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UPLC-PDA-Q/TOF-MS identification of bioactive compounds and on-line UPLC-ABTS assay in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) leaves and rhizomes grown in Poland

Sabina Lachowicz[1] · Jan Oszmiański[2] · Aneta Wojdyło[2] · Tomasz Cebulak[3] · Lidia Hirnle[4] · Maciej Siewiński[5]

Abstract

The activity of polyphenolic compounds, triterpenoids, carotenoids, chlorophylls and antioxidants in leaves and rhizomes of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) grown in Poland was investigated. Leaves and rhizomes were assessed for the presence of bioactive compounds with the ultra-performance liquid chromatography photodiode detector-quadrupole/time-of-flight mass spectrometry (UPLC-PDA-Q/TOF-MS) method, and for antioxidant activity with the on-line UPLC-ABTS screening. Forty-six polyphenolic compounds (15 phenolic acids, 12 flavones and flavonols, 11 flavan-3-ols and 8 stilbenes), were identified in *Fallopia japonica and Fallopia sachalinensis*. Furthermore, accurate mass measurement technique was for the first time in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) in leaves and rhizomes it identified 25 new compounds belonging to carotenoids (9), chlorophylls (13) and triterpenoids (3) as well as rated the antioxidant properties of each polyphenolic compound. Major qualitative differences were found in the profiles. The leaves and rhizomes were found to be a good source not only of (average 20408.18 and 2716.42 mg/100 g dm), but also chlorophylls (average 179.97 and 43.82 mg/100 g dm), carotenoids (average 100.23 and 53.25 mg/100 g dm) and triterpenoids (average 580.87 and 434.05 mg/100 g dm). The content of bioactive compounds in *Fallopia japonica* Houtt was around 8.0, 4.0, 2.0 and 1.3 times higher than the content of polyphenols, chlorophylls, carotenoids and triterpenoids in *Fallopia sachalinensis* (F.Schmidt). The accurate identification of *Fallopia* bioactive compounds is an indispensable detailed knowledge of the profile and step toward better understanding of the medicinal properties of the species and also potentially more extensive use of the plant.

Keywords *Fallopia japonica* Houtt · *Fallopia sachalinensis* (F.Schmidt) · Leaves · Rhizomes · Polymeric procyanidins · Triterpenoids · Carotenoids · Chlorophylls · UPLC-PDA-Q/TOF-MS

✉ Sabina Lachowicz
sabina.lachowicz@upwr.edu.pl

Tomasz Cebulak
tomcebulak@gmail.com

Lidia Hirnle
lidia.hirnle@umed.wroc.pl

Maciej Siewiński
siewinski@op.pl

[1] Department of Fermentation and Cereals Technology, Faculty of Biotechnology and Food Science, Wrocław University of Environmental and Life Sciences, 37 Chełmońskiego Street, 51-630 Wrocław, Poland

[2] Department of Fruit, Vegetable and Plant Nutraceutical Technology, Faculty of Biotechnology and Food Science,

Wrocław University of Environmental and Life Sciences, 37 Chełmońskiego Street, 51-630 Wrocław, Poland

[3] Department of Food Technology and Human Nutrition, Faculty of Biology and Agriculture, Rzeszow University, 4 Zelwerowicza Street, 35-601, Rzeszow, Poland

[4] Department and Clinic of Gynaecology and Obstetrics, Wroclaw Medical University ul. T, Chałubińskiego 3, 50-368 Wrocław, Poland

[5] Department of Basic Sciences, Wroclaw Medical University, ul. Chalubinskiego 4, 50-368 Wrocław, Poland

Introduction

Fallopia—Japanese knotweed—herbaceous perennial, strongly branching. *Fallopia* naturally occurs i.a. in Japan, the Kuril Islands, Sakhalin, Korea, South-West China, Taiwan, Vietnam [1]. It occurs there in river valleys, at the edge of forests and at roadside. Growing up to a height of 3–5 m, rhizomes produce substances that inhibit the growth of other plants. Green leaves, 5–15 cm long, broad in shape is the plant grows very fast—young stems can grow up to 10–15 cm per day. It has thick, wide, dark yellow rhizomes in cross-section, with reddish or brown bark [2–4].

In Korea and China, knotweed has been known for thousands of years as a medicinal plant [5, 6]. In addition, Japanese knotweed is a plant used in phototherapy [7]. In traditional Chinese and Japanese herbal medicine, this raw material is recommended as analgesic, antipyretic, diuretic and expectorant. It is used in the treatment of diseases, including asthma, atherosclerosis, cough, inflammation, hypertension, heart disease, fungal and bacterial infections and tumors [8]. Japanese knotweed also displays several beneficial biological effects such as inhibition of topoisomerases and neuraminidases, anti-oxidancy, anti-tumor activity, neuroprotective properties and inhibition of the development of borreliosis [3, 9–11]. Thanks to these properties, Japanese knotweed can be used as an alternative material of natural origin, which is a source of bioactive compounds in the prevention or treatment of many diseases.

Raw material obtained from the natural state and crops can be used in the pharmaceutical, cosmetic and food industry as well as in phototherapy. In order to better understand the potential of the plant and the possibility of its use, a thorough analysis of compounds with pro-health effects is necessary. Ultra-performance liquid chromatography coupled with a photodiode detector-quadrupole and tandem time-of-flight mass spectrometry (UPLC-PDA-Q/TOF-MS) is proved to be extremely useful for UV–Vis spectrum, peak assignment and further characterization of individual compounds. Particularly, the electrospray ionization mass spectrometry (ESI-MS) has been widely applied in the identification of phenolic compounds. In most instances, particular bioactive compounds can be identified directly by comparison with data reported in previous literature or authentic standards. Additionally, combination of separation and activity measurement, i.e., sensitive on-line HPLC-ABTS assays for analyzing free radical scavenging activity, exhibits a prominent advantage for screening and evaluating antioxidants particular bioactive compounds without loss of active components. Therefore, the methods are focused on the analyses of free radical scavenging activities of complex mixtures, especially the plant extracts [12, 13]. So far, to the best of our knowledge, there have been no reports regarding the properties of individual carotenoids, chlorophylls and triterpenoids as well as the antioxidant properties of each polyphenolic compound of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt), especially in leaves and rhizomes. Therefore, the aim of this study was to identify, quantify and compare a broad range of potential health-promoting components (polyphenols, carotenoids, chlorophylls, triterpenoids) by UPLC-PDA-Q/TOF-MS, and their on-line UPLC-ABTS assay in leaves and rhizomes of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) grown in Poland.

Materials and methods

Chemicals

Acetonitrile, formic acid, methanol, all-*trans*-β-carotene, all-*trans*-lutein, all-*trans*-zeaxanthin, violaxanthin, chlorophyll *a*, chlorophyll *b*, chlorophyllide *b*, pheophytin *a*, pheophytin *b*, betulinic, oleanolic and ursolic acid, ABTS 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), methanol acetic acid, and phloroglucinol were purchased from Sigma-Aldrich (Steinheim, Germany). (−)-Epicatechin, (+)-catechin, procyanidin B2,, caffeic acid, *p*-coumaric acid, 3-*O*-caffeoylquinic, 5-*O*-caffeoylquinic, ferulic acid, galloyl glucose, caftaric acid, luteolin 7-*O*-galactoside, apigenin 7-*O*-glucoside, kaempferol-3-*O*-galactoside, quercetin-3-*O*-rutinoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-arabinoside, quercetin-3-*O*-xyloside, *cis*-piceid, *trans*-piceid, *cis*-resveratrol were purchased from Extrasynthese (Lyon, France). Acetonitrile for ultra-phase liquid chromatography (UPLC; Gradient grade) and ascorbic acid were purchased from Merck (Darmstadt, Germany).

Plant materials

Leaves and rhizomes of *Fallopia japonica* and *Fallopia sachalinensis* were used in the study. Material samples (~ 1.0 kg each) were collected from the Garden of Medicinal Plants herbarium at the Wroclaw Medical University, Poland. The fresh leaves and rhizomes were directly frozen at − 25 °C and then freeze-dried (24 h; Christ Alpha 1–4 LSC; Germany). The homogeneous dry material was obtained by crushing the dried tissues using a closed laboratory mill (IKA A.11, Germany). The powders were kept in a refrigerator (− 80 °C) until extract preparation.

Qualitative and quantitative assessment of polyphenols

The samples (1 g) were extracted with by 10 mL of mixture containing HPLC-grade methanol (30 mL/100 mL), ascorbic acid (2.0 g/100 mL) and acetic acid in an amount of 1.0 mL/100 mL of reagent. The extraction was performed twice by incubation for 20 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland) and with occasional shaking. Next, the slurry was centrifuged at 19,000g for 10 min, and the supernatant was filtered through a Hydrophilic PTFE 0.20 μm membrane (Millex Samplicity Filter, Merck, Darmstadt, Germany) and used for analysis. The content of polyphenols in individual extracts was determined by means of the ultra-performance liquid chromatography-photodiode array detector–mass spectrometry method. All extractions were carried out in triplicate.

Qualitative (LC/MS Q-TOF) and quantitative (UPLC-PDA-FL) estimation of polyphenol (flavan-3-ols, flavonols, flavanone, stilbenes and phenolic acids) of *Fallopia japonica* and *Fallopia sachalinensis* extracts was carried out using an ACQUITY Ultra Performance LC system equipped with a photodiode array detector with a binary solvent manager (Waters Corporation, Milford, MA, USA) series with a mass detector G2 Q/TOF micro-mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in negative and positive modes. Separations of individual polyphenols were carried out using a UPLC BEH C18 column (1.7 mm, 2.1 × 100 mm, Waters Corporation, Milford, MA; USA) at 30 °C. The samples (10 μL) were injected, and the elution was completed in 15 min with a sequence of linear gradients and isocratic flow rates of 0.45 mL min^{-1}. The mobile phase consisted of Solvent A (2.0% formic acid, *v/v*) and Solvent B (100% acetonitrile). The program began with isocratic elution with 99% Solvent A (0–1 min), and then a linear gradient was used until 12 min, reducing Solvent A to 0%; from 12.5 to 13.5 min, the gradient returned to the initial composition (99% A), and then, it was held constant to re-equilibrate the column. The analysis was carried out using full-scan, data-dependent MS scanning from *m/z* 100 to 1500. Leucine enkephalin was used as the reference compound at a concentration of 500 pg/L, at a flow rate of 2 L/min, and the [M–H]$^{-}$ ion at 554.2615 Da was detected. The [M–H]$^{-}$ ion was detected during a 15-min analysis performed within ESI-MS accurate mass experiments, which were permanently introduced via the Lock-Spray channel using a Hamilton pump. The lock mass correction was ± 1.000 for the mass window. The mass spectrometer was operated in negative and positive ion mode, set to the base peak intensity (BPI) chromatograms and scaled to 12,400 counts per second (cps) (100%). The optimized MS conditions were as follows: capillary voltage of 2500 V,

cone voltage of 30 V, source temperature of 100 °C, desolvation temperature of 300 °C and desolvation gas (nitrogen) flow rate of 300 L/h. Collision-induced fragmentation experiments were performed using argon as the collision gas, with voltage ramping cycles from 0.3 to 2 V. Characterization of the single components was carried out via the retention time and the accurate molecular masses. Each compound was optimized to its estimated molecular mass in the negative mode, before and after fragmentation. The data obtained from UPLC–MS were subsequently entered into the MassLynx 4.0ChromaLynx Application Manager software (Waters). On the basis of these data, the software is able to scan different samples for the characterized substances. The runs were monitored at the following wavelengths: flavan-3-ols at 280 nm, phenolic acids at 320 nm, flavonol at 360 nm. The PDA spectra were measured over the wavelength range of 200–600 nm in steps of 2 nm. The retention times and spectra were compared to those of the authentic standards. The quantification of phenolic compounds was performed by external calibration curves, using reference compounds selected based on the principle of structure-related target analyte/standard (chemical structure or functional group).

The calibration curve for 3-caffeoylquinic acid was used to quantify quinic acid, caffeoylquinic acid isomers *cis*-3-*O*-caffeoylquinic acid, *cis*-5-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid. The calibration curve for caffeic acid was used to quantify caffeoyl glucoside. The calibration curve of *p*-coumaric acid was used, besides its own quantification, to quantify p-coumarylquinic acid. The calibration curve for ferulic acid was used to quantify feruloylquinic acid. Galloyl glucose, caftaric acid, 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid were quantified with their own standards. The calibration curve for procyanidin B2 was used to quantify all B-type procyanidins. The calibration curve for (+)-catechin was used to quantify (+)-catechin glucoside and (+)-catechin gallate. (+)-catechin and (−)-epicatechin were quantified with its own standard. The calibration curve for kaempferol 3-*O*-glucoside was used for its own quantification as well as kaempferol rhamnoside. The calibration curve for luteolin 7-*O*-galactoside was used for its own quantification as well as luteolin 7-*O*-glucoside. The calibration curve for apigenin 7-*O*-glucoside standard was used for its own quantification as well as apigenin 7-apiosylglucoside. The calibration curves of quercetin rutinoside, 3-*O*-glucoside and 3-*O*-galactoside were used to quantify quercetin derivatives. The calibration curves of *Cis*-piceid, *trans*-piceid, *cis*-resveratrol were used to quantify piceid and resveratrol derivatives. *Cis*-piceid, *trans*-piceid, *cis*-resveratrol were quantified with its own standard. All measurements were repeated three times. The results were expressed as mg/100 g of dry matter (dm).

Analysis of proanthocyanidins by phloroglucinolysis

Direct phloroglucinolysis of freeze–dried samples was performed as described by Lachowicz et al. [14] and Oszmiański and Lachowicz [15]. Materials lyophilisates were weighed in an amount of 5 mg in 2-mL Eppendorf vials. Subsequently, 0.8 mL of the methanolic solution of phloroglucinol (75 g/L) and ascorbic acid (15 g/L) were added to samples. After addition of 0.4 mL of methanolic HCl (0.3 M), the vials were incubated for 30 min at 50 °C with continuous vortexing in a thermo-shaker (TS-100, BioSan, Riga, Latvia). The reaction was terminated by placing the vials in an ice bath, drawing 0.6 mL of the reaction medium and diluting with 1.0 mL of sodium acetate buffer (0.2 M). The samples were centrifuged immediately at 20,000g for 10 min at 4 °C, and stored at 4 °C before reverse-phase HPLC (RP-HPLC) analysis. All incubations were done in triplicate. Phloroglucinolysis products were separated on a Cadenza CD C18 (75 mm×4.6 mm, 3 μm) column (Imtakt, Japan). The liquid chromatograph was a Waters (Milford, MA) system equipped with diode array and scanning fluorescence detectors (Waters 474) and an autosampler (Waters 717 plus). Solvent A (25 mL aqueous acetic acid and 975 mL water) and solvent B (acetonitrile) were used in the following gradients: initial, 5% B; 0–15 min to 10% B linear; 15–25 min to 60% B linear; followed by washing and reconditioning of the column. Other parameters were as follows: a flow rate of 1 mL/min, an oven temperature of 15 °C, and volume of filtrate injected onto the HPLC system was 20 μL. The fluorescence detection was monitored at 278 nm and 360 nm. The calibration curves were established using (+)-catechin and (−)-epicatechin-phloroglucinol adducts standards. All data were obtained in triplicate. The results were expressed as mg/100 g dm.

Identification and quantification of carotenoids and chlorophylls

For the extraction of carotenoids, a protocol similar to that described previously was applied [16]. The samples (0.5 g) containing 10% of MgCO3 were continuously shaken at 500 rpm (DOS-10L Digital Orbital Shaker, Elmi Ltd., Riga, Latvia) for 30 min in the dark with 5 mL of hexane:acetone:methanol (2:1:1, v/v/v) containing 1% BHT. After the first extraction, the samples were centrifuged at 19,000g for 10 min at 4 °C, and the supernatant was recovered. The samples were re-extracted and centrifuged in the same conditions. Supernatants were combined and evaporated to dryness. The pellet was re-extracted using 2 mL of 100% methanol, filtered through a hydrophilic PTFE 0.20_m membrane (Millex Samplicity Filter, Merck) and used for analysis.

Compounds were separated with an ACQUITY UPLC BEH RP C18 column (1.7_m, 2.1 mm×100 mm, Waters Corp.) at 32 °C. The elution solvents were ACN : MeOH (7:3, v/v) (A) and 0.1% formic acid (B). Samples (10 μL) were eluted according to the linear gradient: 0–0.6 min, 25% B, 0.5 mL/min (isocratic); 0.6– 6.5 min, 4.9% B, 0.5 mL/min (linear gradient); 6.5–7.5 min, 0% B, 0.7 mL/min (linear gradient); 7.5–13.6 min, 0% B, 0.7 mL/ min (isocratic); 13.6–14.1 min, 25% B, 0.5 mL/min (linear gradient); and 14.1–16.6 min, 25% B, 0.5 mL/min (isocratic). Weak and strong needle solvents were ACN–MeOH (7:3, v/v) and 2-propanol, respectively.

Identification of carotenoids was carried out on the basis of fragmentation patterns and on the basis of PDA profiles. Where available, compounds were compared with authentic standards (their fragmentation pathways, retention times and PDA profiles). If standards were not available, fragmentation pathways and PDA profiles were compared with literature data. The runs were monitored at 450, 427 and 650 nm. The PDA spectra were measured over the wavelength range of 200–800 nm in steps of 2 nm. Calibration curves were made from all-*trans*-β-carotene, all-*trans*-zeaxanthin, all-*trans*-lutein, violaxanthin, neoxanthin, chlorophyll *a*, chlorophyll *b*, chlorophyllide *b*, pheophytin *a*, pheophytin *b*. (8′R) neochrome and (8′S) neochrome derivatives were expressed as neoxanthin. Hydrooxypheophytin *a* and *b*, pheophytin a′ and b′ were expressed as pheophytin *a* and *b*. Hydroxychlorophyll a and chlorophyll a′ and b′ were expressed as chlorophyll *a* and *b*.

All incubations were done in triplicate. The results were expressed as mg/100 g of dm.

Identification and quantification of triterpenoids

Sample extraction was performed as described by Farneti et al. [17]. The samples (0.5 g) were extracted with 5 mL of ethyl acetate and 5 mL of hexane. The extraction was performed by incubation for 20 min under sonication (Sonic 6D, Polsonic, Warsaw, Poland) with occasional shaking. After the first extraction, the samples were kept at 4 °C overnight. On the next day the samples were re-extracted in the same conditions. After the first extraction, the samples were centrifuged at 19,000g for 10 min at 4 °C, and the supernatant was recovered. The samples were re-extracted and centrifuged in the same conditions. Supernatants were combined and evaporated to dryness. The pellet was re-extracted using 2 mL of 100% methanol, filtered through a hydrophilic PTFE 0.20_m membrane (Millex Samplicity Filter, Merck) and used for analysis.

Identification and quantification of ursolic, oleanolic, and betulinic acids was done using the ACQUITY Ultra Performance LC system with a binary solvent manager (Waters Corp., Milford, MA, USA), a UPLC BEH C18 column

(1.7 μm, 2.1 mm × 150 mm, Waters Corp., Milford, MA, USA), and a Q-TOF mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source, operating in negative mode. The elution solvents were 100% methanol (A) and 100% acetonitrile (B) (15:85, v/v). Ursolic, oleanolic, and betulinic acids were eluted isocratically at a flow rate of 0.1 mL/min for 10 min at 20 °C. The m/z for betulinic acid was 455.34, for oleanolic acid 455.34, and for ursolic acid 455.33, and the retention times were 6.99, 7.66 and 8.36 min, respectively. The compounds were monitored at 210 nm. All data were obtained in triplicate. The results were expressed as mg/100 g of dm.

HPLC-PDA-on-line-ABTS-based assay

The antioxidant activity of individual HPLC peaks was measured using an on-line HPLC antioxidant detector system based on the TEAC assay of Re et al. [18] and Kusznierewicz et al. [19].

A CADENZA C18 column (75 mm × 4.6 mm i.d., 3 μm; Tokyo, Japan) with a C18 guard column was used. The column temperature was set at 30 °C. The separation was achieved by a gradient elution of 2.0% formic acid solution (solvent A) and acetonitrile (solvent B) at a flow rate of 0.6 mL/min: 0–30 min, 2–40% B; and up to 45 min column was recognition. The injection volume of sample was 10 μL, and the detection wavelength was set at 280 and 734 nm. ABTS radical cation was produced as described previously by Re at al [18]. After the first PDA detector, the mobile phase was mixed to the ABTS radical cation delivered by the second pump at a flow rate of 0.2 mL/min. The mixture was guided through a 25-m-long PTFE reaction coil with 0.25 mm internal diameter at 40 °C to a second UV detector, where ABTS decolourization was detected as a negative peak at 734 nm.

Statistical analysis

Statistical analysis, one-way ANOVA and hierarchal cluster (HA) and principal component analysis (PCA) were conducted using Statistica version 12.5 (StatSoft, Kraków, Poland). Significant differences ($p \leq 0.05$) between mean values were evaluated by one-way ANOVA and Duncan's multiple range test.

Results and discussion

Identification and quantification of phenolics in Fallopia

The identification and quantification of 46 compounds belonging to flavones and flavonols, phenolic acids,

flavan-3-ols and stilbenes was based on a comparison with available standards of their MS, MS/MS data and retention times and literatures [20–23].The results concerning polyphenolic compounds analyzed by a UPLC-PDA-Q/TOF-MS system are summarized in Tables 1, 2, 3 and 4 and Fig. 1.

The main group of phenolics in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) leaves, which contained 37 compounds, were flavan-3-ols (monomers, oligomers and polymeric procyanidins) (~70%) > flavones and flavonols (~15%) > phenolic acid (~13%) ≥ stilbenes (~2%); however, in rhizomes, which contained 34 compounds, the main group were flavan-3-ols (~53%) > phenolic acid (~31%) ≥ flavones and flavonols (each ~8%). The leaves and rhizomes are good source of polyphenolic compounds and their average content in leaves was 20408.18 mg/100 g dm and was around 8.0 times higher than in rhizomes. The leaves and rhizomes of *Fallopia japonica Houtt* were more fertile around 1.5 and 1.2 times than anatomical parts of *Fallopia sachalinensis* (F.Schmidt). According to Shitasue et al. [24], the content of polyphenolics in *Fallopia japonica* grown in Hoshigaoka, Nagoya was around 5.0 and 7.0 times lower and 1.5 and 2.0 times higher than the content of these compounds in leaves and rhizomes of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) after ethanol extract. In comparison to other plants from the same family as *Rumex japonicas and Rumex acetosa*, the content of polyphenolic compounds was around 13 and 1.4 times lower and 4.8 times lower and 1.8 times higher than in leaves and rhizomes of *Fallopia japonica* Houtt [24]. The content of phenolics in leaves of *Allium ursinum* was around 15 and 18 times lower than the content of these compounds in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) [21]. The content of phenolics in leaves of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) was around 1.8 and 2.5 times higher than in Wild Rubus L. species [25]. Similarly, higher levels of phenolic compounds were presented in spinach leaves [26]. The content of individual compounds with antioxidant properties largely depends on factors such as variety, stage of maturity, part of the plant analyzed, climatic conditions, post-harvest handling and storage [26, 27].

Flavan-3-ols constituted a major group of the analyzed extracts and monomers, oligomers accounted for 13% and 20% but polymeric procyanidins accounted for 57% and 33% in leaves and rhizomes, respectively (Table 1). In this group, 11 flavan-3-ols were identified, of which 9 in leaves and 8 in rhizomes. Flavan-3-ols were detected in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) extracts: (+)-catechin and derivatives, (−)-epicatechin, 7 B-type procyanidin (dimer, tetramer, gallate).

The average content of flavan-3-ols (monomers and oligomers) and procyanidin polymers of *Fallopia japonica* Houtt were 1795.81 and 7070.04 mg/100 g dm and were

Table 1 UV and MS spectra data of flavan-3-ol derivatives (mg/100 g dm) in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) leaves and rhizomes

No.	Compounds	Retention time (min)	λ (nm)	MS[M-H]− molecular ion	MS/MS fragments	*Fallopia japonica* Leaves	*Fallopia japonica* Rhizomes	*Fallopia sachalinensis* Leaves	*Fallopia sachalinensis* Rhizomes	References
8	Procyanidin dimer B[b]	3.87	279	577	289	362.49 ± 0.72ea	63.84 ± 0.13f	545.37 ± 3.27b	168.59 ± 1.01b	[20, 27]
9	(+) Catechin[b]	3.96	279	289		145.36 ± 0.29i	8.10 ± 0.02i	110.68 ± 0.66h	21.57 ± 0.13g	[20, 27]
11	Procyanidin dimer B[b]	4.27	279	577	289	247.11 ± 0.49g	nd	338.29 ± 2.03d	nd	[20, 27]
14	Procyanidin dimer B[b]	4.93	279	577	289	641.51 ± 1.28	119.69 ± 0.24b	241.10 ± 1.45g	37.10 ± 0.22d	[20, 27]
15	(−)-Epicatechin[b]	5.24	278	289		329.58 ± 0.66f	74.14 ± 0.15e	338.43 ± 2.03d	168.53 ± 1.01b	[20, 27]
17	(+)-Catechin glucoside[c]	5.70	277	451	289	537.92 ± 1.08c	82.68 ± 0.17d	260.98 ± 1.57f	5.12 ± 0.03h	[20, 27, 28]
23	Procyanidin B gallate[c]	6.00	279	729	577/289	nd	49.81 ± 0.10g	nd	36.22 ± 0.22e	[20, 27]
26	Procyanidin tetramer B[b]	6.25	277	1153	863/575/289	460.03 ± 0.92d	nd	438.91 ± 2.63c	nd	[20, 27]
28	Procyanidin tetramer B[b]	6.53	279	1153	863/575/289	193.66 ± 0.39h	nd	262.42 ± 1.57e	nd	[20, 27]
32	(+)-Catechin gallate[c]	6.95	277	441	289	131.05 ± 0.26j	97.02 ± 0.19c	85.86 ± 0.52i	34.41 ± 0.21f	[20, 27]
35	Procyanidin tetramer B[b]	7.32	277	1153	863/575/289	nd	47.62 ± 0.10h	nd	60.24 ± 0.36c	[20, 27]
	Procyanidin polymers					13272.26 ± 26.54a	867.81 ± 1.74a	9307.93 ± 55.85a	1374.8 ± 8.25a	

Values are means ± standard deviation. $n = 3$

[a]a–j: Means ± SD followed by different letters within the same column represent significant differences ($P < 0.05$)

[b]Identification confirmed by commercial standards

[c]Identification by comparison of MS data with the literature and their identification is tentative

Table 2 UV and MS spectra data of flavones and flavonols derivatives [mg/100 g dm] in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F. Schmidt) leaves and rhizomes

No.	Compounds	Retention time (min)	λ (nm)	MS[M-H]− molecular ion	MS/MS fragments	*Fallopia japonica*		*Fallopia sachalinensis*		References
						Leaves	Rhizomes	Leaves	Rhizomes	
21	Apiin (apigenin-7-apiosylglucoside) [c]	5.78	331	563	269	39.71 ± 0.08ha	nd	27.75 ± 0.17k	nd	[20]
24	Luteolin 7-O-galactoside [b]	6.06	349	447	285	102.88 ± 0.21f	5.23 ± 0.01e	234.48 ± 1.41c	2.62 ± 0.02d	[20]
25	Luteolin 7-O-glucoside [c]	6.21	347	447	285	43.09 ± 0.09j	nd	120.04 ± 0.72e	nd	[20]
27	Apigenin-7-O-glucoside [b]	6.35	334	431	269	36.10 ± 0.07i	nd	23.15 ± 0.14l	nd	[20]
31	Quercetin 3-O-rutinoside [b]	6.86	332	609	463/301	158.04 ± 0.32d	nd	110.42 ± 0.66f	nd	[14, 20]
33	Quercetin 3-O-glucoside [b]	7.02	352	463	301	55.31 ± 0.11i	3.64 ± 0.01f	66.81 ± 0.40g	1.82 ± 0.01e	[14, 25, 29]
34	Quercetin 3-O-glucuronide [c]	7.13	353	477	301	232.81 ± 0.47c	16.93 ± 0.03c	149.28 ± 0.90d	8.47 ± 0.05c	[20, 25, 26]
36	Quercetin 3-O-pentoside [c]	7.42	355	433	301	117.00 ± 0.23e	8.25 ± 0.02d	64.57 ± 0.39h	nd	[20, 25]
38	Quercetin acetylhexoside [c]	7.58	354	505	463/301	93.79 ± 0.19g	nd	58.51 ± 0.35i	nd	[20, 25]
39	Quercetin 3-O-pentoside [c]	7.73	351	433	301	454.26 ± 0.91b	27.60 ± 0.06b	304.07 ± 1.82b	13.80 ± 0.08b	[20, 25]
41	Quercetin 3-O-rhamnoside [c]	7.86	346	447	301	2229.50 ± 4.46a	160.27 ± 0.32a	1164.76 ± 6.99a	80.14 ± 0.48a	[20, 25]
45	Kaempferol rhamnoside [c]	8.77	340	431	285	37.72 ± 0.08k	nd	55.31 ± 0.33j	nd	[20, 25]

Values are means ± standard deviation. $n = 3$

[a–l] Means ± SD followed by different letters within the same column represent significant differences ($P < 0.05$)

[b] Identification confirmed by commercial standards

[c] Identification by comparison of MS data with the literature and their identification is tentative

Table 3 UV and MS spectra data of phenolic acid derivatives [mg/100 g dm] in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) leaves and rhizomes

No.	Compounds	Retention time (min)	λ (nm)	MS[M−H]⁻ molecular ion	MS/MS fragments	*Fallopia japonica* Leaves	Rhizomes	*Fallopia sachalinensis* Leaves	Rhizomes	References
1	Galloyl glucose[b]	1.55	277	331	169	31.63 ± 0.06ha	8.3 ± 0.02l	15.12 ± 0.09i	4.36 ± 0.03i	[20, 27]
2	Galloyl glucose[b]	1.70	277	331	169	4.12 ± 0.01i	4.90 ± 0.01k	22.87 ± 0.14h	0.51 ± 0.00m	[20, 27]
3	Galloyl glucose[b]	1.97	277	331	169	nd	0.57 ± 0.00m	nd	1.53 ± 0.01j	[27, 29]
4	3-*O*-Caffeoylquinic acid[c]	3.06	324	353	191/179	118.86 ± 0.24f	nd	126.14 ± 0.76d	nd	[20, 27]
5	Cis 3-*O*-Caffeoylquinic acid[c]	3.32	326	353	191	507.97 ± 1.02c	36.56 ± 0.07g	468.17 ± 2.81c	237.01 ± 1.42a	[20]
6	Caftaric acid[b]	3.41	328	311	179	1013.67 ± 2.03a	54.43 ± 0.11d	1338.67 ± 8.03a	0.88 ± 0.01l	[20]
7	Caffeoyl-glucose[c]	3.50		341	179	nd	13.89 ± 0.03h	nd	9.22 ± 0.06h	[20]
10	*p*-Coumaroylquinic acid[b]	4.05	310	337	191/163	184.36 ± 0.37e	9.72 ± 0.02j	104.28 ± 0.63f	10.96 ± 0.07g	[10, 29]
12	5-*O*-Caffeoylquinic acid[c]	4.36	324	353	191	732.36 ± 1.46b	386.61 ± 0.77a	630.64 ± 3.78b	176.24 ± 1.06b	[20]
13	Cis 5-*O*-Caffeoylquinic acid[c]	4.52	324	353	191	62.87 ± 0.13g	10.15 ± 0.02i	55.53 ± 0.33g	3.88 ± 0.02k	[20]
16	3-*O*-*p*-Coumaroylquinic acid[c]	5.34	312	337	191/163	318.13 ± 0.64d	nd	120.55 ± 0.72e	nd	[20]
19	Feruloylquinic acid[c]	5.76	320	367	191	nd	37.13 ± 0.07f	nd	43.34 ± 0.26c	[20, 27]
37	3,4-Di-*O*-caffeoylquinic acid[c]	7.56	324	515	353/191	nd	90.73 ± 0.18c	nd	34.66 ± 0.21e	[20]
40	3,5-Di-*O*-caffeoylquinic acid[c]	7.82	326	515	353/191	nd	121.91 ± 0.24b	nd	46.57 ± 0.28d	[20]
44	4,5-Di-*O*-caffeoylquinic acid[c]	8.30	326	515	353/191	nd	48.07 ± 0.10e	nd	18.36 ± 0.11f	[20]

Values are means ± standard deviation. $n = 3$

[a]a–m: Means ± SD followed by different letters within the same column represent significant differences ($P < 0.05$)

[b]Identification confirmed by commercial standards

[c]Identification by comparison of MS data with the literature and their identification is tentative

Table 4 UV and MS spectra data of stilbene derivatives [mg/100 g dm] in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) leaves and rhizomes

No.	Compounds	Retention time (min)	λ (nm)	MS[M-H]⁻ molecular ion	MS/MS fragments	*Fallopia japonica*		*Fallopia sachalinensis*		References
						Leaves	Rhizomes	Leaves	Rhizomes	
18	Astringin[b]	5.71	328	405	243	20.95 ± 0.04fa	28.29 ± 0.06c	33.57 ± 0.20d	45.33 ± 0.27a	[31]
20	Piceatannol-glucoside[c]	5.77	330	405	243	91.26 ± 0.18b	21.88 ± 0.04d	38.29 ± 0.23c	5.47 ± 0.03f	[31]
22	Trans-Resveratroloside[c]	5.90	303	389	227	nd	21.3 ± 0.04e	nd	42.08 ± 0.25c	[31]
29	Trans-Piceid[b]	6.80	317	389	227	138.76 ± 0.28a	104.50 ± 0.21a	89.04 ± 0.53b	45.85 ± 0.28b	[31]
30	Resveratrol-galloyl-glucoside[c]	6.83	333	541	227	49.74 ± 0.10d	nd	31.92 ± 0.19e	nd	[31]
42	Cis-Resveratroloside[c]	7.93	322	389	227	25.69 ± 0.05e	27.14 ± 0.05b	16.49 ± 0.10f	21.85 ± 0.13d	[20]
43	Cis-Piceid[b]	8.10	285	389	227	61.57 ± 0.12c	2.97 ± 0.01f	110.76 ± 0.66a	8.31 ± 0.05e	[20, 30]
46	Cis-Resveratrol[b]	8.84	285	227	227	12.93 ± 0.03g	0.64 ± 0.01g	9.17 ± 0.06g	0.70 ± 0.00g	[20, 30]

Values are means ± standard deviation. $n = 3$

[a]–[g]: Means ± SD followed by different letters within the same column represent significant differences ($P < 0.05$)

[b]Identification confirmed by commercial standards

[c]Identification by comparison of MS data with the literature and their identification is tentative

1.2 and 1.4 times higher than in *Fallopia sachalinensis* (F.Schmidt). The average content of flavan-3-ols in leaves of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) were 2835.37 mg/100 g dm of monomers and oligomers and 11290.10 mg/100 g dm of procyanidin polymer, which was around 6 and 10 times higher than in rhizomes (Table 1). The major compounds in flavan-3-ols (monomers and oligomers) were procyanidin type-B dimer and (+)-catechin glucoside. These compounds constituted an average of 22% and 18% of all flavan-3-ols. (+)-Catechin gallate in leaves and rhizomes constituted just 4% and 18%, but this compound is responsible for blocking the cell cycle of cancer in the G_0/G_1 phase by inhibiting topoisomerase I activity [28].
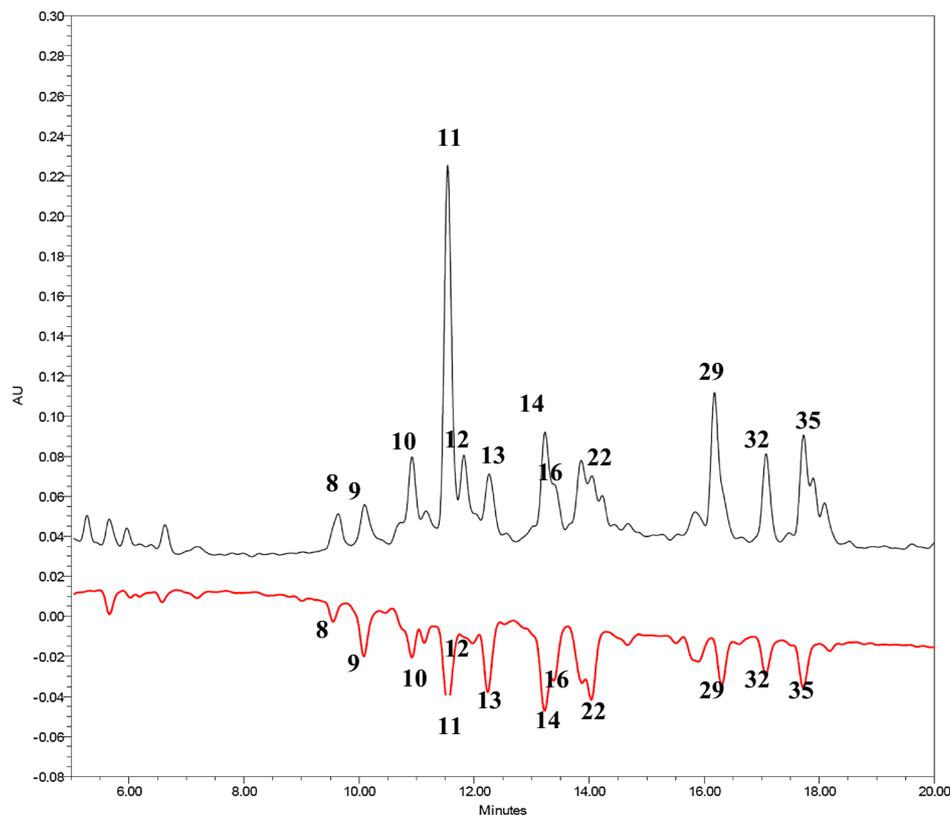
The next group belonging to polyphenols was flavones and flavonols. In this group, 12 compounds were detected in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt), of which 12 in leaves and 6 in rhizomes (Table 2).

The average content of flavones and flavonols in *Fallopia japonica* Houtt was 1911.07 mg/100 g dm and was 2.0 times higher than in *Fallopia sachalinensis* (F.Schmidt). The average content of flavones and flavonols in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) leaves amounted to 2989.68 mg/100 g dm and the content was around 19 times higher than in rhizomes (Table 2). Quercetin rhamnoside and pentoside were the major components of *Fallopia japonica* Houtt leaves and rhizomes extracts (62–72% and 12%, respectively, of all flavones and flavonols). The content of flavones and flavonols in leaves of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) was around 1.7 and 2.2 times lower than in Wild Rubus L. Species [25]. Similarly, higher levels of phenolic compounds were presented in spinach leaves [26, 29].

The second group belonging to polyphenolic compounds were phenolic acids. In this group, 15 compounds were detected in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt), of which 9 in leaves and 13 in rhizomes (Table 3).

The average content of phenolic acids in *Fallopia japonica* Houtt was 1898.47 mg/100 g dm and was 1.2 times higher than in *Fallopia sachalinensis* (F.Schmidt). The average content of phenolic acids in leaves of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) was 2927.97 mg/100 g dm and it was around 4 times higher than in rhizomes (Table 3). Caftaric and 5-*O*-caffeoylquinic acids were the major components in *Fallopia japonica* Houtt leaves and rhizomes extracts (34–7% and 25–47%, respectively, of all phenolic acids). The results regarding phenolic acids in leaves of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) were similar to the results in Wild Rubus L. Species [25].

Fig. 1 HPLC-UV-ABTS coupled chromatograms of the *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) rhizomes extracts (at 280 nm and 734 nm). For extract compound refer to Tables 1, 2, 3 and 4

The last group of phenolic compounds was stilbenes. In this group, 8 compounds were detected in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt), of which 7 in leaves and rhizomes (Table 4).

The average content of stilbenes in *Fallopia japonica* Houtt was 303.81 mg/100 g dm and was 1.2 times higher than in *Fallopia sachalinensis* (F.Schmidt). The average content of stilbenes in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) leaves amounted to 329.24 mg/100 g dm and it was around 2.0 times higher than in rhizomes (Table 4). Trans-piceid was the major component in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) leaves and rhizomes extracts (34–50% of all stilbenes). The leaves and rhizomes of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) are an excellent source of resveratrol and piceid. Similar results regarding resveratrol and piceid in Japanese knotweed leaves and rhizomes were presented by Beňova et al. [22]. According to Surguladze et al. [30], the content of resveratrol and piceid identified in red grape wine was around 24.0 and 7.0 times lower than in leaves and 9.0 and 5.0 times lower than in rhizomes of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt). Additionally, the content of piceid in grape cv. Casteao from Portugal was around 15.0 times lower than in leaves and rhizomes of *Fallopia japonica* Houtt and 16.0 times of *Fallopia sachalinensis* (F.Schmidt) [31, 32].

HPLC-UV-ABTS chromatograms (according to the formed negative inactivated ABTS•+ radical cation peaks) of the *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) rhizome extracts revealed that flavan-3-ols derivatives such as monomers and oligomers (Fig. 1, compounds no. 8, 9, 11, 14, 32 and 35) and trans-piceid and trans resveratrol (compounds 22 and 29) possess greater radical scavenging capacity than phenolic acids (Fig. 1, compounds no. 10, 12, 13, 16). Flavan-3-ols as catechins and procyanidins have two ortho-dihydroxy functional groups, which determine high antioxidant activity of these compounds. Additionally, Fig. 1 revealed that especially: (+)-catechin, procyanidin dimer B and trans-resveratroloside possess the strongest radical scavenging capacity. Furthermore, the on-line HPLC-ABTS assay used enables to evaluate the quality of materials and choice of species rich in antioxidants. The test allows to determine the antioxidant properties of unknown compounds, the precise antiradical activity of known compounds and effects on total antioxidant capacity of a medicinal preparation or medicinal herbal raw material [13, 29, 33–35].

Identification and quantification of chlorophylls and carotenoids

The results regarding carotenoids and chlorophylls analyzed by the UPLC-PDA-Q/TOF-MS system are summarized

Table 5 UV and MS spectra data of chlorophylls and their derivatives (mg/100 g dm) in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) leaves and rhizomes

No.	Compounds	Retention time (min)	λ (nm)	MS[M-H]⁻ molecular ion	ms/ms fragments	*Fallopia japonica*		*Fallopia sachalinensis*		References
						Leaves	Rhizomes	Leaves	Rhizomes	
3	Chlorophyllide b[b]	4.00	407/505/663	813		8.91 ± 0.02fa	1.79 ± 0.02e	5.79 ± 0.03f	1.16 ± 0.01d	[26]
4	Chlorophyllide a[b]	4.16	411/663	811		1.52 ± 0.00j	1.04 ± 0.01i	0.99 ± 0.01k	0.68 ± 0.00i	[23]
9	Chlorophyll b[b]	7.39	462/600/648	907	687/629/597/571/569/533	63.19 ± 0.13b	2.44 ± 0.02c	41.07 ± 0.25b	1.59 ± 0.01c	[26]
10	Hydroxypheophytin b[c]	7.57	434/522/598/652	901		21.29 ± 0.04d	1.22 ± 0.01g	13.84 ± 0.08d	0.79 ± 0.00g	[23, 27]
11	Chlorophyll b'[c]	7.66	462/600/648	907	687/629/597/571/569/533	4.45 ± 0.01g	0.47 ± 0.00l	2.89 ± 0.02g	0.31 ± 0.00k	[26]
12	Hydroxychlorophyll a[c]	7.85	422/614/660	909		2.34 ± 0.02i	0.56 ± 0.01k	1.52 ± 0.01i	0.36 ± 0.00j	[26, 35]
13	Chlorophyll a[b]	8.33	430/618/664	893	639/ 589/555	22.91 ± 0.05c	14.44 ± 0.13b	14.89 ± 0.09c	9.39 ± 0.06b	[23, 26]
14	Chlorophyll a'[c]	8.52	430/618/664	893	639/ 589/555	1.00 ± 0.01l	1.15 ± 0.01h	0.65 ± 0.00m	0.75 ± 0.00g	[23, 26]
15	Hydroxypheophytin a[c]	8.99	406/502/532/610/666	887		3.74 ± 0.01h	2.17 ± 0.02d	2.43 ± 0.01h	1.41 ± 0.01e	[23, 35]
19	Pheophytin a[b]	9.37	408/506/536/608/666	871	593/533	1.48 ± 0.01k	0.67 ± 0.01j	0.96 ± 0.01j	0.44 ± 0.00h	[26, 35]
20	Pheophytin a'[c]	9.64	408/506/536/608/666	871	593/533	0.68 ± 0.01m	1.45 ± 0.01f	0.44 ± 0.00l	0.94 ± 0.01f	[26, 35]
21	Pheophytin b[b]	9.99	436/528/598/652	885	607	75.13 ± 0.15a	24.51 ± 0.22a	48.83 ± 0.29a	15.93 ± 0.10a	[35]
22	Pheophytin b'[c]	10.28	436/528/600/656	885	607	11.51 ± 0.02e	1.21 ± 0.01g	7.48 ± 0.04e	0.79 ± 0.00g	[35]

Values are means ± standard deviation. $n = 3$

[a] a–m: Means ± SD followed by different letters within the same column represent significant differences ($P < 0.05$)

[b] Identification confirmed by commercial standards

[c] Identification by comparison of MS data with the literature and their identification is tentative

Table 6 UV and MS spectra data of carotenoids and their derivatives [mg/100 g dm] in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) leaves and rhizomes

No.	Compounds	Retention time (min)	λ (nm)	MS[M-H]⁻ molecular ion	MS/MS fragments	*Fallopia japonica*		*Fallopia sachalinensis*		References
						Leaves	Rhizomes	Leaves	Rhizomes	
1	(8′R) Neochrome[c]	2.82	422/450	601		1.91 ± 0.00ga	0.83 ± 0.01e	1.24 ± 0.01g	0.54 ± 0.01e	[23, 38]
2	(8,S) Neochrome[c]	2.98	400/422/450	601		2.31 ± 0.00f	0.11 ± 0.00g	1.50 ± 0.01f	0.07 ± 0.00g	[23, 38]
5	Lutein-5,6 epoxide[c]	4.60	430/455	585		0.84 ± 0.00h	0.10 ± 0.00g	0.55 ± 0.00h	0.07 ± 0.00g	[38]
6	all-trans-Lutein[b]	5.04	267/445/474	569	551/533/416/376	24.08 ± 0.05b	5.07 ± 0.05c	15.65 ± 0.09b	3.30 ± 0.02b	[23]
7	all-trans-Zeaxanthin[b]	5.11	451/478	568	551	4.46 ± 0.01e	0.56 ± 0.01f	2.90 ± 0.02e	0.36 ± 0.01f	[23]
8	Trans-Violaxanthin[b]	5.67	441/466	601	583/221/181	0.68 ± 0.00i	0.03 ± 0.00h	0.44 ± 0.01i	0.02 ± 0.00h	[38]
16	*all-trans-β*-Carotene[b]	9.04	452/479	537	445/203/177/149/137	63.70 ± 0.13a	49.24 ± 0.44a	41.41 ± 0.25a	32.01 ± 0.19a	[38]
a17	9-*cis-β*-Carotene[c]	9.10	345/447/475	537	444/430/269	19.08 ± 0.04c	5.60 ± 0.05b	12.40 ± 0.07c	3.64 ± 0.02c	[38]
18	13-*cis-β*-Carotene[c]	9.21	338/444	537	269	4.43 ± 0.01d	3.01 ± 0.03d	2.88 ± 0.02d	1.96 ± 0.01d	[38]

Values are means ± standard deviation. n = 3

[a-i]Means ± SD followed by different letters within the same column represent significant differences ($P < 0.05$)

[b]Identification confirmed by commercial standards

[c]Identification by comparison of MS data with the literature and their identification is tentative

in Tables 5 and 6. Twenty-two compounds, of which 13 belong to chlorophylls and nine to carotenoids, were identified in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) of leaves and rhizomes. There were twice as many chlorophylls in the analyzed leaves but 1.5 times more carotenoids in rhizomes. These compounds have not been found in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) so far.

The average content of chlorophylls in *Fallopia japonica* Houtt was 135.64 mg/100 g dm and was 1.6 times higher than in *Fallopia sachalinensis* (F.Schmidt). The average amount of chlorophylls in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) of leaves was 179.97 mg/100 g dm and it was 4 times higher than in rhizomes (Table 5). The major compounds in the analyzed extracts were pheophytin *b*, chlorophyll *a* and *b* (~ 34–46%, 29–5% and 11–27% of all compounds). The same results were obtained in wild garlic leaves by Lachowicz et al. [23] and *Moringa oleifera* leaves by Sreelatha et al. [36]. According to Lachowicz et al. [23], the presented content of chlorophylls in wild garlic leaves was around 2.6 times higher than in *Fallopia japonica* Houtt leaves. In broccoli or guava, the content of chlorophylls was 1.7 and 6.0 and 1.4 and 4.9 times lower than in leaves of *Fallopia japonica* Houtt *Fallopia sachalinensis* (F.Schmidt) [37, 38].

The average content of carotenoids in *Fallopia japonica* Houtt was 93.02 mg/100 g dm and was 1.6 times higher than in *Fallopia sachalinensis* (F.Schmidt). The average content of carotenoid in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) leaves was 100.23 mg/100 g dm which was 2.0 times higher than in rhizomes. The major compounds in the analyzed extracts were *all-trans-β*-carotene and *all-trans*-lutein (~ 52–76% and 20–8% of all carotenoids). The same results were obtained in wild garlic by Lachowicz et al. [23] and *Zea mays* by Drążkiewicz et al. [39] In the leaves of *Allium ursinum*, the content of carotenoids was 5.0 and 3.9 times higher than in the leaves of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) [23], but the content of carotenoids in broccoli and coriander leaves was around 2.5 and 1.3 times and 1.4 and 1.1 times lower than in leaves of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) [40]. The content of carotenoids in *Moringa oleifera* leaves was 1.4 and 1.1 times lower than the content of these compounds in leaves of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) [37].

Identification and quantification of triterpenoid compounds

The results of identification and quantification of triterpenoids in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) (leaves and rhizomes) are presented in Table 7. Three triterpenoids were identified as betulinic

Table 7 UV and MS spectra data of triterpenoids derivatives [mg/100 g dm] in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) leaves and rhizomes

No.	Compounds	Retention time (min)	λ (nm)	MS[M-H]⁻ molecular ion	*Fallopia japonica*		*Fallopia sachalinensis*		Literature
					Leaves	Rhizomes	Leaves	Rhizomes	
1	Betulinic acid[b]	6.99	210	455.3452	133.72 ± 0.27ca	29.02 ± 0.26b	32.20 ± 0.26b	96.97 ± 0.78c	[23]
2	Oleanolic acid[b]	7.66	201	455.3496	289.91 ± 0.58b	83.72 ± 0.75a	92.88 ± 0.74a	210.24 ± 1.68b	[23]
3	Ursolic acid[b]	8.36	201	455.3365	592.66 ± 1.19a	18.36 ± 0.17c	20.37 ± 0.16c	429.78 ± 3.44a	[23]

Values are means ± standard deviation. $n = 3$

[a]a–c: Means ± SD followed by different letters within the same column represent significant differences ($P < 0.05$)

[b]Identification confirmed by commercial standards

[c]Identification by comparison of MS data with the literature and their identification is tentative

(Rt = 6.99 min), ursolic (Rt = 7.66 min) and oleanolic acids (Rt = 8.36 min). The value of their molecular ion [M–H]⁻ was *m/z* 455.3 [23]. Triterpenoids have not been found in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) so far.

The average content of triterpenoids in *Fallopia japonica* Houtt was 573.70 mg/100 g dm and was 1.3 times higher than in *Fallopia sachalinensis* (F.Schmidt). The average content of triterpenoids in *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) leaves amounted to 580.87 mg/100 g dm and it was 1,5 times higher than in rhizomes. The major compound in leaves was ursolic acid (~58% of all triterpenoids), followed by oleanolic and betulinic acid (~29 and 13%). Similarly, the results regarding individual triterpenoids were presented in *Olea europaea* leaves [41]. In rhizomes, the major compound was oleanolic acid (~64%) and betulinic and oleanolic acids constituted 22% and 14%. According to Lachowicz et al. [23], the content of triterpenoids, namely betulinic, ursolic and oleanolic acids in wild garlic leaves was, respectively, 1.2 and 7.4 times lower and 1.2 times higher than in leaves of *Fallopia japonica* Houtt. In *Allium ursinum*, the major compound was oleanolic acid as it was in rhizomes of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt). The triterpenoids are dependent on several factors, such as environmental conditions, climate and degree of fruit maturity [41].

Cluster analysis

Cluster analysis (HCA) is a data analysis method, meaning that prior knowledge of the sample is not required. Cluster analysis enables interpretation of the results in a fairly intuitive, graphic way. HCA of bioactive compounds such as the polyphenols, carotenoids, chlorophylls, triterpenoids analyzed in samples, was used as an additional tool to assess heterogeneity among leaves (B) and rhizomes (A) of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) grown in Poland. Generally, cluster analysis presented three clear similarity clusters (Fig. 2). The highest correlation of

bioactive compounds from rhizomes was obtained for polymeric procyanidins and total phenolic compounds. The lowest concentration was obtained for phenolic acids and flavan-3-ols (monomeric and oligomeric). The difference between the rhizomes and leaves was that the leaves were richer in flavan-3-ols (monomeric and oligomeric) and contained a lower concentration of stilbenes and flavones and flavonols.

Principal component analysis (PCA)

The differences between anatomical parts of *Fallopia japonica* (Houtt.) and *Fallopia sachalinensis* (F.Schmidt) species in their polyphenolic profiles and antioxidant properties were emphasized during principal component analysis (PCA). Two major PCs for the researched anatomical parts of species *Fallopia japonica* (Houtt.) and *Fallopia sachalinensis* (F.Schmidt) for 96.70% of total variability: for PC1 81.63%, and for PC2 15.03% (Fig. 3).

PC1 indicated the differences between the concentrations of flavan-3-ols (monomers and oligomers), polymeric procyanidins, flavonols, phenolic acids, stilbenes, total triterpenoids, betulinic, oleanolic, ursolic acid, total carotenoids and chlorophylls and their derivatives. PC2 showed the comparison of feruloylquinic acid. The research results showed some differences between the species *Fallopia japonica* (Houtt.) and *Fallopia sachalinensis* (F.Schmidt) and their leaves and rhizomes. For example, rhizomes of *Fallopia sachalinensis* (F.Schmidt) showed the highest feruloylquinic acid. Leaves of *Fallopia japonica* (Houtt.) and *Fallopia sachalinensis* (F.Schmidt) demonstrated higher concentrations of total bioactive compounds and their derivatives. Figure 3 shows that rhizomes of *Fallopia japonica* (Houtt.) showed the lowest content of analyzed parameters.

Fig. 2 Hierarchical cluster analysis of bioactive compounds in rhizomes (A) and leaves (B) of *Fallopia japonica* and *Fallopia sachalinensis* grown in Poland
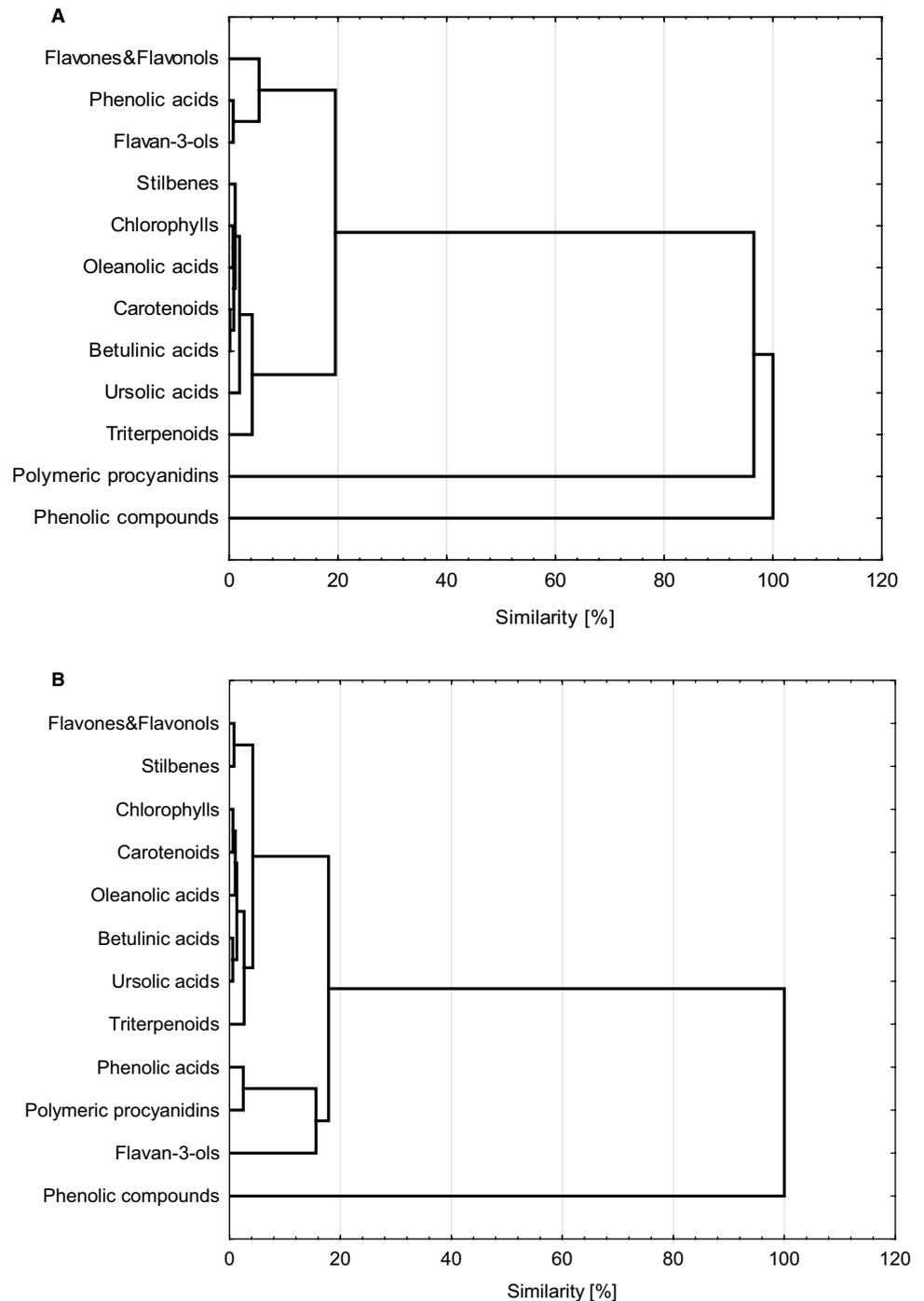
Conclusion

The results presented the significant effect of the measured anatomical parts of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) on the composition of bioactive compounds. In this study, 71 potential health-promoting compounds were identified, including for the first time 25 belonging to chlorophylls (13 compounds), carotenoids (nine compounds) and triterpenoids (three compounds) in *Fallopia*

using the UPLC-PDA-Q/TOF-MS method. The leaves and rhizomes of *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) were found to be a good source not only of phenolics (average 20408.18 and 2716.42 mg/100 g dm), but also chlorophylls (average 179.97 and 43.82 mg/100 g dm), carotenoids (average 100.23 and 53.25 mg/100 g dm) and triterpenoids (average 580.87 and 434.05 mg/100 g dm). The content of bioactive compounds in *Fallopia japonica* Houtt was around 8.0, 4.0, 2.0 and 1.3 times higher than
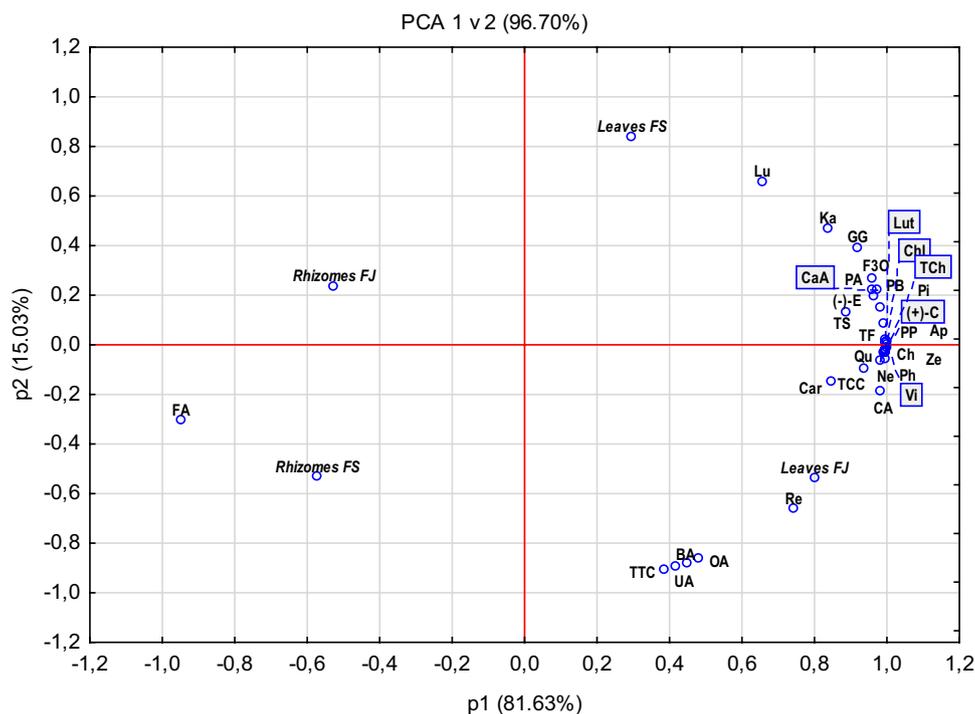
Fig. 3 PCA mean showing the relationship among phenols, carotenoids, chlorophylls, triterpenoids in leaves and rhizomes of *Fallopia japonica* (FJ) and *Fallopia sachalinensis* (FS). UA, ursolic acid; OA, oleanolic acid; BA, betulinic acid; TF, total flavonols; PA, phenolic acid; F3O, flavan-3-ols; PP, polymeric procyanidins; TS, total stilbenes; (+)-C, (+)-Catechin; (−)-E, (−)-Epicatechin; PB, procyanidin B; Lu, luteolin; Ap, apigenin; Qu, quercetin; Ka, kaempferol; GG, galloyl glucose; Ca, caffeoylquinic; Co, coumaroylquinic; Fe, feruloylquinic; Pi, piceid; Re, resveratrol; TCC, total carotenoid compounds; Lu, lutein; Ze, zeaxanthin; Vi, violaxanthin; Ca, carotene; Ne, neochrome; TCh, total chlorophyll compounds; Chl, chlorophyllide; Ch, chlorophyll; Ph, pheophytin; TTC, total triterpenoid compounds

the content of polyphenols, chlorophylls, carotenoids and triterpenoids in *Fallopia sachalinensis* (F.Schmidt). Used HPLC-UV-ABTS chromatograms of the *Fallopia japonica* Houtt and *Fallopia japonica* Houtt and *Fallopia sachalinensis* (F.Schmidt) rhizomes extracts revealed that (+)-catechin, procyanidin dimer B, Cis 5-*O*-caffeoylquinic, 3-*O*-p-coumaroylquinic acids and *trans*-resveratroloside possess the strongest radical scavenging capacity. Furthermore, the on-line HPLC-ABTS assay used enabled to evaluate the quality of materials and demonstrated the differences between the leaves and the rhizomes rich in antioxidants. The study showed that *Fallopia japonica* Houtt leaves and rhizomes can be considered a significant source of bioactive components. Overall, *Fallopia japonica* Houtt leaves and rhizomes can be an excellent source of individual bioactive compounds showing a broad spectrum of biological activity. This raw material will certainly an interesting product to use in the pharmaceutical, cosmetic and food industry as well as in phototherapy (as a medicinal preparation or medicinal herbal raw material). However, it is necessary to constantly monitor and collect information on the spread of the species.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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