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Research Article

HPLC-DAD analysis of flavonoids and hydroxycinnamic acids in *Aster novi-belgii* L.

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Abstract

Aster novi-belgii is a perennial ornamental herb native to eastern Canada and the United States of America, cultivated in Ukraine. This species should be considered a possible source of phenolic compounds, principally hydroxycinnamic acids and flavonoids. Therefore, in this study, the aim was to determine these compounds in *Aster novi-belgii* by HPLC-DAD analysis, and validation of this chromatographic method and lay a scientific and technical basis for the utilization and development of the plant resources of the cultivated plants of the genus *Aster*. The HPLC-DAD method determined the flavonoids and hydroxycinnamic acids composition and content in the herb of *Aster novi-belgii* L. The HPLC-DAD method allowed the detection of 13 phenolic compounds, namely 6 hydroxycinnamic acids (chlorogenic, sinapic, caffeic, syringic, trans-cinnamic, trans-ferulic acids), and 7 flavonoids (kaempferol 3-O-beta-D-glucoside, naringin, quercetin, luteolin, rutin, kaempferol, rhamnetin). The quantitative detection showed that the main hydroxycinnamic acids were chlorogenic acids (15069.21 ± 0.34 μg/g) and sinapic acids (949.95 ±0.22 μg/g). Concerning flavonoids, the largest amounts were kaempferol 3-O-beta-D-glucoside (8989.79 ±0.31 μg/g) and naringin (2092.02 ± 0.26 μg/g). HPLC-DAD method was evaluated in terms of linearity, precision, accuracy, limits of quantification, and limits of detection. The calibration curves of reference substances were linear ($R^2 \geq 0.997$), the LODs were in the range of 0.21–1.71 μg/mL, and the LOQs – of 0.48–5.19 μg/mL, respectively. Our phytochemical research confirms that the study material is a rich source of hydroxycinnamic acids and flavonoids. Findings mean that *Aster novi-belgii* is a promising plant because of the important role of these phenolic compounds in many biological processes.

Keywords

HPLC-DAD, flavonoids, hydroxycinnamic acids, phenolic compounds, *Aster novi-belgii*

Introduction

Concerns over possible adverse health effects of commonly used synthetic antioxidants have driven research interests toward finding antioxidants from natural sources, mainly from widely consumed foods (Hawke 2013; Damašius et al. 2014; Marchyshyn et al. 2021c). Plants constitute one of the most valuable sources of natural antioxidants and other health-promoting phytochemicals (Šulniute et al. 2017; Tzima et al. 2018; Marchyshyn et al. 2021a; Slobodianiuk et al. 2022). According to WHO statistics, up to 80% of the world's population prefers drugs of natural origin. In particular, herbs have shown antioxidant solid activities due to their high content of phenolic compounds

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(Babovic et al. 2010; Budniak and Vasenda 2021). As ubiquitous constituents of most medicinal plants, phenolic compounds have been the subject of endless studies describing a variety of health effects like an antioxidant, cardiovascular protectors, and antitumoral effects (Quideau et al. 2011; Falcone Ferreyra et al. 2012; Zhang et al. 2016).

Polyphenols are a group of small organic molecules synthesized by plants as secondary metabolites. Among these compounds, special attention has been given to the most prominent classes of polyphenols, namely flavonoids and hydroxycinnamic acids in plants of medicinal importance as antioxidant, anti-inflammatory, antifungal, or antibacterial compounds (Quideau et al. 2011; Feshchenko et al. 2021b; Slobodianiuk et al. 2021a).

For example, Asteraceae, one of the largest herbal families worldwide (1300 genera and approximately 21000 species) (Hawke 2013), has been the subject of numerous studies that demonstrated the high radical scavenging capacity (RSC) of its extracts.

Aster is a large genus of the family Asteraceae comprising more than 200 species distributed around the world (Yu et al. 1997). *Aster* is a genus of perennial flowering plants. Many *Aster* plants are ornamental and have been used in traditional medicine for the treatment of fever, cold, tonsillitis, snake bite, and bee sting (Shao et al. 1995). The genus *Aster* species have been widely used for thousands of years in the Qinghai-Tibetan plateau for the clearing of heat, detoxification, and the treatment of seasonal pandemic diseases (Li et al. 2022). Phytochemical analysis of *Aster* spp. evidenced that these species are characterized by different classes of secondary metabolites, including phenylpropanoids (Liu et al. 2010) caffeoylquinic acids (Nugroho et al. 2009), and saponins (Corea et al. 2004). These compounds are reported to exert valuable pharmacological activities including hemolytic, anticholesterolemic, immunostimulatory, vaccine adjuvant, and anticarcinogenic properties (Sparg et al. 2004).

Aster novi-belgii is an ornamental herbaceous perennial native to the eastern United States and Canada (Kristiansen et al. 1997; Ibrahim et al. 2006). The native species of North America, *Aster novi-belgii* L. has been introduced and naturalized in most parts of Europe, including Ukraine. In Ukraine, it is cultivated as an ornamental plant. The *Aster novi-belgii* is one of the least understood plants of the groups in the *Aster* genus. In this work, we report a phytochemical analysis of *Aster novi-belgii*, which, to the best of our knowledge, has no citations in the literature, showing the investigation of flavonoids and hydroxycinnamic acids.

The pharmacological effect of the *Aster novi-belgii* is caused by a complex of biologically active substances, the composition of which has not yet been studied in Ukraine. The primary focus of this study is to establish a platform for the analysis of identified flavonoids and hydroxycinnamic acids from *Aster novi-belgii*. The objective is to provide preliminary data for a comprehensive HPLC-DAD analysis of these compounds in *Aster novi-belgii* and to lay a scientific and technical basis for the utilization and development of the plant resources of the cultivated plants of genus *Aster*.

Materials and method

Plant material

Aster novi-belgii L. herb was selected as the object of study. Plant raw material was collected at the M. M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Marchyshyn et al. 2021d; Slobodianiuk et al. 2021b) in the summer of 2021. The raw material was authenticated by Prof. Svitlana Marchyshyn Department of Pharmacognosy and Medical Botany (TNMU, Ternopil, Ukraine) (Budniak et al. 2021c; Marchyshyn et al. 2021b). The voucher specimen of herbal raw material has been deposited in the departmental herbarium for future records. The study plant material was dried using the conventional oven with the forced air circulation method and stored in paper bags in a dry place (Budniak et al. 2021b; Feshchenko et al. 2021a).

Standards and chemicals

High-performance liquid chromatography (HPLC)-grade acetonitrile, trichloroacetic acid, and methanol (E. Merck, Darmstadt, Germany) were used for the HPLC analysis. Deionized water was purified using a Milli-Q system (Millipore, Bedford, MA, USA) (Budniak et al. 2022). Standards of kaempferol, kaempferol 3-O-beta-D-glucoside, naringin, luteolin, rhamnetin, quercetin, rutin, chlorogenic acid, caffeic acid, *trans*-ferulic acid, syringic acid, sinapic acid, and *trans*-cinnamic acid, were of primary reference standard grade (≥ 96% purity HPLC) and were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA).

High-performance liquid chromatography-diode array detection analysis

High-performance liquid chromatography-diode array detection (HPLC DAD) analysis was performed using an Agilent Technologies 1200 (USA) equipped with a G1311A quaternary pump, a G1315B diode array detector, a G1313A autosampler, and a G1316A column compartment (Pyrzynska and Sentkowska 2019). Data acquisition was done using copyrighted Agilent Technologies software.

Separation was performed using a Zorbax SB-C18 column (5 µm, 150 mm × 4.6 mm, i.d., Agilent, USA). The temperature of the column was set at 30 °C and the flow rate was 1.0 ml/min, 20 µl of injection. Mobile phase A – 0.1% trichloroacetic acid, mobile phase B – acetonitrile was used as shown in Table 1.

Table 1. HPLC-DAD gradient solvent system for hydroxycinnamic acids and flavonoids separation.

Time/min	Solvent A (%)	Solvent B (%)
0–2	98	2
2–25	90	10
25–40	85	15
40–48	80	20
48–68	75	25

Peak purity and absorbance were automatically detected by a DAD (UV–Vis) detector at 200–400 nm. Purified fractions of hydroxycinnamic acids and flavonoids were identified by matching retention time and spectra of standards with unknown peaks. External standards were used for the identification and quantification of compounds (Kuppusamya et al. 2018).

Extraction procedure

The sample of herbal raw materials was ground into a powder by a laboratory mill, and then about 400 mg (accurately weighed) was selected and placed into a flask with 10 ml of 60% methanol (v/v). The extraction was carried out in an ultrasonic water bath at 80 °C for 4 hours. The resulting extract was centrifuged at 3000 rpm and filtered through disposable membrane filters with pores of 0.22 μm (Sumere et al. 2018).

Stock solutions

Quantification of hydroxycinnamic acids and flavonoids was performed by using different external standards. Stock solutions of hydroxycinnamic acids and flavonoid standards were prepared at 1 mg/ml after dissolving in DMSO and methanol, respectively. For quantification of hydroxycinnamic acids, four mass concentrations (1, 0.5, 0.25, and 0.125 mg/ml) were prepared separately for chlorogenic acid, caffeic acid, *trans*-ferulic acid, syringic acid, sinapic acid, and *trans*-cinnamic acid. They were then mixed to make a final concentration of 1, 0.5, 0.25, or 0.125 mg/ml. Likewise, four concentrations (1, 0.5, 0.25, and 0.125 mg/ml) of flavonoid standards such as kaempferol, kaempferol 3-O-beta-D-glucoside, naringin, luteolin, rhamnetin, quercetin, rutin were prepared separately. They were then mixed to obtain a final concentration of 1, 0.5, 0.25, or 0.125 mg/ml for all standards. Then 10 μl of the sample was analyzed in the HPLC-DAD system.

Method validation

Limits of quantification (LOQ) and limits of detection (LOD) were calculated for each sample in triplicates (Budniak et al. 2021a). Calibration curves were obtained consecutively by plotting concentration against peak area. The mean of the slope (S) and standard deviation of intercept (σ) were calculated from the standard curve of three replicates. LOD and LOQ were calculated with the following equations: LOD = 3.3 * s / Slope; LOQ = 10 * s / Slope. The accuracy was determined through recovery (%) by preparing two different concentrations (1 and 10 mg/ml) of reference standard with 10 mg of *Aster novi-belgii* sample. The percentage of recovery of each standard was calculated based on the ratio of the standard concentration after and before HPLC (changed amount minus original amount).

A standard curve was constructed for each external standard by plotting the concentration of the standard (mg/ml) against the peak area at a specific wavelength. All external standards and samples gave almost linear calibration curves through zero points.

Method linearity was calculated by plotting peak area ratio (A) vs. analyte concentration (C in mg/ml) to obtain calibration curves (Zhang et al. 2013).

Linearity testing was repeated with the same samples after a complete restart of the system with removal and re-installation of the column. Repeatability precision was determined by five-fold injection of the same sample in a row in a day. For the resulting relative peak area the relative standard deviation (RSD) was calculated. To determine intra-day precision, three standard preparations of each reference standard with the same concentration were single injected and the resulting relative peak areas were used to calculate the RSD. Inter-day precision for the day of sample preparation and the two following days was specified by injecting three standard samples of each CRS solution once each on all three days. RSD values for all retention times ranged from 0.44–1.68.

Linearity was performed by injecting a series of standard solutions of each chemical reference substances (50–300 μg/mL). As can be seen in Table 2, the linearity of HPLC-DAD method was good, since R^2 was in the range of 0.997–0.999.

Table 2. Validation parameters for HPLC-DAD method.

Compound	Correlation coefficient R^2	Recovery, %	Limit of detection LOD, μg/ml	Limit of quantification LOQ, μg/ml
luteolin	0.999	95.18	1.71	5.19
quercetin	0.997	90.08	0.39	1.20
rutin	0.998	93.98	1.02	3.10
kaempferol	0.999	90.53	0.21	0.65
kaempferol 3-O-beta-D-glucoside	0.998	91.02	0.44	1.36
naringin	0.999	96.87	1.60	4.86
rhamnetin	0.999	90.63	0.67	2.05
chlorogenic acid	0.998	92.14	1.33	4.04
caffeic acid	0.997	94.42	1.15	3.48
sinapic acid	0.999	90.26	0.15	0.48
syringic acid	0.998	94.45	0.38	1.16
trans-ferulic acid	0.999	90.63	0.75	2.28
trans-cinnamic acid	0.998	99.98	1.06	3.21

Results and discussion

The reverse phase HPLC-DAD analysis of the *Aster novi-belgii* L. herb shows the presence of some phenolic compounds (Table 3). The HPLC chromatogram of hydroxycinnamic acids of the *Aster novi-belgii* L. herb is specified in Fig. 1, of flavonoids in Fig. 2. Thirteen phenolic compounds were detected in the raw material, namely: hydroxycinnamic acids (chlorogenic, caffeic, syringic, *trans*-ferulic, sinapic, *trans*-cinnamic acids), and flavonoids (rutin, kaempferol 3-O-beta-D-glucoside, naringin, quercetin, luteolin, rhamnetin, kaempferol).

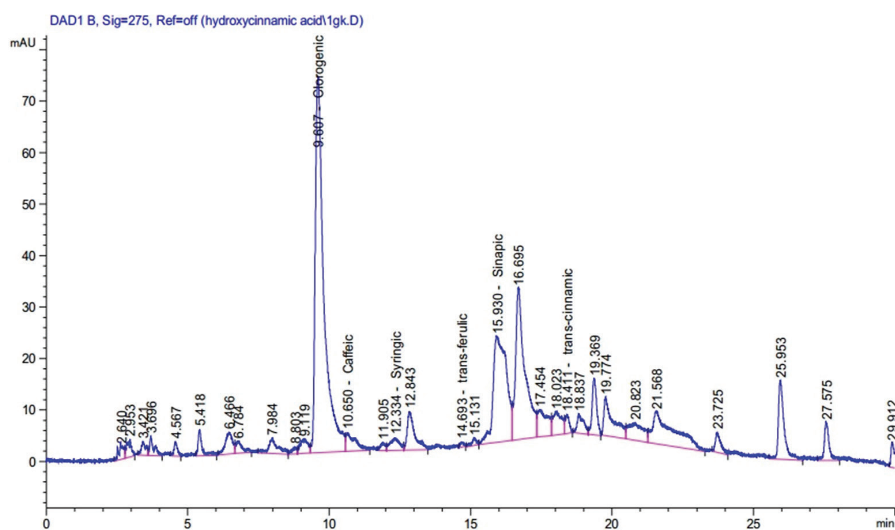

Figure 1. HPLC-DAD chromatogram of hydroxycinnamic acids identified in *Aster novi-belgii* L.

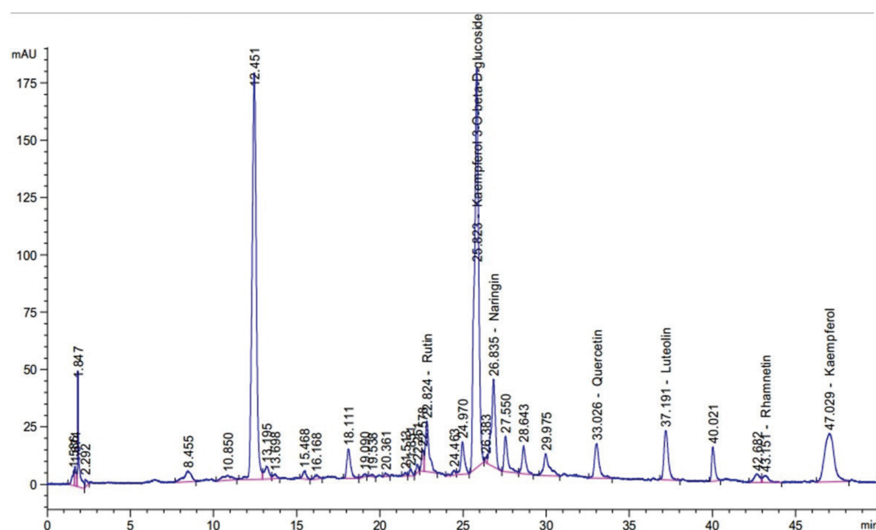

Figure 2. HPLC-DAD chromatogram of flavonoids identified in *Aster novi-belgii* L.

The quantitative content of hydroxycinnamic acids and flavonoids as determined by the HPLC method in *Aster novi-belgii* raw material is presented in Table 3. The highest content among hydroxycinnamic acids and the most abundant was chlorogenic acid (15069.21 µg/mg). Chlorogenic acid (known as 3-(3,4-dihydroxycinnamoyl) quinic acid) is one of the most available molecules concerning other phenolic acids due to its abundance in different foods (Farah and Lima 2019; Contardi et al. 2021). Chlorogenic acid is an important and biologically active dietary polyphenol, playing several important and therapeutic roles such as antioxidant activity, hepatoprotective, antibacterial, anti-inflammatory, cardioprotective, antipyretic, antiobesity, neuroprotective, antiviral, antihypertension, antimicrobial, free radicals scavenger, and a central nervous system stimulator (Naveedab et al. 2018). It is thought that chlorogenic acid can perform decisive roles in lipid and glucose metabolism regulation and thus help to treat many disorders such as cardiovascular disease, hepatic steatosis, diabetes, and obesity as well (Santana-Gálvez et al. 2017).

Table 3. Results of HPLC-DAD analysis of phenolic compounds in *Aster novi-belgii* L.

No.	Retention time	Common name of identified compound	Quantitative content of phenols, µg/g
1	9.61	chlorogenic acid	15069.21 ±0.34
2	10.65	caffeic acid	262.22 ±0.12
3	12.33	syringic acid	88.23 ±0.11
4	14.69	*trans*-ferulic acid	20.37 ±0.09
5	15.93	sinapic acid	949.95 ±0.22
6	18.41	*trans*-cinnamic acid	24.74 ±0.11
7	22.82	rutin	727.79 ±0.23
8	25.82	kaempferol 3-O-beta-D-glucoside	8989.79 ±0.31
9	26.84	naringin	2092.02 ±0.26
10	33.03	quercetin	822.34 ±0.18
11	37.19	luteolin	787.91 ±0.15
12	43.15	rhamnetin	377.93 ±0.11
13	47.03	kaempferol	493.10 ±0.17

Among hydroxycinnamic acids also the most abundant was sinapic acid (949.95 µg/mg). Sinapic acid is common in the plant world (vegetables, fruits, cereal grains, and medicinal plants) and as such is common in the human

diet. Sinapic acid shows antimicrobial, antioxidant, anticancer, and anti-inflammatory activity. 4-Vinylsyringol (a decarboxylation product of sinapic acid) is a potent antioxidative agent which suppresses carcinogenesis and the induction of inflammatory cytokines (Nićiforović and Abramovič 2014).

Flavonoids are an important group of secondary metabolites and a source of bioactive compounds in plants (Ghasemzadeh and Jaafar 2013). Table 3 shows the concentration of flavonoids in *Aster novi-belgii* L. The highest amount of flavonoids detected was kaempferol 3-O-beta-D-glucoside (8989.79 μg/mg), and naringin (2092.02 μg/mg). Kaempherol-3-O-β-d-glucoside, known as astragalin, is one of the flavonoids found in a variety of plants. It has antioxidant, anticancer, anti-inflammatory, and anticoagulant activities (Jun et al. 2011). It has also been reported that astragalin could inhibit the production of prostaglandin and angiotensin-converting enzyme activity, increase endogenous estrogen and progesterone, and reduce inflammation induced by lipopolysaccharide by inhibiting the signal pathway of NF-κB (Desai et al. 2014; Zhang et al. 2017). A literature survey has revealed that naringin has antioxidant, anti-apoptotic, anti-inflammatory, anti-osteoporotic, anti-ulcer, and anti-carcinogenic properties (Wang et al. 2013; Chen et al. 2016).

Conclusions

As omnipresent constituents of most medicinal plants, polyphenols have been the subject of endless studies describing a variety of health effects like cardiovascular protectors, an antioxidant, and antitumor effects. The present describes the investigation of particular members of the two most prominent classes of polyphenols, namely flavonoids and hydroxycinnamic acids in the herb of *Aster novi-belgii* L. The HPLC-DAD assay of phenols revealed that *Aster novi-belgii* represents important sources of bioactive compounds with a wide range of pharmacological activities. The quantitative detection showed that the main hydroxycinnamic acids chlorogenic acids and sinapic acids. Regarding flavonoids, the largest amounts were kaempferol 3-O-beta-D-glucoside and naringin. The obtained results will be useful in the development of quality control methods for *Aster novi-belgii* herb and the manufacture of drug preparations on its basis.

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