METHODS FOR QUANTITATIVE DETERMINATION OF TOTAL FLAVONOIDS IN QUERCUS ROBUR L. BUDS

N.A. Ryabov, V.M. Ryzhov, V.A. Kurkin

Samara State Medical University
89, Chapayevskaya St., Samara, Russia, 443099

E-mail: v.a.kurkin@samsmu.ru

Received 24 May 2021             Accepted 05 Oct 2021

Currently, the actual task of modern pharmacy is to study the chemical composition and pharmacological properties of plant objects. Within the framework of this concept, it seems interesting to study Quercus robur L. buds. One of the promising groups of biologically active compounds of Quercus robur L. buds are flavonoids. This group of substances has a wide range of a pharmacological activity, which is significant in the creation of new medicines based on medicinal plant raw materials.

The aim of the article was to work out methods for quantitative determination of total flavonoids in Quercus robur L. buds.

Materials and methods. The research materials were aqueous-alcoholic extracts from Quercus robur L. buds with 70% ethyl alcohol which were analyzed by differential UV spectrophotometry on spectrophotometer “SF 2000” (Russia).

Results. The methods for quantitative determination of total flavonoids in Quercus robur L. buds by differential UV spectrophotometry, has been developed using a standard sample of cynaroside at the analytical wavelength of 400 nm. The optimum parameters for the extraction of total flavonoids from Quercus robur L. buds have been determined. They are: the optimum extractant is 70% ethyl alcohol; the “raw material-extractant” ratio is 1:50; the extraction time is 120 min, the degree of atomization is 2 mm.

The content of total flavonoids for Quercus robur L. buds has been determined; it varies from 0.27%±0.01 to 0.44%±0.02.

Conclusion. The data obtained in the course of the experiment, makes it possible to conclude that a further study of Quercus robur L. buds is promising, and it also contributes to the implementation of medicinal plant raw materials “Quercus robur L. buds” in the State Pharmacopoeia (Russia).

Keywords: Quercus robur L.; buds; flavonoids; cynaroside; differential spectrophotometry; standardization

Abbreviations: BASs – biologically active substances; HPLC – High Performance Liquid Chromatography; SP (Russia), XIVth ed. – State Pharmacopoeia of the Russian Federation, XIVth edition; GM – general monograph; SS – standard sample; UV spectroscopy – ultraviolet spectroscopy; PM – pharmacopoeial monograph; SD – Standard Deviation; RSD – Relative Standard Deviation.
INTRODUCTION

The genus Quercus L. (Fagaceae) is represented by more than 500 species, most of which are the most important producers of broad-leaved and mixed coniferous-broad-leaved forests in the European part of Russia and Western Europe1-2. In Russia, 19 species grow wild, and about 60 species have been introduced [1].

Quercus robur L. is a large tree with a wide-pyramidal tent-like crown, reaching more than 50 meters in height3. The economic importance of Quercus robur L. is quite great, so it is used in many areas: in the furniture and leather industries, in forestry, etc. The bark of Quercus robur L. is used in the world medical practice, it is found in such pharmacopoeias as Russian, British, European and others [6, 7]. Its bark is also used in the production of various complex medicines, such as "Stomatophyt", "Tonsilgon N", "Dentos" and others3,5,6.

Quercus robur L. is rather widely used in folk medicine as a remedy for the prevention and treatment of gastrointestinal, gynecological, as well as otorhinolaryngological and dermatological diseases [1].

A complex of biologically active substances (BASs), which include flavonoids, is present in plant objects, particularly, in the oak bark. This group of substances is one of the most common groups of all phenolic plants compounds, in the chemical structure of which there is a C6-C3-C6 carbon skeleton [2-6]. These are the substances of a phenolic nature with valuable pharmacological properties such as anti-inflammatory, diuretic, choleretic, antispasmodic, antiviral, antioxidant, antimicrobial, etc., ones6-8 [2-6]. The oak bark also contains tannins (gallic acid, ellagic acid), triterpenes (fridelin, fridelinol, etc., ones9), and a number of other valuable substances [6-12].

Besides studying Quercus robur L. bark, the buds of this plant are of interest as a source of flavonoids. An important concept in the study of Quercus robur L. buds and their implementation into pharmaceutical and medical practice, is to solve the problem of standardization of raw materials, as well as the development of methods for the quantitative analysis of BASs in the raw materials. As a type of a medicinal plant raw material, buds are included in SP (Russia), XIVth ed., as a general monograph (GM). It should be noted that the attention of domestic and foreign scientists used to be attracted to the study of some plants’ buds [13-16]. Currently, for the quantitative determination of flavonoids compounds, rath-

---

1 State Pharmacopoeia of the Russian Federation. Ministry of Health of the Russian Federation. XIV ed. Vol. 1-4. M., 2018. Available from: http://femb.ru/femb/pharmacopea.php
2 Assessment report on Quercus robur L., Quercus petraea (Matt.) Liebl., Quercus pubescens Willd., cortex EMA/HMPC/3206/2009.
3 Ibid.
4 State Pharmacopoeia of the Russian Federation. Ministry of Health of the Russian Federation. XIV ed. Vol. 1-4. M., 2018.
5 Assessment report on Quercus robur L., Quercus petraea (Matt.) Liebl., Quercus pubescens Willd., cortex EMA/HMPC/3206/2009.
6 European Pharmacopoeia – 8th. "01/2008:1887 corrected 6.0". 2013. Available from: http://pharmeuro.epdm.eu
7 British Pharmacopoeia 2009. British Pharmacopoeia Herbal Drugs and Herbal Drug Preparations // Oak Bark. 2009;37:203.
er a wide list of analytical methods is used. The most frequently used methods are high-performance liquid chromatography (HPLC) and UV spectroscopy [14–17]. The UV spectroscopy method makes the quantitative determination of total flavonoids of biologically active substances in plant objects possible, whereas the HPLC method, as a rule, is used to determine individual components of the studied objects [14, 17].

Thus, the research was conducted on the study of *Aesculus hippocastanum* L. buds, which resulted in the development of a method for the quantitative determination of rhamnocitrin in *Aesculus hippocastanum* L. buds by HPLC [14, 15]. The same scientists studied the chemical composition of *Aesculus hippocastanum* L. buds by differential spectrophotometry, which resulted in the identification of the dominant substance in the raw material [15]. The study of new antimicrobial agents of the plant origin for the suppression of a microbial biofilm formation was also conducted to identify and quantify phenolic compounds extracted from *Populus nigra* and *Populus alba* L. buds. It was also done to evaluate their antimicrobial and antibiotic activity by HPLC [13]. Besides studying *Populus nigra* L. and *Populus alba* L. buds, the research of *Populus balsamifera* L. buds was conducted to determine the optimal way of extraction by a barothermic method, with ethanol and supercritical carbon dioxide, the isolation and purification of flavonoid components of *Populus balsamifera* L. buds [13].

The method of differential UV spectroscopy is widely used for the qualitative and quantitative assessment of BASs in plant raw materials [1, 15, 17–21]. The essence of differential spectrophotometry is the complex formation of aluminum cation, carbonyl and hydroxyl groups of flavonoid resulting in the stable complex formation, due to which the so-called bathochromic shift occurs [1, 19]. The differential spectrophotometry method was used in the development of methods for the quantitative determination of flavonoids in *Leonotodon autumalis* L. raw materials after the formation of a stained complex with an aluminum chloride solution [20]. This method was also used in the process of the development of the quantitative determination method of total flavonoids in *Juglans regia* L. leaves using the rutin standard sample at the analytical wavelength of 416 nm in order to solve the issues of the new type standardization of medicinal plant raw materials [22]. Differential spectrophotometry was used in the development of methods for the quantitative determination of flavonoids in *Tagetes patula* flowers using a patulitrin standard sample (7-O-β-D-glucopyranoside 3,5,7,3’4’-pentahydroxy-6-methoxylflavone) at the analytical wavelength of 428 nm [22]. As a result of the analysis of the above mentioned studies, it can be concluded that the method of differential spectrophotometry is in demand in modern pharmaceutical practice in the standardization of medicinal plant raw materials [22].

The method of differential spectrophotometry in the quantitative analysis of flavonoids has significant advantages, such as simplicity, availability, accuracy, small amounts of time spent on the analysis. Proceeding from the fact that the method of differential spectrophotometry makes it possible to determine the content of flavonoids, their total or the individual substance in the analyzed raw materials; it is logical to use this method in the development of regulatory documentation on the raw materials – *Quercus robur* L. buds [19].

In the course of the literature review regarding the study of *Quercus robur* L. buds, the data on the research of morphological and anatomical signs of *Quercus robur* L. buds, an important link in the standardization of new medicinal plant raw materials, were found [23]. A study of the alcoholic extracts based on *Quercus robur* L. buds, which revealed an antimicrobial activity against a number of pathogenic strains of microorganisms *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus cereus, Candida albicans*, had been conducted [24]. A further direction in the study of *Quercus robur* L. buds, is the development of methods for the quantitative determination of the BASs in the raw materials.

**THE AIM** of the article was to work out methods for the quantitative determination of total flavonoids in *Quercus robur* L. buds.

**MATERIALS AND METHODS**

The objects of the study were three samples of *Quercus robur* L. buds, harvested in the winter-spring period from late February to early April 2021. Sample No. 1 was collected in the Samara region (Pohvistevesky district, Pervomaisk village); sample No. 2 – in the Botanical Garden of Samara University (Samara); sample No. 3 – in the Nature Forest Park “Dubki” (Samara, Russia). The species specificity of the analyzed objects was confirmed by the determinants of the central part of Russia [1].

Morphologically, *Quercus robur* L. buds are obovate, multilobed, dense, dark brown topping off in the center at the end of the shoot with one or three apical (terminal) buds [1, 23]. Both vegetative and generative buds from three representatives of this species were selected for the analysis. After harvesting, the buds were crumbled in a thin layer and dried without heating in a well-ventilated room without direct sunlight. The end of drying was determined by the brittleness of the buds. A differential spectrophotometry method was used to develop the methodology, which was carried out in accordance with the Pharmacopoeia Monograph of the State Pharmacopoeia (Russia), XIVth ed. (SP (Russia), XIVth ed.) [10]. A solution of cynaroside in 70% alcohol was used as a standard sample (Fig. 1). The cynaroside standard

---

[10] State Pharmacopoeia of the Russian Federation. Ministry of Health of the Russian Federation. XIV ed. Vol. 1–4. M., 2018.
sample complies with the requirements of the Pharmacopoeia Monographs (PMs) and was provided for the research by the Research Equipment Sharing Center of SamSMU Institute of Pharmacy.

During the analysis of aqueous-alcoholic extractions of Quercus robur L. buds and standard samples of cynaroside solutions, the device “SF 2000” (Russia) was used. The aqueous-alcoholic extractions were prepared using 96% alcohol (Trademark OOO “Hippocrates”, Russia, Samara, series: 360917). The alcohol concentrations of 40%, 50%, 60%, 70%, 80%, 90% and 96% were obtained by diluting 96% alcohol according to Table No. 5 of Appendix to SP (Russia), XIVth ed.11.

Method of quantitative determination of total flavonoids in Quercus robur L. buds

Preparation of aqueous-alcoholic extractions from Quercus robur L. buds

The analytical sample of the raw material is ground to a particle size, passing through a sieve with a diameter of 2 mm. About 1 g of the crushed raw material (a precisely weighed amount) is placed in a conical heat-resistant flask (Erlenmeyer flask) with a 100 ml slotted volume, and 50 ml of 70% alcohol added. The flask is closed with a stopper and weighed on Sarto GOSM laboratory balance (LV 210-A (Ru-LV-210-A), No. 23425181; 2008; Russia) with an accuracy of ±0.001. The flask is attached to a reflux condenser and heated in a boiling water bath (moderate boiling) for 120 min. Then the flask is cooled to a room temperature, its volume is brought to the mark, and a selected aliquot of aqueous-alcoholic extractions of Quercus robur L. buds to the mark, the alcohol of this concentration was also used to bring the cynaroside A solution to the mark.

The content of total flavonoids equivalent to cynaroside and absolutely dry raw materials in percent (X), is calculated by the formula:

\[ X = \frac{D \cdot m \cdot 50 \cdot 25 \cdot 2 \cdot 100 \cdot 100}{D_0 \cdot m \cdot 5 \cdot 25 \cdot 25 \cdot (100 - W)} \]

where: \( D \) – the optical density of the test solution; \( D_0 \) – the optical density of the cynaroside standard sample; \( m \) – the mass of raw materials; \( g; \) \( m_0 \) – the mass of the cynaroside standard sample, g; \( W \) – the mass loss in drying, %.

In the absence of a cynaroside standard sample, it is advisable to use the theoretical value of the specific absorption index, 334.

\[ X = \frac{D \cdot 50 \cdot 25 \cdot 100}{m \cdot 334 \cdot 5 \cdot (100 - W)} \]

where: \( D \) – the optical density of the test solution; \( m \) – the mass of raw materials; \( g; \) 334 – specific absorbance \( E_{1%}^{1%} \) of cynaroside standard sample at 400 nm; \( W \) – the weight loss in-drying, %.

The value of the specific absorption index \( E_{1%}^{1%} \) for the cynaroside standard sample at 400 nm was calculated experimentally by the formula:

\[ E_{1%}^{1%} = \frac{D \cdot V_1 \cdot V_2}{100 \cdot g \cdot m_0} \]

where: \( D \) – the optical density of the test solution; \( m_0 \) – the mass of the cynaroside standard sample, g; \( V_1 \) – the volume of flask 1, ml; \( V_2 \) – the volume of flask 2, ml; \( q \) – the volume of the aliquot, ml;

Validation of analytical methods

Validation of the developed methods was carried out according to the following indicators: specificity, linearity, precision (a repeatability level), intralaboratory precision, correctness in accordance with SP (Russia),

11 Ibid. 12 Ibid.
RESULTS AND DISCUSSIONS

In the course of the experiment, a method for the quantitative determination of total flavonoids in *Quercus robur* L. buds has been developed. As a result, the optimum conditions for the extraction have been determined, and the choice of the optimal extractant has been substantiated.

Since at present, the component composition of the buds has not been studied, the total substances (flavonoids) in the studied extracts were determined.

The development of the methods was carried out stage by stage. At the first stage, the absorption spectra of aqueous-alcoholic extractions on the basis of *Quercus robur* L. buds were studied. During the analysis of the obtained extracts by differential spectrophotometry, the absorption maxima of spectral curves characteristic for the substances of the flavonoid nature, were determined (Fig. 2). A bathochromic shift of the electronic absorption spectrum of the aqueous-alcoholic extractions of *Quercus robur* L. buds with an absorption maximum similar to that of the standard sample of a cynaroside solution (400 nm) was recorded (Fig. 3). Therefore, when carrying out the quantitative determination of total flavonoids in the aqueous-alcoholic extractions based on *Quercus robur* L. buds, cynaroside was chosen as a standard sample (Fig. 4 and 5). The observed similar picture of spectral absorption curves in the analysis of the studied samples of raw materials and the cynaroside standard sample solution, makes it possible to assert that in aqueous-alcoholic extractions of *Quercus robur* L. buds flavonoids are present, and the method of differential spectrophotometry makes it possible to carry out their quantitative determination.

At the second stage of the methods development, it was found out that the complete extraction of flavonoids from *Quercus robur* L. buds is achieved with the extraction of 70% alcohol. The next stage was an experiment to determine the optimal ratio “raw material-extractant” (1:50). Then the extraction time parameters were determined: it was found out that the maximum extraction of flavonoids from raw materials occurs during 120 minutes. The final step was to determine the degree of atomization of raw materials (2 mm), contributing to the full extraction of flavonoids by the extractant (Table 1).

On the basis of the obtained results, the conditions for the quantitative determination methods have been determined: the extraction of flavonoids from *Quercus robur* L. buds crushed to 2 mm, in 70% ethanol, in the ratio of “raw material-extractant” 1:50, within 120 min in a boiling water bath. The quantitative determination of total flavonoids equivalent to cynaroside, is carried out by differential spectrophotometry at the analytical wavelength of 400 nm, using a standard sample or value of the specific absorption index of the cynaroside standard sample (334). The criterion for evaluating the analytical methods is the validation assessment. The validation of the methods was performed in accordance with SP (Russia), XIVth ed. The methods specificity was determined by the correspondence of the absorption maxima of the *Quercus robur* L. buds flavonoid complex and the solution of the standard cynaroside sample with aluminum chloride and the differential peak of the standard cynaroside sample.

The methods linearity was determined for a series of 10 cynaroside solutions (with the concentrations ranging from 0.00225 to 0.0225 mg/ml: 0.00225; 0.00325; 0.00425; 0.00525; 0.00625; 0.00725; 0.00825; 0.00925; 0.0125; 0.0225) with aluminum chloride at the wavelength of 400 nm. Based on the data obtained, a dependence graph of the optical density values of cynaroside solutions with aluminum chloride on the concentration of cynaroside was constructed, and then a linear regression equation was calculated (Fig. 6; Table 2).

While studying the linear dependence of the kind of \( y = bx + a \), the correlation coefficient made 0.99957, hence, the given methods can be used for the analysis of total flavonoids in *Quercus robur* L. buds equivalent to cynaroside in the specified range of concentrations (Fig. 6; Table 2).

The precision of the methods (a repeatability level) was estimated by analyzing the studied sample of medicinal plant raw materials in a 10-fold replication (Table 3).

To assess the in-laboratory precision, the analysis of the test sample was performed by another analyst on other days using the same equipment (Table 4). For each sample, the studies were carried out in six replications. Table 4 shows that the calculated value of Fisher’s F-criterion 1.19 is less than the tabulated value of 5.05. Consequently, the variance of the analysis results of both chemistries are statistically equivalent, and the differences between the values obtained are random. Thus, the developed methods meets the validation requirements for the index of in-laboratory precision.

The correctness of the methods was determined by the addition method. Cynaroside solutions with the known concentration (80%, 100% and 120%) were added to the aliquot of the test sample. The average opening percentage was 100.30±2.12% (Tables 5 and 6). Three determinations were performed for each concentration. The error determined for the samples with additives of the standard samples, was within the error of a single determination, indicating that there was no systematic error. The value of the average opening percentage of the experiment 100.30±2.12% was within the normalized range of values and within 100±5% (Tables 5 and 6).

13 State Pharmacopeia of the Russian Federation. Ministry of Health of the Russian Federation. XIV ed. Vol. 1—4. M., 2018.

14 Ibid.
Figure 1 – Cynaroside formula

Figure 2 – Electronic spectra of solutions of aqueous-alcoholic extraction from Quercus robur L. buds
Note: 1 – extraction solution (direct spectrophotometry); 2 – extraction solution with addition of aluminum chloride; 3 – differential curve

Figure 3 – Electronic spectra of aqueous-alcoholic solutions of cynaroside standard sample
Note: 1 – initial cynaroside solution (direct spectrophotometry); 2 – cynaroside solution with addition of aluminum chloride; 3 – differential curve of cynaroside (bathochromic shift of short- and long-wave bands)
Table 1 – Optimal extraction rates of total flavonoids from *Quercus robur* L. buds at wavelength of 400 nm

| No. | Extractor | “Raw materials: extractant” ratio | Extraction time, min | Degree of atomization, mm | Optical density value, D | Total flavonoids content per cyanaroside and absolutely dry raw materials, % |
|-----|-----------|----------------------------------|-----------------------|---------------------------|-------------------------|------------------------------------------------------------------------------------------------|
| 1   | 40% ethanol | 1:30                             | 60 min                | 2                         | 0.3918                  | 0.23±0.012                                                                                     |
| 2   | 50% ethanol | 1:30                             | 60 min                | 2                         | 0.4116                  | 0.24±0.012                                                                                     |
| 3   | 60% ethanol | 1:30                             | 60 min                | 2                         | 0.4417                  | 0.24±0.012                                                                                     |
| 4   | 70% ethanol | 1:30                             | 60 min                | 2                         | 0.4705                  | 0.25±0.013                                                                                     |
| 5   | 80% ethanol | 1:30                             | 60 min                | 2                         | 0.4866                  | 0.24±0.012                                                                                     |
| 6   | 90% ethanol | 1:30                             | 60 min                | 2                         | 0.4771                  | 0.22±0.011                                                                                     |
| 7   | 96% ethanol | 1:30                             | 60 min                | 2                         | 0.4725                  | 0.23±0.012                                                                                     |
| 8   | 70% ethanol | 1:30                             | 30 min                | 2                         | 0.4845                  | 0.20±0.01                                                                                      |
| 9   | 70% ethanol | 1:30                             | 45 min                | 2                         | 0.5236                  | 0.21±0.01                                                                                      |
| 10  | 70% ethanol | 1:30                             | 60 min                | 2                         | 0.4742                  | 0.23±0.012                                                                                     |
| No. | Extractor | “Raw materials: extractant” ratio | Extraction time, min | Degree of atomization, mm | Optical density value, D | Total flavonoids content per cyanaroside and absolutely dry raw materials, % |
|-----|-----------|----------------------------------|----------------------|---------------------------|------------------------|---------------------------------------------------------------|
| 11  | 70% ethanol | 1:30                             | 90 min               | 2                         | 0.383                  | 0.23±0.012                                                     |
| 12  | 70% ethanol | 1:30                             | 120 min              | 2                         | 0.388                  | 0.26±0.013                                                     |
| 13  | 70% ethanol | 1:30                             | 150 min              | 2                         | 0.388                  | 0.25±0.013                                                     |
| 14  | 70% ethanol | 1:20                             | 120 min              | 2                         | 0.3169                 | 0.14±0.01                                                     |
| 15  | 70% ethanol | 1:30                             | 120 min              | 2                         | 0.6121                 | 0.16±0.01                                                     |
| 16  | 70% ethanol | 1:50                             | 120 min              | 2                         | 0.4399                 | 0.27±0.012                                                     |
| 17  | 70% ethanol | 1:100                            | 120 min              | 2                         | 0.6121                 | 0.26±0.013                                                     |
| 18  | 70% ethanol | 1:50                             | 120 min              | 1                         | 0.5843                 | 0.23±0.012                                                     |
| 19  | 70% ethanol | 1:50                             | 120 min              | 2                         | 0.6649                 | 0.27±0.013                                                     |
| 20  | 70% ethanol | 1:50                             | 120 min              | 3                         | 0.6063                 | 0.23±0.011                                                     |

**Table 2** — Input data for assessing methods linearity

| No. | Concentration of cynaroside standard sample solution, mg/ml | The optical density value, o.d.u (average of three consecutive measurements) |
|-----|-----------------------------------------------------------|---------------------------------------------------------------------|
| 1   | 0.00225                                                   | 0.078411                                                            |
| 2   | 0.00325                                                   | 0.112547                                                            |
| 3   | 0.00425                                                   | 0.146935                                                            |
| 4   | 0.00525                                                   | 0.181541                                                            |
| 5   | 0.00625                                                   | 0.216048                                                            |
| 6   | 0.00725                                                   | 0.250947                                                            |
| 7   | 0.00825                                                   | 0.275401                                                            |
| 8   | 0.00925                                                   | 0.318974                                                            |
| 9   | 0.0125                                                    | 0.440864                                                            |
| 10  | 0.0225                                                    | 0.780564                                                            |

**Table 3** — Precision estimation results of quantitative determination methods of total flavonoids in *Quercus robur* L. buds (repeatability level)

| Metrological characteristics | f | X, % | S² | SD | RSD | P, % | t (tab.) | ΔX, % | ε, % |
|------------------------------|---|-----|----|----|-----|------|---------|-------|-----|
| Values                       | 9 | 0.24| 0.00011738 | 0.011738 | 4.81% | 95 | 2.262 | ±0.01 | ±3.44 |

**Table 4** — Validation of laboratory precision of methods for determining total flavonoids in *Quercus robur* L. buds

| Analyst 1 | Analyst 2 | Metrological characteristics |
|-----------|-----------|-------------------------------|
| X, %      | X, %      | X, %                          |
| 0.24      | 0.26      | 0.24                          |
| 0.24      | 0.25      | 0.24                          |
| 0.23      | 0.26      | 0.24                          |
| 0.25      | 0.24      | 0.24                          |
| 0.23      | 0.24      | 0.24 ± 0.01                   |
| 0.24      | 0.25      | 0.25 ± 0.01                   |
| 0.24      | 0.25      | 0.25 ± 0.01                   |

Notes: t_calculated = 2.44 < t (95%; 10); F_calculated = 1.19 < F (95%; 5; 5), differences between the results obtained are random

**Table 5** — Preparation scheme of aqueous-alcoholic extractions from *Quercus robur* L. buds with solutions addition of cynaroside standard sample

| Initial cynaroside content, mg/ml aqueous-alcoholic extraction | Cyanaroside additive, mg/ml | Total calculated cynaroside content, mg/ml | Concentration level relative to nominal, % |
|--------------------------------------------------|-----------------------------|---------------------------------------------|-------------------------------------------|
| 2.30                                              | 1.84                        | 4.14                                        | 80                                        |
| 2.30                                              | 2.30                        | 4.60                                        | 100                                       |
| 2.30                                              | 2.76                        | 5.06                                        | 120                                       |
Table 6 – Assessment results of correctness of quantitative determination method of total flavonoids in *Quercus robur* L. buds

| Injected cyanaroside, mg/ml | Found, mg/ml | Openness, % | Characteristics calculated for opening value, % |
|-----------------------------|--------------|-------------|-----------------------------------------------|
| 0.84                        | 0.80         | 95.24       |                                               |
| 0.84                        | 0.86         | 102.38      |                                               |
| 0.84                        | 0.83         | 98.81       |                                               |
| 2.30                        | 2.32         | 100.87      |                                               |
| 2.30                        | 2.26         | 98.26       |                                               |
| 2.30                        | 2.38         | 103.48      |                                               |
| 2.76                        | 2.81         | 101.81      |                                               |
| 2.76                        | 2.72         | 98.55       |                                               |
| 2.76                        | 2.85         | 103.26      |                                               |

\[ \bar{X} = 100.30\% \]
\[ SD = 2.76\% \]
\[ RSD = 2.75\% \]

Table 7 – The content of total flavonoids in *Quercus robur* L. buds samples (in %) equivalent to cynaroside

| No. | Characteristics of raw material sample | Content of total flavonoids in absolutely dry raw materials (in %) calculated on cynaroside |
|-----|----------------------------------------|---------------------------------------------------------------------------------------------|
| 1   | Samara region, Pokhvistnevsky district, Pervomaysk village (March 2021) | 0.27±0.01                                                                                  |
| 2   | Botanical Garden of Samara University, Samara (March 2021)              | 0.44±0.02                                                                                  |
| 3   | Natural forest park "Dubki", Samara (March 2021)                        | 0.35±0.02                                                                                  |

The results obtained testify to the satisfactory precision of the proposed quantitative determination methods of total flavonoids in *Quercus robur* L. buds equivalent to cynaroside at the levels of repeatability and in-laboratory precision.

It was found out that the average content of flavonoids in the studied sample of the raw materials was 0.24 ± 0.01% (the relative error of the determination was ±3.60%).

Thus, based on the experimental results validation, it can be concluded that this method is suitable for the quantitative estimation of total flavonoids equivalent to cynaroside.

Using this method, three samples of *Quercus robur* L. buds, harvested at the same time (May-June 2021), were analyzed (Table 7). It was determined that the content of total flavonoids in the analyzed samples varies from 0.27±0.01 to 0.44±0.02 depending on its habitat (Table 7).

The presence of the flavonoid cynaroside in *Quercus robur* L. buds makes it possible to position them as a medicinal plant raw material. The medicines based on *Quercus robur* L. buds can be prescribed in diseases of chronic glomerulonephritis and pyelonephritis, complicated by a renal failure with hyperazotemia [25].

The results obtained correlate with the data obtained for the buds of other plant species. If we take into account the fact that the determined total flavonoids in the buds of different species are converted to different substances, total flavonoids in *Aesculus hippocastanum* L. buds are equivalent to rhamnocitrin and varies from 1.24% to 2.31%. The content of total flavonoids in *Populus balsamifera* L. buds is equivalent to dihydroquercetin and ranger from 7.5% to 11.1%) [13–15].

Thus, the data obtained during the experiment suggest the feasibility of using the method of differential spectrophotometry for the quantitative determination of total flavonoids in *Quercus robur* L. buds. These results make it possible to recommend not less than 0.25% of total flavonoids for this type of raw material as a lower limit.

CONCLUSION

Thus, as a result of the study, the quantitative determination methods of total flavonoids in *Quercus robur* L. buds has been developed by differential spectrophotometry using a standard sample of cynaroside at the analytical wavelength of 400 nm. The content of total flavonoids has been determined for *Quercus robur* L. buds, which ranges from 0.27±0.01 to 0.44±0.02. The error of a single determination with a 95% confidence level is ±3.6%. The optimum values of the total flavonoids’ extraction from *Quercus robur* L. buds, have been established. For *Quercus robur* L. buds, the total flavonoids content not less than 0.25%, can be recommended as the lower limit.

A validation assessment of the developed methods
by the indicators of specificity, linearity, precision (a repeatability level), in-laboratory precision, correctness in accordance with SP (Russia), XIVth ed., has been carried out. Based on the results of the validation assessment of the experimental results, these methods can be suitable for the quantitative assessment of total flavonoids calculated on cynaroside.

This study has laid the foundation for the study of Quercus robur L. buds chemical composition, a quantitative assessment of total flavonoids of BAS in them by differential spectrophotometry. The results of the study can be used in the creation of herbal medicines based on Quercus robur L. buds and used in the treatment of kidney and dermatological diseases due to the content of total flavonoids in the raw material of biologically active substances and the substance of cynaroside alone.

The results obtained contribute to the development of the normative documentation for the promising species of the raw materials “Quercus robur L. buds” for the introduction to the State Pharmacopoeia (Russia).

**FUNDING**

This study did not have any external support.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**AUTHORS’ CONTRIBUTION**

Nikolay A. Ryabov – data collection, conducting the experiment, analysis and interpretation of the data obtained, preparation of the draft manuscript, literature analysis, writing the manuscript; Vitaly M. Ryzhov – study planning, participation in the development of study concept and design, collection of plant material for analysis; Vladimir A. Kurkin – final approval of the manuscript publication, processing of the obtained results, checking the critical intellectual content, statistical processing of the obtained results.

**REFERENCES**

1. Maevsky PF. Flora of the middle zone of the European part of Russia. 11th ed. M.: Partnership of scientific publications KMK, 2014; 200-1. Russian
2. Grotewold E. The Science of Flavonoids. New York: Springer. 2006;35. DOI: 10.1007/978-0-387-28822-2.
3. Cushnie TP, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob Agents. 2011 Aug;38(2):99–107. DOI: 10.1016/j.ijantimicag.2011.02.014.
4. Magnus S, Gazdik F, Anjum NA, Kadlecova E, Lackova Z, Cerni N, Brtnicky M, Kynicky J, Klejduis B, Necas T, Zitka O. Assessment of Antioxidants in Selected Plant Rootstocks. Antioxidants (Basel). 2020 Mar 3;9(3):209. DOI: 10.3390/antiox9030209.
5. Dureshahwar K, Mubashir M, Upaganlwar AB, Sangshetti J, Upasani C, Une H. Quantitative assessment of tactile allodynia and protective effects of flavonoids of Ficus carica Lam. leaves in diabetic neuropathy. Pharmacognosy Magazin. 2019; 15 (Issue 62): 128–34. DOI: 10.4103/ppm.pm_553_18.
6. Makarova N, Ignatova D, Eremeeva NB. Influence of extraction technology on the content of phenols, flavonoids and antioxidant activity for hips (Rosa L.), Oak bark (Quercus robur L.), root Rhena (Rheum officinale), Ginseng root (Panax L.), buds birch (Betula L.). Chemistry of plant raw material. 2020;3:271–8. DOI: 10.14258/jcprm.2020036608
7. Eaton E, Caudullo G, Oliveira S, de Rigo D. Quercus robur and Quercus petraea in Europe: distribution, habitat, usage and threats. European Atlas of Forest Tree Species. Publ.: Public Office of the European Union, Luxembourg. 2016:162–3.
8. Budantsev AL., Plant resources of Russia: Wild flowering plants, their component composition and biological activity. Vol. 1. Families Actinidaceae-Malvaceae, Euphorbiaceae-ae-Haloragaceae. Ed. by A.V. Budantsev. Saint Petersburg; Moscow, Scientific Publishing House KMK, 2009; 158.
9. Okuda T. Systematics and health effects of chemically distinct tannins in medicinal plants. Phytochemistry. 2005; 66:2012–31.
10. Elansary OH, Szopa A, Kubica P, Ekiert H, Mattar AM, Al-Yafraisi MA, et al. Polyphenol Profile and Pharmaceutical Potential of Quercus spp. Bark Extracts. Plants. 2019; 8(11):486. DOI: 10.3390/plants8110486.
11. Milton Prabu S. Quercetin: a flavonol with universal therapeutic use and its interactions with other drugs. Nonvitamin and Nonmineral Nutritional Supplements. 2019;106:256–71. DOI: 10.1016/B978-0-12-812491-8.00010-2.
12. Pérez AJ, Pecio L, Kowalczyk M, Kontek R, Gajek G, Stopinsek L, Mirt I, Oleszek W, Stochmal A. Triterpenoid Components from Oak Heartwood (Quercus robur) and Their Potential Health Benefits. J Agric Food Chem. 2017 Jun 14;65(23):4611–23. DOI: 10.1021/acs.jafc.7b01396.
13. Nassima B, Behid-Benyounes N, Ksouri R. Antimicrobial and antibiofilm activities of phenolic compounds extracted from Populus nigra and Populus alba buds (Algeria). Brazilian Journal of Pharmaceutical Sciences. 2019;55. DOI: 10.1590/s2175-97902019000218114/
14. Kurkin VA, Belov PV, Ryzhov VM. The quantitative determination of the amount of flavonoids in the buds of the horse chestnut. Pharmaceutical Chemistry Journal. 2019;53(2):47–51. DOI: 10.30906/0023-1134-2019-53-2-47-51. Russian
15. Kurkin VA, Belov PV, Ryzhov VM, Braslavsky VB. Determination of the content of rhamnocitrin in the buds of horse chestnut by HPLC. Pharmaceutical Chemistry Journal. 2019; 53(12):21–5. DOI: 10.30906/0023-1134-2019-53-12-21-25. Russian
16. Kurkina AV, Savelyeva AE, Kurkin VA. The quantitative determination of the amount of flavonoids in the flowers of rejected marigolds. Chemical and pharmaceutical journal.
17. Lysiuk R, Hudz N. Differential Spectrophotometry: Application for Quantification of Flavonoids in Herbal Drugs and Nutraceuticals Editorial. International Journal of Trends in Food and Nutrition. 2017; 1:102.
18. Adekenov SM, Baisarov GM, Khabarov IA, Polyakov BB. Flavonoids in the buds of Populus balsamifera L. and methods of their isolation. Chemistry of vegetable raw materials. 2020;2:181–8. DOI: 10.14258/jcprm.2020027602.
19. Bunaciu AA, Vu Dang H, Hassan Y. Aboul-Enein. Applications of Differential Spectrophotometry in Analytical Chemistry. Critical Reviews in Analytical Chemistry. 2013; 43(3): 25–130. DOI: 10.1080/10408347.2013.803357.
20. Bubenchikov RA, Goncharov NN. Working out and validation of the method of the quantitative determination of flavonoids in the herb of Leontodon autumnalis L. Pharmacy & Pharmacology. 2016; 4(1(14)):26–35. (In Russ.) DOI: 10.19163/2307-9266-2016-4-1(14)-26-35.
21. Bubenchikova VN, Starchak YuA. Validation of the method for the quantitative determination of the amount of flavonoids in thyme herb. Scientific Bulletin of Belgorod State University. 2011;16/2(22):203–6. Russian

AUTHORS

Nikolay A. Ryabov – Postgraduate student of the Department of Pharmacognosy with Botany and the basics of Phytotherapy, Samara State Medical University. ORCID ID: 0000-0002-1332-953X. E-mail: ryabov.nikolay.2014@mail.ru

Vitaly M. Ryzhov – Candidate of Sciences (Pharmacy), Associate Professor of the Department of Pharmacognosy with Botany and the basics of Phytotherapy, Samara State Medical University. ORCID ID: 0000-0002-8399-9328. E-mail: lavr_rvm@mail.ru

Vladimir A. Kurkin – Doctor of Sciences (Pharmacy), Professor, Head of the Department of Pharmacognosy with Botany and the basics of Phytotherapy, Samara State Medical University. ORCID ID: 0000-0002-7513-9352. E-mail: kurkinvladimir@yandex.ru