METHODS

A New Method for Express Analysis of Flavonoid Fraction in Nephrophite Plant Extract and Prediction of Its Antioxidant Effects

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 6, pp. 715-717, June, 2006
Original article submitted October 12, 2005

A new chromatographic procedure is proposed for rapid (35 min) complete fractionation of flavonoids in plant raw material; the method well discriminates between components with antioxidant activity (rutin, apigenin, quercetin, hesperidin). The method is based on reverse-phase high performance liquid chromatography using ODS-S5CN carrier and linear 10-60% pyridine elution gradient.

Key Words: *flavonoids; antioxidants; Nephrophite; high pressure liquid chromatography*

Antioxidant characteristics of extracts from medicinal plants and complex plant preparations are used with good effect for the treatment of toxic nephropathies [1], untimely myocyte aging syndromes [5], side effects of antibiotics, phenylcyanocreatine phosphate derivatives [3], and hydroxythiasoline immunosuppressors [6]. These effects are determined by the presence of oligo/polyphenol [1,3] and flavonoid [5,13] components in extracts, serving as "traps" for free radicals and peroxide ions [5] and as competitive inhibitors of FAD (FMN)-dependent dehydrogenases [6,13].

Material obtained *in vitro* (purified enzymes, isolated mitochondria, microsomes, and peroxisomes) helps to evaluate the antioxidant potential of the majority of the most prevalent flavonoids from medicinal plants [5,6,12-14], this rendering special significance to the data on the composition of the flavonoid pool [14]. This circumstance necessitates the development of reliable and simple (better single-stage) laboratory procedures for fractionation, identification, and measurement of the studied group of bioactive substances. Creation of this method is an important task for studies of the Nephrophite plant composition, whose antioxidant properties were described previously [1].

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MATERIALS AND METHODS

Measurement of the summary flavonoid fraction in Nephrophite complex preparation was carried out by standard O'Farrell method using aluminum chloride treatment of 95% ethanol extract and plotting the calibration curve of rutin ethanol solution (Bio-Rad) spectrophotometry at λ=414 nm [9]. The total content of flavonoids in the studied preparation in conversion to reference rutin was 2.86±0.31% (by weight; n=6).

Flavonoids were extracted with 95% ethanol (1:10) at room temperature for 6 h, after which the sample was concentrated to 2-3 ml in a rotor evaporator. Fractionation was carried out in an Altex-2000BE column (1.2×18.0 cm) with the stationary phase ODS-S5CN, at ambient temperature and pressure of 2000 p.s.i. Linear 10-60% pyridine concentration gradient in 10% methanol was delivered to the column for the mobile phase. Shimadzu

LD600-2XT high pressure liquid chromatograph was used in all cases. Calibration of the columns was carried out using pure flavonoid markers (RJ-410 Kit; Bio-Rad); this was the initial stage of fraction identification. Precise identification of the fractions was carried out by field ionization mass-spectrometry (Varian RX4-MS Analytic System; Varian) and Sigma-2000-BioData library with subsequent processing in an Olivetti QW410-Orion digital analyzer (Olivetti) [11].

The content of fractions was determined planimetrically by computer analysis of chromatogram profiles recorded by UV light absorption at λ=280 nm in the Shimadzu—AC2E block. In order to select the optimal fractionation protocol, each variant was tested 5-6 times.

RESULTS

Our result was characterized by 100% reproducibility and optimal separation of the components (Fig. 1).

Apigenin, rutin, quercetin, and hesperidin make the greatest contribution to the composition of the flavonoid pool in the studied complex preparation (26.8±4.3, 18.8±4.8, 16.3±2.8, and 12.4±1.8%, respectively). These components exhibited pronounced and selective antioxidant effect in L-cell culture [7], in experiments on perfused myocardium under conditions of hypoxia [4], on mammalian lymphoid tissue cells after a single exposure to high-dose γ-radiation [12], and stabilized the membranes in cells with low oxygen and glucose deficiency tolerance [4,5,12].

We suggest pyridine elution gradient for fractionation of flavonoids by reverse phase HPLC. Our method differs significantly from few analogs: apigenin and quercetin, luteolin and gallic acid fractions can be separated (some authors failed to attain these results [10]), and all the main flavines can be separated in one experiment taking (together with extraction) no more than one working day. This circumstance is an important feature of our method, because, according to published data, complete separation of 12 tea leaf flavonoids required multistaged chromatographic purification and fractionation schemes [8]. The method is simple (single-staged), its resolving capacity is high (Fig. 1), due to which it stands apart from laboratory technologies used for studies of natural flavonoid compositions [2,8,10].

Hence, this method can be effectively used in plant biochemistry and physiology, pharmaceutical chemistry, pharmacology, theoretical chromatography and bioorganic chemistry.

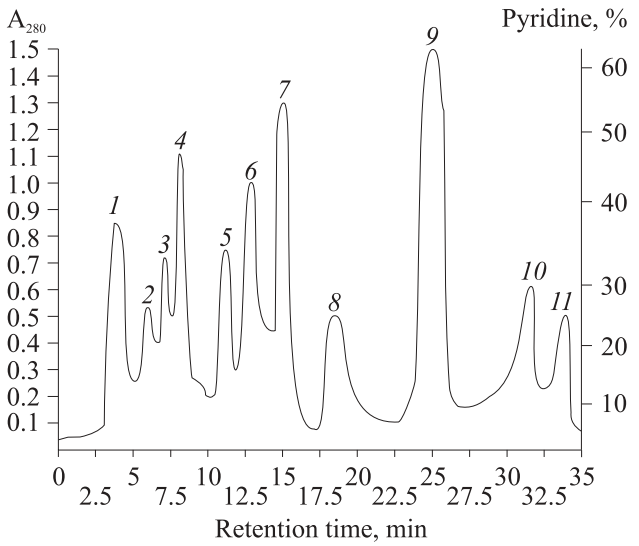

Fig. 1. Percentage of flavonoid components in Nephrophite dry extract according to reverse phase HPLC (ODS-S5CN/10-60% linear pyridine elution gradient). *1*) arbutin; *2*) gallic acid; *3*) caffeic acid; *4*) luteolin; *5*) chlorogenic acid; *6*) luteolin-7-glycoside; *7*) hesperidin; *8*) hyperoside; *9*) rutin; *10*) apigenin; *11*) quercetin.

The authors are grateful to Professor D. A. Kuznetsov, Doct. Biol. Sci., leading scientist of Institute of Chemical Physics, Russian Academy of Sciences, for valuable discussion of our work.

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