SOME REMARKS ON FLAVONOID QUANTITATION IN *RHODODENDRON TOMENTOSUM*

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Introduction. Flavonoids are a group of phenolic compounds with various bioactivities and are widely distributed in plants. In various *in vitro* and *in vivo* models, they have exhibited diverse biological activities including anti-inflammatory, antiatherosclerotic, antitumor, antithrombogenic, antiosteoporotic, and antiviral effects [1]. *Rhododendron tomentosum* is considered by scientists primarily as an ethereal medicinal plant, but it also contains flavonoids. A common method for quantitative determination of the total flavonoid content in plant raw materials is the spectrophotometric method, based on the reaction of flavonoids with aluminum chloride [2,3]. The calculation is carried out on a standard substance of flavonoids. For quantitatively determining flavonoids, it is important to determine the appropriate standard substance (marker). In the case of using an incorrect standard, the results of quantitative calculations of the flavonoid content may be overestimated or, conversely, underestimated [4].

Aim of the study. This work aims to justify the choice of a standard substance for quantitatively determining the flavonoid content in *Rhododendron tomentosum*.

Research methods. *Sample preparation for raw materials:* about 5.0 g shoots of *Rhododendron tomentosum* (exact weight), crushed to the size of a diameter of 2 mm, were placed in a 200 ml round-bottom flask, 50 ml of 50% ethanol was added. The flask was connected to a reflux condenser and heated in a water bath for 30 min. The hot extract was filtered through cotton wool. Extraction was carried out twice more under the conditions described above, filtering the extracts into the same flask. The combined extracts were evaporated to 1/4 of the previous volume. The evaporated extract was quantitatively transferred in a 50.0 ml volumetric flask and fill it up to 50.0 ml with 50% ethanol (solution B). 2.0 ml of solution B was placed in a 25 ml volumetric flask, 2.0 ml of 3% aluminum chloride in 50% ethanol was added, the volume was fill it up to the mark with 50% ethanol and mixed. After 30 min, the solution was filtered through a paper filter and was measured on a spectrophotometer (U-3900/3900H Hitachi) in the range of 280 to 500 nm in a cuvette with a layer thickness of 10 mm. The reference solution was a solution containing 2.0 ml of solution B, fill it up to the mark in a 25.0 ml volumetric flask with 50% ethanol.

Preparation of the rutin solution: 0.01 g (exact weight) of rutin was placed in a 25 ml volumetric flask, dissolved in 96% ethanol, fill it up to the mark and mixed.

Sample preparation for the reference solution: 1.0 ml of 3% ethanol solution of aluminum chloride was added to 1.0 ml of the rutin solution and fill it up to 25.0 ml with 50% ethanol. As a reference solution, a rutin solution was fill it up to the mark in a 25.0 ml volumetric flask with 50% ethanol. Hyperoside and quercetin solutions were prepared similarly.

Main results. Absorption spectra of the test samples are shown in Fig. 1. The difference in the spectral behaviour of rutin, quercetin and hyperoside can be explained, the reaction of complexation with $AlCl_3$ can potentially have several centers for the course, the localization of which is determined by the presence of a

nearby hydroxyl and carbonyl group in the C-4 position of the C ring, also A and B rings [3]. The maximum absorption of quercetin is in the range of 430-433 nm, while rutin has a maximum at a wavelength of 418-420 nm, hyperoside − 418-414 nm. As can be seen from Fig. 1, which shows the absorption spectra of the studied samples, the maximum absorption of the extract corresponds to the maximum absorption of rutin and hyperoside.

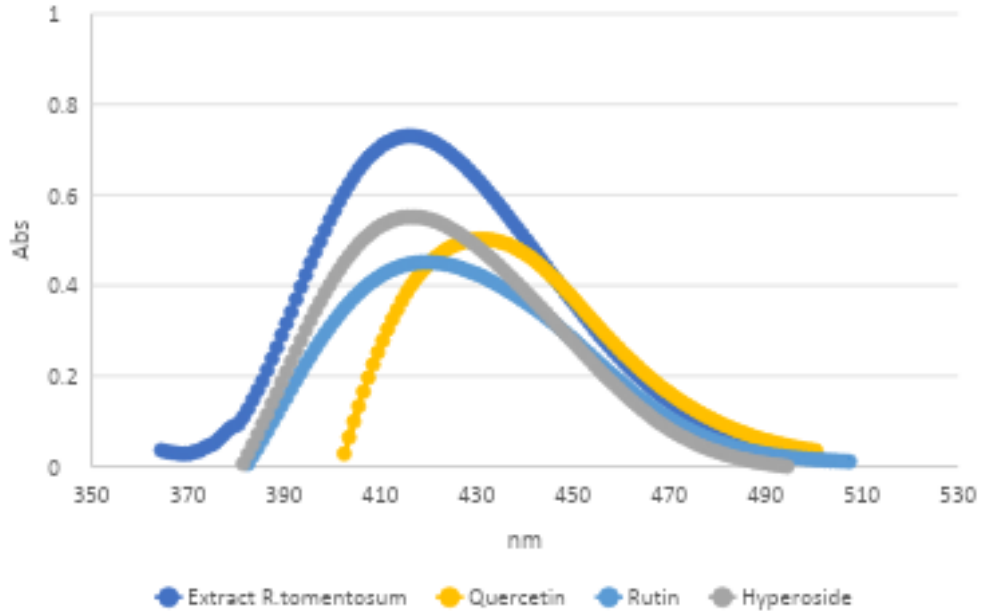

Fig. 1 Absorption spectra of the test samples

Calculations of the quantitative content of flavonoids showed that the shoots of the *Rhododendron tomentosum* contains 32.19 ± 0.2 mg rutin equivalents per 1 g raw materials, 21.78 ± 0.2 mg hyperoside equivalents per 1 g raw materials, 18.24 ± 0.2 mg quercetin equivalents per 1 g raw materials.

Conclusions. The results of calculations of the quantitative content of flavonoids are highly dependent on the standard substance used for calculations.

References.

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