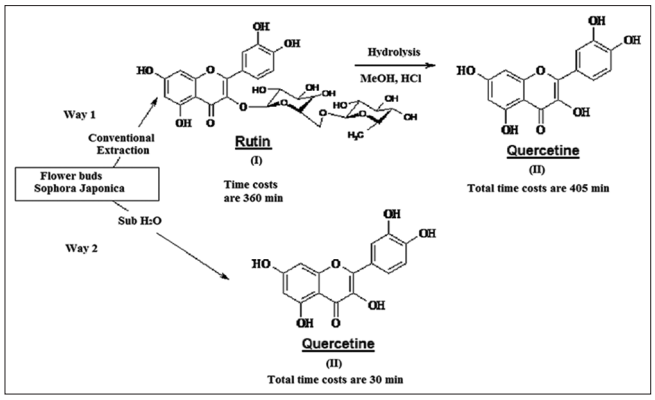

Figure 1: The principal reaction schemes for preparation of quercetin from flower buds of Sophora Japanese

apply the "one-pot" strategy for numerous tasks related to the extraction and transformation of plant's secondary metabolites. The successive implementation of this strategy could open up a perspective for the future development of inexpensive and environmentally friendly technologies to the production of new plant-based substances for pharmaceutical, nutritional, and cosmetic industries.

In the present work, as a model source of flavonoids, we used flower buds of Japanese Sophora flowers. Sophora Japanese (*Sophora japonica* L.) is often used as a plant material for the production of flavonoids and, in particular, rutin [Figure 1] from the legume family, which is widespread in Asia and cultivated in Southern regions of Russia. Leaves, flowers, and flower buds of Japanese Sophora are widely employed in traditional Chinese medicine because they contain a wide range of biologically active compounds:[4] flavone glycosides, isoflavones, chromones of coumarins, saponins, triterpene glycosides, phospholipids, alkaloids, amino acids, polysaccharides, and fatty acids. For the extraction of flavonoids from the plant matrix, including buds of Sophora, various two-step methods[5] have been developed.

As already noted above, the two basic steps are required to obtain of the natural antioxidant QR from plants, including the step of extracting QR glycosides (for example, rutin) and subsequent hydrolysis thereof. Typically, traditional extraction of buds of Sophora is carried out using various organic solvents. The most common technique is the extraction with 70%–95% ethanol usually taken from 20 min to 4 h[6] and affording rutin in the yields from 0.2% to 18%. Also common is extraction with methanol occurring under reflux[7] and requiring infusion for 48 h[8] or 18 h with preliminary shaking for 6 h.[9]

The process can be accelerated by the ultrasound irradiation. The yields of rutin in the methanol extracts are higher (15%–18%) than those achieved in the ethanol extraction procedures.[10] The conventional synthesis of QR from the plant involves the acidic hydrolysis of the glycosides usually catalyzed by mineral acids (phosphate, sulfate, and hydrochloric acids). The reactions are lasting –3 h.[2] Often used is also enzymatic hydrolysis of (reaction time [RT] is in the range of 12–24 h).[11,12] It should be noted that conventional acid hydrolysis of RT requires 2–3 h. The products of the hydrolysis are needed of careful purification. Thus, the conventional methods of preparation of RT require significant time costs.

An alternative to the conventional methods based on using acids to catalyze hydrolysis is to perform the reactions in SBW.[13] In recent years, SBW has been used as a cheap, environmentally friendly solvent for extraction, synthetic transformations, and the recycling of different organic wastes.[5] The hydrothermal reaction has been attracting much attention because of the fascinating physical and chemical characteristics of water near its critical point. In these conditions, water exhibits a much lower dielectric constant and a much larger ion product. The ion product or dissociation constant is about 3 orders of magnitude higher near the critical point (in the temperature, range from 220°C to 270°C) than it is for ambient liquid water. Under these conditions, there is a high H_3O^+ and OH^- ion concentration. As such, some acid-catalyzed organic reactions can be carried out without the addition of acids. However, the ion product decreases greatly above the critical point. This fact makes subcritical water an ideal reaction medium for the hydrolysis of organic compounds.

In this study, it is for the first time proposed to perform extraction of QR glycosides simultaneously with the subsequent hydrolysis as a "one-pot" process in a medium of subcritical water (SBW). This approach makes possible to avoid the use of flammable and costly, toxic organic solvents. In this regard, the purpose of the presented paper was the development of an expedient eco-friendly procedure for producing of QR in good yields starting from flowers buds of Sophora Japanese (*S. japonica* L.).

Materials and Methods

The flowers buds of *S. japonica* were purchased from JSC "Azbuka trav" (Russia). Acetonitrile (high-performance liquid chromatography [HPLC] grade) and water for the mass -spectrometry were purchased from JSC "Vekton" (Russia). Rutin ($C_{27}H_{30}O_{16}$, Mw 610.25 ≥96.6%) was purchased from "Merk." QR ($C1_5H_{10}O_7$, Mw 302.10, ≥98.2%) was purchased from JSC "Diaem" (Russia).

HPLC analysis was performed using a Agilent 1200 LC system, including a quaternary pump, a temperature controlled column compartment, an autosampler, and a diode-array detector. Eclipse XDB reversed-phase column C8 150 mm × 2.1 mm, 3.5 μm, was used for HPLC analysis. The mobile phase composition was as follows: CH_3CN: 0.5% H_3PO_4, 78:22; column temperature 30°C; mobile phase flow rate, 0.14 mL/min; ultraviolet-detector wavelengths, 360 nm.

The conventional way for preparation of QR from the buds of *S. japonica* included two basic steps. The step one is to obtain of rutin from the flower buds through extraction with using the organic solvents and subsequent purification. The step two involves the procedure of hydrolysis of the extraction

of rutin that obtained from the flower buds, to release the QR using the organic solvents and the mineral acids and subsequent purification. The extraction of rutin from the buds of *S. japonica* included the several stages. A 1 g sample of the dry buds of *S. japonica* (particle size: 0.5–1.0 mm) was boiled four times under reflux. At the first stage, dry buds were boiled with hexane 30 ml for 90 min. At the next three stages, 30 mL of 80% aqueous solution of ethanol was boiled for 90 min. The obtained extracts were filtered, combined, and analyzed by HPLC.

The hydrolysis of RT from extract of *S. japonica* buds was performed using hydrochloric acid. A sample of 0.10 g of extract (content of RT 38.6%) was dissolved in 2 ml of 80% methanol and 0.25 ml of concentrated hydrochloric acid (density 1.179) is added. The hydrolysis is carried out for 45 min at 100°C. At the end of the hydrolysis, the obtained precipitate is filtered through paper and washed with distilled water to a neutral pH. The washed precipitate is dried at 80°C for 2–3 h; the weight of the precipitate is 0.0268 g. Quantity of the QR in precipitate is 55.6%.

The "one-pot" way for preparation of QR from the buds of *S. japonica* by SBW, development in this paper, included the one single step: treatment of the flower buds using the medium of SBW.

The treatment's procedure of the flower buds in the SBW was performed using self-made reactor (autoclave).[14] The reactor has inner volume 10 mL. The buds (0.1 g) were put into self-made stainless steel reactor. Into reactor was filled with 7 mL of the water's solution of sulfuric acid (0.25%). The reactor was hermetically closed and put into a drying oven, where it was kept at a certain temperature (accuracy ± 1°C) for 30 min. After that, the reactor was cooled down to room temperature (15 min) in a tank filled with cold water. Its content was quantitatively transferred into a paper filter, filtered, and washed with distilled water to a neutral pH and after that washed with 80% EtOH until the color disappears. The aliquots of the solution obtained were diluted to the concentration required for analysis by HPLC/MS.

Results and Discussion

In accordance with the tasks of this study, the products containing QR were obtained from flowers buds of Sophora Japanese using two different schemes [Figure 1]. The scheme 1 conforms to the conventional two-step way of preparation of QR and includes traditional extraction by ethanol following by the hydrolysis of the obtained extract with HCl as the catalyst. The second scheme pictures the "one-pot" production of QR with the use of SBW. The obtained targeted products were analyzed for the contents of QR.

At the first stage, the amounts of RT and QR contained in the test sample were determined using a conventional extraction by ethanol. It was found that 1 g of the used raw material of the flower buds contains 191.1 mg of RT and 4.8 mg of QR. After treatment of the extract obtained by the two-step procedure with the use of HCl, the content of QR was increased to 91.1 mg/g.

At the next stage, the yields of QR were studied using SBW for realization of the "one-pot" technique. Our previous studies showed that the conversion of RT into QR, using the SBW medium, was most complete at the temperatures range of 100°C–250°C.[5,14] Therefore, here, we studied in detail the temperature dependence of the yield of the RT and QR in medium of SBW in this temperature range. Also studied was the composition of the products obtained by the conventional procedure, starting from the flower buds of Sophora Japanese.

The dependence of the amount of RT and QR in the products obtained from flower buds of Sophora Japanese in SBW (without any additives) demonstrates an increase in the yield of QR (from 0.6 to 27.3 mg/g) when elevating the temperature to 200°C and corresponding decrease in the yield of RT. The decrease in the amount of RT, as was previously shown, is caused by the hydrolysis processes in which SBW serves as the catalyst. The further increase in temperature led to a decrease in the amount of QR. Analysis of the data obtained showed that at the temperatures range of 200°C–210°C, the yield of QR was the highest but still lower than the theoretical yield (if the stoichiometry of hydrolysis is taken into account in this case). At a temperature of 230°C, RT was not detected by HPLC in either the precipitate or solution. Hence, the hydrolysis was complete, and the decrease in the yield of QR at the temperature above 210°C is caused by its thermal destruction. The QR yield in the temperature range of 220°C–250°C does not exceed 30 mg/g, whereas they should grow to 90 mg/g. It can be assumed that an increase in temperature above 210°C leads to a significant decrease in the yield of QR due to its thermal destruction. The results obtained are in accord with the previous data obtained in the study of the conversion of rutin to QR in SBW.[5,14]

These data allow selecting the temperature of 200°C as the temperature at which the effect of the thermal degradation of QR on its yield is negligible. However, since the dissociation constant of SBW reaches a maximum in the higher temperature (between 220°C and 270°C), the acidity of SBW at 200°C is insufficient for complete hydrolysis of RT. Therefore, we assumed that increase in the yield can be achieved with the use of catalytic amounts of strong mineral acids. To determine the effect of trace amounts of acids, the dependence of the QR yield from buds of Sophora on the acid concentration (H_2SO_4) and the processing time (in the interval of time from 10 to 60 min) was studied [Figure 2].

As can be seen from the data pictured in Figure 2, the use of trace amounts of acid made it possible to achieve the yield of QR obtained by the proposed "one-pot" technique comparable to that obtained by the employment of conventional techniques at the significantly (by 10 times) decreased temporary costs. The important advantage of the proposed procedure free of the use of toxic organic solvents is that it allows preparation of the final

Table 1: Comparison of the effectiveness of the different techniques for preparing quercetin from flower buds of Sophora Japanese (sample 1 g)

Technique of obtaining QR	Total time costs (min)	Temperature (°C)	Yield of QR (mg in 1 g sample)	Yield of QR (%)
Way 1 two-stage conventional procedure (extraction and acid hydrolysis [HCl])	405	100	91.1	95
Way 2 "one-pot" technique treatment by SBW (0.25% H_2SO_4)	30	200	89.9	94

SBW: Subcritical water, QR: Quercetin

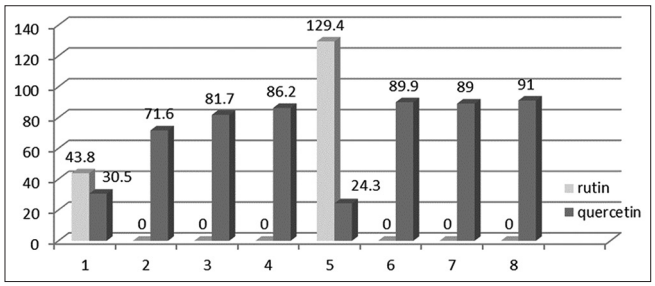

Figure 2: The yields of reaction time and quercetin starting from 1 g of the flower buds of Sophora Japanese (mg). Conditions: (1) Subcritical water 200°C, *t* = 60 min, (2) subcritical water 200°C +0.1% H+, *t* = 60 min, (3) subcritical water 200°C +0.1% H+, *t* = 30 min, (4) subcritical water 200°C +0.1% H+, *t* = 20 min, (5) subcritical water 200°C +0.25% H+, *t* = 10 min (6) subcritical water 200°C + 0.25% H+, *t* = 30 min, (7) subcritical water 200°C +0.25% H+, t = 40 min, (8) Two-stage conventional procedure

products in sufficiently pure state which excludes an additional stage of their purification. The comparison of the effectiveness of the developed and traditional methods is given in Table 1.

Conclusions

A novel eco-friendly "one-pot" facile technique was proposed for the preparation of the natural antioxidant QR from flower buds of *S. japonica* using SBW.

The method way requires no use of toxic and flammable organic solvents. The suggested procedure is significantly (by 10 times) faster than the commonly used conventional techniques.

The proposed technique has a potential for the future development of low cost and enviromentally friendly technologies for the production of the new plant-based substances for the pharmaceutical, nutritional, and cosmetic industries.

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Conflicts of interest

There are no conflicts of interest.

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