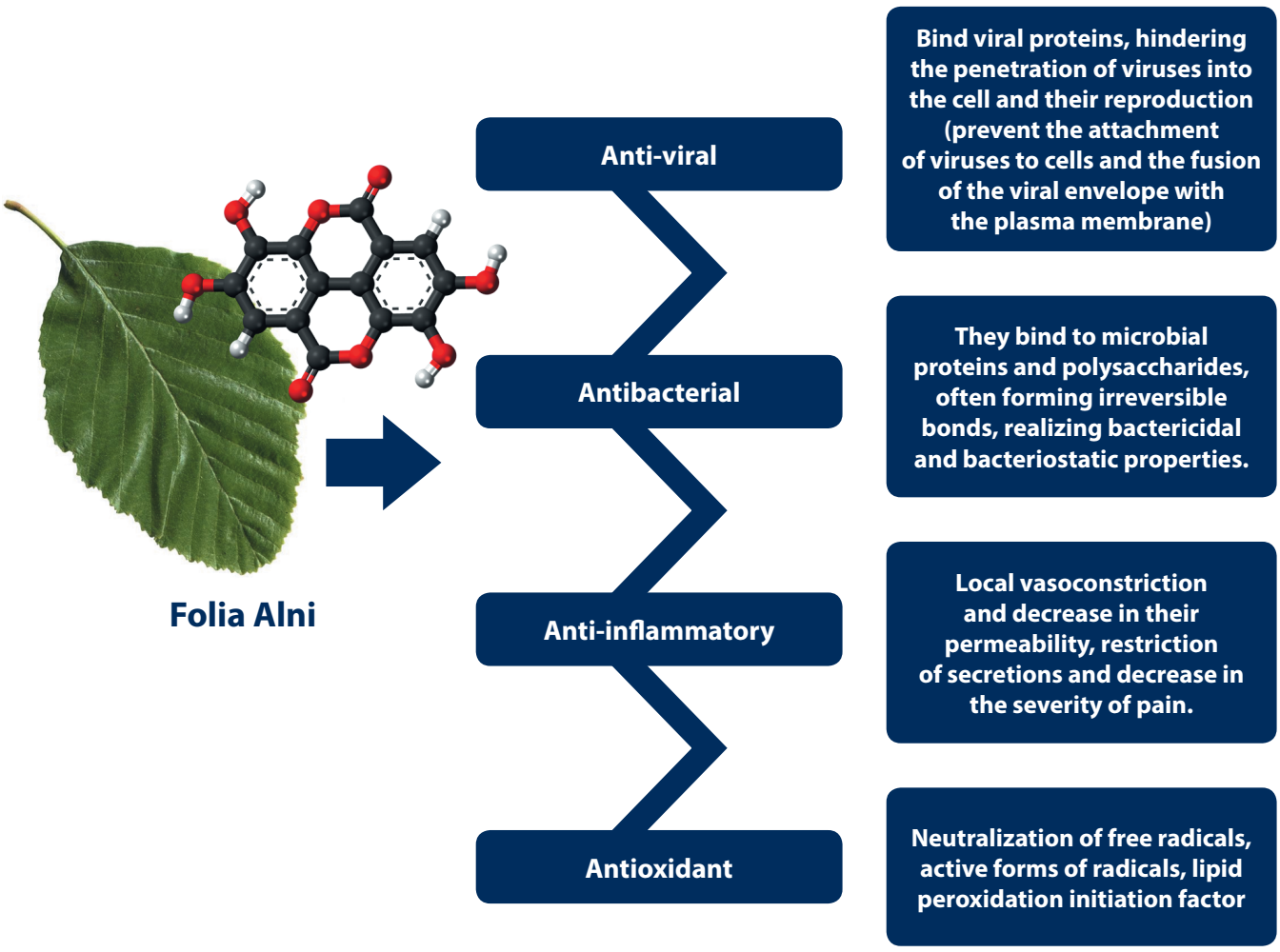

FIG. 1. *Pharmacological effects of alder leaves*

and herbal medicinal products". The quantitative content of flavonoids was carried out by spectrophotometric method. In order to study the effect of particle size on the quality of water extraction, the alder leaves were crushed to a particle size of 2, 3, 5, 7 and 10 mm. The prepared aqueous extracts were evaluated for the content of dry residue, tannins and flavonoids. The total content of flavonoids in terms of rutin in aqueous extracts from alder leaves was evaluated according to the following method: 5 ml of aqueous extract from alder leaves was placed in a 25 ml volumetric flask, after which the volume in the flask was brought to the mark with 70% ethyl alcohol stirring carefully (solution A). Aliquots of 5 ml were accurately measured from solution A and transferred to 25 ml volumetric flasks.

6 ml of 2% alcohol aluminum chloride solution was poured into one of the flasks and the same volume of 70% ethyl alcohol was poured into the other. The amount of flavonoids in the studied aqueous extracts was calculated in terms of rutin.

Table 1

ANALYSIS OF THE INFLUENCE OF FINENESS OF ALDER LEAVES ON THE QUALITY PARAMETERS OF WATER EXTRACT

Object of study	Particle size, mm	Dry residues, %	Tannin content, %	Flavonoid content, %
Alder leaves Alnus incana (L.) Moench (Moscow Region)	2	2.18 ±0.07	0.827±0.001	0.075±0.002
	3	2.18±0.05	0.831±0.005	0.076±0.003
	5	2.18±0.04	0.826±0.003	0.076±0.001
	7	2.14±0.06	0.817±0.004	0.073±0.004
	10	2.11±0.02	0.816±0.003	0.071±0.003
Alder leaves Alnus incana (L.) Moench (Tver Region)	2	2.16±0.07	0.829±0.005	0.079±0.002
	3	2.17±0.02	0.830±0.003	0.079±0.003
	5	2.17±0.06	0.829±0.002	0.078±0.001
	7	2.13±0.02	0.818±0.004	0.074±0.002
	10	2.12±0.05	0.815±0.006	0.071±0.002
Alder leaves A. glutinosa (L.) Gaerth (Moscow Region)	2	2.34±0.03	0.811±0.003	0.071±0.002
	3	2.33±0.03	0.812±0.006	0.071±0.001
	5	2.33±0.02	0.812±0.002	0.070±0.005
	7	2.31±0.06	0.807±0.002	0.067±0.002
	10	2.31±0.04	0.803±0.004	0.065±0.002
Alder leaves A. glutinosa (L.) Gaerth (Tver Region)	2	2.27±0.03	0.812±0.004	0.069±0.001
	3	2.28±0.02	0.813±0.003	0.070±0.001
	5	2.27±0.02	0.813±0.003	0.069±0.003
	7	2.24±0.03	0.806±0.004	0.065±0.001
	10	2.23±0.04	0.801±0.002	0.064±0.002
Mixture of leaves	2	2.24±0.02	0.811±0.004	0.071±0.001
	3	2.24±0.04	0.812±0.003	0.073±0.001
	5	2.23±0.04	0.812±0.003	0.072±0.002
	7	2.21±0.03	0.810±0.004	0.068±0.002
	10	2.20±0.02	0.809±0.002	0.067±0.002

RESULTS AND DISCUSSION

According to the description, water extracts from the studied alder leaves are brown or greenish-brown liquids with a faint odor and astringent taste.

In the course of assessing the influence of particle size on the quality of water extract from alder leaves, the data were obtained characterizing the optimal degree of grinding to a particle size of 3 mm, providing maximum yield of active substances. The results of the analysis are presented in Table 1.

As one can see from the data in Table 1, the maximum yield of dry residue and tannins is characteristic of aqueous extract prepared using alder leaves crushed to size of 3 and 5 mm, with particle sizes of 2 and 7 mm, these parameters change slightly, and when using larger particles, there is a slight decrease in their content. The content of flavonoids practically does not change when the fineness factor of raw materials changes.

Thus, in the production of aqueous extracts from alder leaves of pharmacopoeia species, it is optimal to use raw materials with particle sizes of 3–5 mm, since this degree of fineness ensures

Table 2

ANALYSIS OF THE EFFECT OF THE INFUSION CONDITIONS ON THE QUALITY PARAMETERS OF WATER EXTRACT FROM ALDER LEAVES

Conditions of preparing the water extract	Description	pH	Dry residues, %	Tannin content, %	Flavonoid content, %
In accordance with the recommendations of the OFS.1.4.1.0018.15 "Infusions and decoctions"	Brown transparent liquid with weak opalescence, with a specific herbaceous smell and astringent taste	3.76	2.240±0.06	0.812±0.001	0.076±0.003
Heating at 100 °C for 15 minutes, followed by cooling for 45 minutes	Brown transparent liquid with weak opalescence, with a specific herbaceous smell and astringent taste	3.78	2.270±0.03	0.834±0.004	0.0078±0.005
Infusion in a thermos flask for 12 hours	Greenish-brown transparent liquid with a specific herbaceous smell and astringent taste	3.81	2.230±0.04	0.768±0.005	0.072±0.001
Pouring of raw materials with boiling water, followed by infusion until cooling	Greenish-brown transparent liquid with a specific herbaceous smell and astringent taste	3.79	2.180±0.07	0.761±0.002	0.073±0.002

maximum yield of substances for preparing aqueous extracts.

In order to assess the effect of the infusion conditions on the quality of the resulting aqueous extract, infusions from alder leaves were prepared using the following conditions:

- In accordance with OFS.1.4.1.0018.15 "Infusions and decoctions";
- heating at 100°C for 15 minutes, followed by cooling for 45 minutes;
- infusion in a thermos flask for 12 hours;
- pouring of raw materials with boiling water, followed by infusion until cooling.

The results of the analysis are presented in Table 2.

The quality of water extracts was assessed by the yield of dry residue and the content of tannins and flavonoids. The maximum yield of dry residue was obtained by heating at 100 °C, however, the yield of tannins and flavonoids varies slightly. An increase in the yield of dry residue in the high-temperature conditions can be due to the simultaneous extraction of excipients, therefore, for preparing the aqueous extract from alder leaves, the conditions recommended by the State Pharmacopoeia can be considered optimal.

CONCLUSION

In the course of the analysis, the influence of the fineness and the conditions of infusion of new medicinal plant raw materials such as black and gray alder leaves on the quality of the infusions prepared was studied. The optimal fineness factor was determined, which provides the greatest yield of biologically active substances from raw materials to water extract. The following parameters were used as criteria for the quality of water extracts: dry residue, tannin content, total flavonoid content, and the value of the hydrogen value. The results obtained can be used in the development of regulatory documentation for alder leaves.

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