The quantitative analysis of rutin in the roots of wild yams (Dioscorea caucasica)

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Abstract. The present study represents spectrophotometric method of rutin determination in the dry roots of wild yams (D. caucasica Lipsky), growing in Abkhazia. Obtained results have proven the presence and quantity of flavonoid rutin in the prepared extracts of this medicinal plant. The differential spectrophotometric method was used to confirm the presence of flavonoids and determine their amount in ethanol extracts from Dioscorea caucasica roots. Rutin was applied as a standard sample. A solution of aluminum chloride with rutin had a significant maximum at a wavelength of 0.576 nm. The present data have shown that flavonoids from wild yams roots formed a complex compound with an analytical maximum of intrinsic absorption at 0.565 nm in the aluminum chloride presence. A method of differential spectrophotometry has been developed for the quantitative analysis of the flavonoid rutin, which was found with value of 0.343 mg / g.

1. Introduction
Wild yam (Dioscorea) has long been used by native-born Americans due to curative properties, in particular as an expectorant, as well as a cure for intestinal cramps, rheumatic pain, and some gynecological symptoms, inclusive of dysmenorrhea and problems associated with the menstrual cycle, childbirth, and menopause [1].

In the 1960s, roots and tubers of yams (Dioscorea) were the primary sources of diosgenin used to synthesize progesterone, androgens, and cortisone. Currently, this plant is recommended for the prevention of various diseases, such as rheumatism, irritable bowel syndrome, diverticulitis, etc. However, the natural source of progesterone is found to be the most effective treatment of symptoms of menopause, dysmenorrhea, and menstrual cramps [2]. Diosgenin is considered to be more essential due to its active properties [3]. Other chemical compounds contained in yams are not so much of interest from point of view of pharmaceutical applications. There is a lack of studies concerning flavonoids content in Dioscorea caucasica.

Flavonoids are a widely distributed group of polyphenolic compounds with higher antioxidant activity. These properties can be applicable for human health maintenance, in particular, the usage active compounds have been found to include anticancer, antiviral, anti-inflammatory activities.

Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is one of the important flavonoids in plants, which has various pharmacological activities, such as antibacterial, antiprotozoal, antitumor, anti-inflammatory, antiallergic, antiviral, antiplatelet, antispasmodic, antihypertensive, and etc [4].

There are numerous studies are represented pharmacological properties in plants such as D.belophylla, D.versicolor, D.deltoides [5,6]. However, presented results in these papers do not
contain methodological details, that cause to difficult evaluation of obtained data.

A large number of studies were focused to find out the effective quantitative and qualitative methods of analysis to identify flavonoids, in particular, rutin in plant sources. For instance, the colorimetric method [7] and thin-layer chromatography (TLC) [8], which were used for qualitative analysis of flavonoids, were assessed as inadequate technics to explore this class of phenols [9]. High-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE), and micellar electrokinetic chromatography (MECC), are used extensively as robust methods due to their high efficiency to isolate and characterize bioflavonoids [10]. However, ultraviolet-visible spectroscopy is the most affordable, convenient and accurate process for quantifying bioflavonoids in plant extracts. This process is based on the complexation of the vicinal hydroxyl groups of bioflavonoids structures with aluminum chloride (AlCl₃) with subsequent spectrophotometric dimension due to bathochromic offset and hyperchromic impact resulting from the complex formation [11].

In contrast to cultivated tubers, little is known about the composition of Dioscorea caucasica roots, as well as methods to assess the amount of rutin in extracts.

The present paper reports the amount of rutin in the dry root of Dioscorea caucasica, the origin of which are mountain forests on the southern slopes of the Caucasian ridge in the Krasnodar Territory, as well as in northwestern Abkhazia.

2. Materials and methods

Preparation of yam extracts
Yams roots (D. caucasica Lipsky) were obtained from Abkhazia. Wild yams roots were assayed for rutin determination. Briefly, 100 g of finely ground raw material was extracted with a single extraction in 100 ml of 70% ethanol in a reflux condenser, with following shaking and heating for 30 minutes. After cooling to ambient temperature, the blend was filtered through filter paper (Whatman no. 4) into a 100 ml flask.

Determination of rutin content
20 ml of prepared extract was placed in a volumetric flask with a capacity of 25 ml by adding 5 ml of aluminum chloride (AlCl₃) solution with a mass fraction of 2% in 95% ethanol. After 30 minutes, the intrinsic absorption spectra of bioflavonoids of D. caucasica roots were analysed at 410 nm using a Shimadzu UV-2600 spectrometer. Differential spectrophotometry was applied for the quantitative determination of flavonoids in fresh extracts. The present method is based on absorbance spectrum of reaction products of with aluminium chloride. In order to compare, a standard solution that consisted of 20 ml of extraction was used, brought with ethyl alcohol with a volume fraction of 95% to the mark in a 25 ml volumetric flask. Standard solution of rutin was prepared for the expression of results by following: 0.05 g of rutin was dissolved in 100 ml of 95% ethanol and heated in a water bath, stirred and then cooled to room temperature.

Calibration tests were performed using three solutions with a content of 0.2, 0.5 and 1 ml of a standard rutin solution, which were mixed in a 25 ml volumetric flask with 5 ml of a 2% solution of AlCl₃ and ethyl alcohol.

3. Results and discussion
Phenolic compounds are poorly soluble in water and relatively thermostable. Therefore, water-alcohol extraction was used to determine bioflavonoid in the plant extract. The most effective and optimal ratio for solvent extraction was found with 70% ethanol, which represented better extraction of bioflavonoids.

The chemical structure of bioflavonoids is two benzene rings (ring A and ring B) that are connected to the ring C (the pyran heterocyclic system) (Figure 1). Flavonoids are divided into 9 classes
depending on the difference in the bond between the benzene rings and the degree of hydroxylation of the functional groups in the heterocyclic pyran system (ring C).

Figure 1. General chemical structure of flavonoids

Commonly, phenolic compounds have two absorption bands in the UV area of the spectrum. A significant absorption peak at 565 nm indicates the predominance of flavonols and flavones in the root extracts. The spectrophotometric test is one of the most commonly used processing, as well as one of the fastest and most affordable methods for the total flavonoid determination. The present method is based on optical density determination of the studied substances having a specific wavelength.

According to research referenced, the maximum of absorption spectrum was observed at 410-415 nm during the total determination of flavonoids content [12, 13]. In this case, qualitative reactions to the presence of rutin were carried out with AlCl₃ at an analytical wavelength of 410 nm in the obtained extract, which conforms to the maximum difference in the optical densities of the extract after the addition of AlCl₃ and standard solution.

In fact, those reactions to flavonoids are based on the formation of coloured complex compounds. Most commonly, assays for total flavonoid determination are reactions of complexes formation with AlCl₃ [14, 15]. The yellow discolouration was observed during mixing AlCl₃ with fresh extract of wild yams roots, that shows the presence of flavonoids.

A standard absorption rate was required to quantify the rutin flavonoid. Results in Table 1 are represented correlation of optical density (AlCl₃ solution) with rutin concentrations at a wavelength of 0.576 nm.

Table 1. Dependence of optical density (A) on the concentration of rutin in solution (mg / ml)

| The concentration of rutin, mg / ml | Optical density, A |
|-------------------------------------|-------------------|
| 0.004                               | 0.180             |
| 0.01                                | 0.308             |
| 0.02                                | 0.579             |

Calculation of the flavonoid quantity of rutin was assayed according to a calibration curve, which is expressed optical density dependence on the concentration of rutin in solution (Figure 2).

UV spectra of complex compounds in extracts of wild yams roots (*Caucasian dioscorea*) were studied the quantity of rutin.

The presence of a larger number of benzene rings in the flavonoid molecules determines the ability of the filtrate to absorb a certain wavelength of optical density and allows to set their number. In the present study, the absorption peak of the complex compound of rutin flavonoid in *Dioscorea caucascica* was at 0.565 nm in the presence of aluminum chloride. Obtained results showed, that rutin concentration in the root extract was 0.019 mg / ml, and flavonoid concentration in 1 gram in terms of dry matter was 0.343 mg/g.
4. Conclusion

The method for the quantitative analysis of the rutin flavonoid in the dry root of wild yams (*Dioscorea caucasica*) was developed using differential spectrophotometry, which is considered to be the fastest and most affordable method for flavonoids determination. It was found that a bathochromic shift of the absorption band of flavonoids, in particular rutin, is formed during the formation of flavonoids complexes with aluminum chloride (AlCl₃), which can be determined in the UV spectrum as the maximum absorption in the range of 560–580 nm. This area of the spectrum is relatively distant from the absorption maxima of other compounds present, that made it possible to make the quantitative determination of rutin flavonoid the most selective.

According to the results of the present study, 1 gram of crushed dry root contains 0.343 mg / g of rutin flavonoid. To our best knowledge, the presented concentration can be considered insignificant, but its presence suggests the potential use of medicinal plant (*Dioscorea caucasica*) for the extraction of rutin, which belongs to the vitamin P group and plays a significant role in human health.

References

[1]  Bhandari M R and Kawabata J 2004 *Food Chemistry* 88 163–8
[2] Narula A, Kumar S and Srivastava P S 2007 *Biotechnol. Lett.* 29 623–9
[3] Patel K, Gadewar M, Tahilyani V and Patel D K 2012 *Nat. Prod. Bioprospect.* 2 46–52
[4] Ganeshpurkar A and Saluja A K 2017 *Saudi Pharmaceutical Journal* 25 149–64
[5] Poomima G and Ravishankar R 2009 *African Journal of Biotechnology* 8
[6] Bhandari M 2003 *Food Chemistry* 82 619–23
[7] Naczek M and Shahidi F 2006 *Journal of Pharmaceutical and Biomedical Analysis* 41 1523–42
[8] Stalikas C D 2007 *J. Sep. Sci.* 30 3268–95
[9] Ignat I, Volf I and Popa V I 2011 *Food Chemistry* 126 1821–35
[10] Zhang S, Dong S, Chi L, He P, Wang Q and Fang Y 2008 *Talanta* 76 780–4
[11] Fernandes A J D, Ferreira M R A, Randau K P, de Souza T P and Soares L A L 2012 *The Scientific World Journal* 1–7
[12] Pękal A and Pyrzynska K 2014 *Food Anal. Methods* 7 1776–82
[13] Eghdami A and Sadeghi F 2010 *Org. Chem. J.* 2
[14] Mazandarani M and Ghafourian M 2017 *Trends in Phytochemical Research* 1 33–8
[15] Bilto Y 2015 *International Journal of Science and Research (IJSR)* 4 197–200