Determination of caffeic acid in root and rhizome of Black cohosh (*Cimicifuga racemosa* (L.) Nutt.)

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ARTICLE INFO	ABSTRACT
	Cimicifuga racemosa, is a plant with a diverse and long history of medicinal use. Caffeic acid, bioactive compound, which often occurs with other polyphenols can influence the biological activity of this plant. The aim of our work was quantitative analysis of caffeic acid in roots and rhizomes of two varieties of *C. racemosa*. Analysis was performed by HPLC method. The extracts were separated on C18 reversed-phase column using mixture of methanol, water and formic acid (25:75:0.5 v/v/v) as a mobile phase. The flow rate of eluent was 1.0 ml·min⁻¹. The obtained validation parameters such as linearity, linear regression equation and precision expressed as a relative standard deviation were adequate for quantitative determination. Caffeic acid was found in all tested extracts. The highest total amount of caffeic acid was determined in *C. racemosa var. racemosa* (255.3 µg·g⁻¹) while its concentration in *C. racemosa var. cordifolia* was significantly lower (213.0 µg·g⁻¹).

INTRODUCTION

Phenolic compounds are secondary metabolites naturally present in nearly all plant materials, mostly in fruits, vegetables, grains and in food products of plant origin such as tea, wine or coffee [2]. Caffeic acid (3,4-dihydroxycinnamic acid) (Fig. 1) belonging to hydroxycinnamic acid derivatives is one of the most known plant polyphenol. This compound has a broad spectrum of pharmacological activities including anti-inflammatory, antibacterial, anti-tumor, antiviral and immunomodulatory effects [5,10,12,13]. Its antioxidant and neuroprotective properties have also been proven. Pharmacological studies have shown that caffeic acid protects the brain against oxidative brain damage induced by hydrogen peroxide [1,6] and might protect against cardiovascular diseases [8].
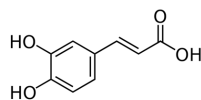

Figure 1. Caffeic acid

Cimicifuga racemosa (L.) Nutt. (*Actaea racemosa,* also known as black cohosh), is a perennial herb belonging to the buttercup family (*Ranunculaceae*) [3]. The roots and rhizomes of *C. racemosa* are traditionally used to treat a variety of disorders, e.g. abnormalities in kidney function, malaria, rheumatism, general malaise, sore throat, menstrual irregularities and for the relief of menopause-related symptoms [4,7]. Clinical studies suggest that black cohosh is effective in relieving emotional symptoms, especially depression or anxiety, and hot flushes during menopause [3]. The chemical constituents of *C. racemosa* include two classes of secondary metabolites: triterpene glycosides and phenolics compounds [4]. Triterpenes (fraction specified name cimicifugin) and isoflavones are mainly responsible for estrogenic activity. However, phenolic compounds, e.g. phenolic acids can also influence on biological activity of black cohosh.

The aim of our work was quantitative analysis of caffeic acid in roots and rhizomes of black cohosh and comparison of its content in two varieties: *C. racemosa (L.) Nutt. var. racemosa* and *C. racemosa (L.) Nutt. var. cordifolia (Pursh) Gray*.

MATERIALS AND METHODS

Chemicals and reagents. HPLC-grade methanol was purchased from Merck (Darmstadt, Germany). Water for chromatography was deionized and purified by ULTRA-PURE Milipore Direct-Q® 3UV-R (Merck, Darmstadt, Germany). Caffeic acid standard was from Sigma (St. Louis, MO, USA).

Standard and sample preparation. Caffeic acid standard was accurately weighted (1.25 mg) and dissolved in 25 mL

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of methanol (final concentration was 50 µg·mL⁻¹). Standard solutions were prepared by dilution of stock solution in methanol to appropriate concentrations.

Rhizomes and roots *of C. racemosa var. racemosa* and *C. racemosa var. cordifolia* were collected in Botanical Garden of Maria Skłodowska-Curie University (UMCS) of Lublin (September 2013) and next, dried and pulverized. 1 g of each plant material was weighed for the research. Samples were extracted with 20 mL of methanol in ultrasonic bath (3 × 15 min). The combined extracts were concentrated to 10 mL.

Chromatography. Chromatographic determination was performed on VWR Hitachi Chromaster 600 chromatograph (Merck, Darmstadt, Germany) with pump (5160), a degasser, thermostat (5310), autosampler (5260), DAD detector (5430) and EZChrom Elite software.

The extracts were analyzed on C18 reversed-phase column (25 cm × 4.0 mm i.d., 5 µm particle size), LiChrospher 100 (Merck), at temperature of 25°C. Twenty µL of each sample was injected. Mixture of methanol, water and formic acid (25:75:0.5 v/v/v) was used as a mobile phase. The flow rate of eluent was 1.0 ml·min⁻¹. The data were collected in the range of wavelength from 200 to 400 nm.

RESULTS AND DISCUSSION

Many factors can affect the amount of biologically active compounds in plants, e.g. composition of soil, environmental conditions, as well as a time of harvest. In our research, the content of caffeic acid in two varieties of C. *racemosa* harvesting in the same period and growing under the same conditions was determined.

The extraction conditions for isolation of caffeic acid and chromatographic conditions of separation of extracts from roots and rhizomes of *C. racemosa* were established on the basis of literature [9]. Phenolic acids are polar group of plant metabolites, thus the mobile phases containing methanol or acetonitrile with high amount of water, from 75 to 85% are recommended for their analysis [11,14]. In our experiments, eluent consisting of methanol and 75% of water enabled the appropriate separation of investigated compound from the other components of extract (Fig. 2). The small addition of formic acid was necessary to improve the shape of chromatographic peaks. Caffeic acid was identified by comparison of retention time (t$_R$ = 11.13 ± 0.06 min) and obtained UV spectrum with standard (Fig. 3).
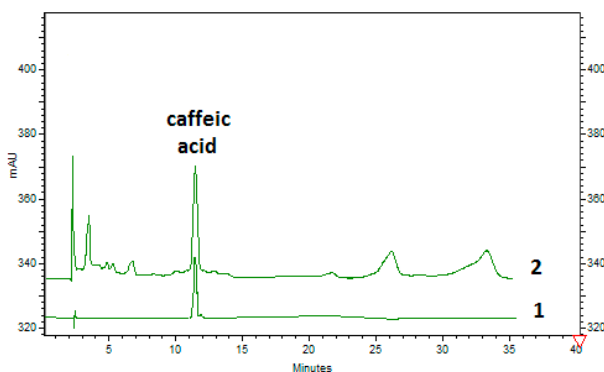
Figure 2. Chromatogram: 1 – standard of caffeic acid, 2 – *Cimicifuga racemosa var. cordifolia* rhizome extract
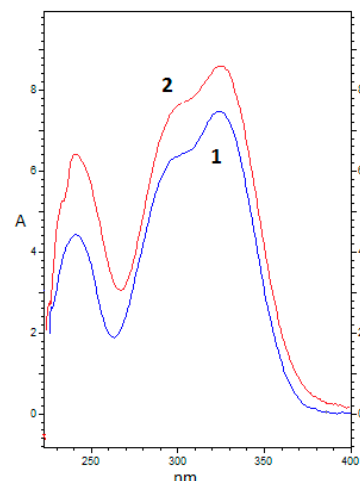
Figure 3. Spectrum: 1 – standard of caffeic acid, 2 – caffeic acid from *Cimicifuga racemosa extract*

The quantitative analysis was performed at λ = 320 nm. The amounts of caffeic acid in extract samples were calculated from the calibration plot. Five standard solutions in concentrations: 5.0, 12.5, 25, 37.5 and 50 µg·µL⁻¹ were used for the construction of calibration curve (n = 3). The quantification was conducted on the basis of linear regression least square model. The obtained parameters such as linearity (r = 0.9998), linear regression equation (y = 412357x - 674814) and precision expressed as a relative standard deviation (RSD range: 0.54-1.66%) were sufficient for quantitative determination.

Investigated compound was found in all tested extracts. However, it can be seen that the amount of caffeic acid strongly depends on the variety of plant. Only slight difference of CA content was noted in rhizomes of both varieties, the amount of CA was only about 3.5% higher in *var. cordifolia*. However, its concentration determined in root of *var. cordifolia* was significantly lower (approximately 41.8%). As our experiments showed, both of the varieties had tendency to accumulate CA in rhizomes. Moreover, the *var. racemosa* produced the higher total amount of caffeic acid. The results of quantification are presented in Table 1.

Table 1. The results of caffeic acid quantification in *Cimicifuga racemosa* extrats

Plant material	Caffeic acid content (µg·g⁻¹ of dry plant material)		
	rhizome ± SD	root ± SD	total amount
C. racemosa var. racemosa	141.7 ± 2.1	113.6 ± 2.4	255.3
C. racemosa var. cordifolia	146.9 ± 0.4	66.1± 1.2	213.0

SUMMARY

The caffeic acid content was investigated in roots and rhizomes of two varieties of C. *racemosa*. The chromatographic system consisting of methanol, water and formic acid (25:75:0.5 v/v/v) provided its appropriate separation from the other components in analysed samples. The highest total amount of caffeic acid was determined in C. *racemosa var. racemosa* (255.3 µg·g⁻¹) while its concentration in C. *racemosa var. cordifolia* was significantly lower (213.0 µg·g⁻¹). This compound can influence the biological activity of investigated plant extracts.

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