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Total phenolic compounds and antioxidant capacity of six species of *Actaea* L. (Ranunculaceae)

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Abstract: Six species of the genus *Actaea* (Ranunculaceae) were analyzed for contents of soluble phenolic compounds, including flavonols, catechins, phenolic acids, coumarins, and tannins. Additionally, the contents of saponins, carotenoids, and chlorophylls a and b were determined. Extracts from the aboveground and underground parts of the studied plant species were tested for antioxidant activity using the DPPH assay. Based on the total phenolic content, four categories could be distinguished, regardless of species: "stems" (up to 22.7 mg/g), "roots" (up to 33.3 mg/g), "leaves" (up to 50.6 mg/g), and "inflorescences" (59.9 mg/g). An exception was the leaf extract of *Actaea erythrocarpa*, which had the highest concentration of phenolic compounds among all the plant samples, at 60.6 mg/g. The analysis of antioxidant activity revealed the same categories: the highest antiradical activity was shown by extracts from the leaves and inflorescences of *A. erythrocarpa* and *A. cimicifuga*. Thus, our assays indicate that extracts from the leaves and inflorescences of *A. erythrocarpa* and *A. cimicifuga* contain high concentrations of phenolic compounds, which correlate with their antiradical activity.

INTRODUCTION

The tribe *Cimicifugeae* Torrey et Gray is a part of the family Ranunculaceae Juss. and is represented by four genera and more than 49 plant species (Compton *et al.*, 1998a; Wang *et al.*, 2005; Yuan *et al.*, 2006; Erst *et al.*, 2023). This tribe includes *Anemonopsis* Siebold et Zucc. (1 species), *Actaea* L. (32 species), *Eranthis* Salisb. (14 species), and *Beesia* Balf. f. et W. W. Sm. (2 species), all of which exhibit a circumboreal distribution (Kostikova *et al.*, 2024; Erst *et al.*, 2023; Ling *et al.*, 2023). *Actaea* is a herbaceous genus belonging to Ranunculaceae.

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Traditionally, this genus has been thought to contain only eight species with fleshy berries (Tamura, 1995). J. Compton *et al.* (1998a) first used molecular data (*ITS*) to investigate its circumscription, where sampled species of *Actaea*, *Cimicifuga* Wernisch., and *Souliea* Franch. formed a clade. Based on two molecular and morphological datasets, the authors (Compton *et al.* 1998b) confirmed the result and accordingly redefined *Actaea* to include *Cimicifuga* and *Souliea*. This approach has been accepted by subsequent investigators (e.g., Xiang & Wang, 2018). The three genera (*Actaea*, *Cimicifug*a, and *Souliea*) are currently recognized as belonging to the genus *Actaea*, which exhibits a perennial habitus, racemose inflorescences, actinomorphic flowers with free carpels, follicular or baccate, multiseeded fruits, and follicles with distinct transverse or reticulate venation (Compton *et al.*, 1998; Erst *et al.*, 2023). According to the integrative morphological and molecular data, the genus is subdivided into eight sections (Compton *et al.*, 1998b; Luo *et al.*, 2016). However, species with wide geographic ranges, such as *A. racemosa* L., *A. rubra* (Aiton) Willd., *A. spicata* L., and *A. cimicifuga* L., may encompass different species, including cryptic species (Erst *et al.*, 2020). In contrast, some subspecies listed in Compton's revision (Compton *et al.*, 1998b) may have the taxonomic rank of a species, for example, *A. spicata* var. *acuminata* (Wall. ex Royle) Girke and *A. cimicifuga* var. *racemosa* (Regel) J. Compton. In our work, we use the generally accepted names of the species *Actaea erythrocarpa*, *A. cimicifuga*, *A. racemosa*, *A. rubra*, *A. podocarpa*, and *A. asiatica* (Luferov, 2004).

Some *Actaea* species are used as teas and food products (Mao, 1996; Zhang *et al.*, 1998). Their extracts contain triterpene saponins, phenylpropanoids, and other biologically active substances (Disch *et al.*, 2017; Shi *et al.*, 2020; Rashid *et al.*, 2023). Species of the genus *Actaea* have traditionally been included in the Chinese Pharmacopoeia and Japanese Pharmacopoeia (Hashida, 2011; Chinese Pharmacopoeia Commission, 2015; Bittner *et al.*, 2016). Additionally, pharmacological studies have proven the validity of extracts of *Actaea* species for treating gynecological disorders (Foster, 1999; Borrelli & Ernst, 2002). In this regard, attention has increasingly been paid to previously unstudied representatives of the genus *Actaea*.

It has been repeatedly proven that different species of the same genus can differ significantly in the profile and concentration of biologically active substances. Therefore, it is advisable to begin large-scale studies not with the isolation of individual compounds but with obtaining data on the total content of individual classes of substances, with simultaneous testing of the extracts for relevant types of activity.

This work is the first step toward preparing extracts from the underground and aboveground parts of six *Actaea* species, investigating the total content of phenolic compounds (including flavonols, catechin, phenolic acids, coumarins, and tannins) and other biologically active substances, and assessing their antioxidant activity.

METHOD

Plant material.

For the study, the leaves, inflorescences, stems, and roots of *A. erythrocarpa* and *A. cimicifuga* were collected during the flowering phase in Russia (Krasnoyarsk Krai and the Republic of Khakassia, Table 1). The leaves of *A. racemosa*, *A. rubra*, *A. podocarpa*, and *A. asiatica* were collected during the fruiting phase in England, the Royal Botanic Gardens, Kew. The plants were collected in dry sunny weather and were dried in the shade in well-ventilated rooms. After drying, the raw materials were divided into organs, crushed, and passed through a 3 mm sieve to obtain a representative sample.

Table 1. Descriptions of the analyzed plant samples.

№	ID	Species	Plant part	Sampling site	Collection date; collector
1.	AEL	*A. erythrocarpa*	Leaves	Russia, Krasnoyarsk Krai, Minusinsk district, vicinity of village Znamenka; 53°32’43.4” N, 91°56’11.1” E, 461 m a.s.l.	15.07.2023; T.V. Leonova
2.	AEI	*A. erythrocarpa*	Inflorescences	Russia, Krasnoyarsk Krai, Minusinsk district, vicinity of village Znamenka; 53°32’43.4” N, 91°56’11.1” E, 461 m a.s.l.	15.07.2023; T.V. Leonova
3.	AES	*A. erythrocarpa*	Stems	Russia, Krasnoyarsk Krai, Minusinsk district, vicinity of village Znamenka; 53°32’43.4” N, 91°56’11.1” E, 461 m a.s.l.	15.07.2023; T.V. Leonova
4.	AER	*A. erythrocarpa*	Roots	Russia, Krasnoyarsk Krai, Minusinsk district, vicinity of village Znamenka; 53°32’43.4” N, 91°56’11.1” E, 461 m a.s.l.	15.07.2023; T.V. Leonova
5.	ACL	*A. cimicifuga*	Leaves	Russia, Republic of Khakassia, Bogradsky district, vicinity of village Bograd; 54°14’28.4’’N, 90°46’26.1’’E 544 m a.s.l.; birch forest.	19.08.2023 T.V. Leonova
6.	ACI	*A. cimicifuga*	Inflorescence	Russia, Republic of Khakassia, Bogradsky district, vicinity of village Bograd; 54°14’28.4’’N, 90°46’26.1’’E 544 m a.s.l.; birch forest.	19.08.2023 T.V. Leonova
7.	ACS	*A. cimicifuga*	Stems	Russia, Republic of Khakassia, Bogradsky district, vicinity of village Bograd; 54°14’28.4’’N, 90°46’26.1’’E 544 m a.s.l.; birch forest.	19.08.2023 T.V. Leonova
8.	ACR	*A. cimicifuga*	Roots	Russia, Republic of Khakassia, Bogradsky district, vicinity of village Bograd; 54°14’28.4’’N, 90°46’26.1’’E 544 m a.s.l.; birch forest.	19.08.2023 T.V. Leonova
9.	AC2L	*A. cimicifuga*	Leaves	Russia, Republic of Khakassia, Tashtypskiy district, near Abaza city; 52°35’13.18” N, 90°04’09.0” E; 502 m a.s.l.; mixed forest.	04.08.2023; A.S. Erst , S.V. Smirnov, V.O. Tolmachev, I.A. Shestakov
10.	AC2I	*A. cimicifuga*	Inflorescence	Russia, Republic of Khakassia, Tashtypskiy district, near Abaza city; 52°35’13.18” N, 90°04’09.0” E; 502 m a.s.l.; mixed forest.	04.08.2023; A.S. Erst , S.V. Smirnov, V.O. Tolmachev, I.A. Shestakov
11.	AC2S	*A. cimicifuga*	Stems	Russia, Republic of Khakassia, Tashtypskiy district, near Abaza city; 52°35’13.18” N, 90°04’09.0” E; 502 m a.s.l.; mixed forest.	04.08.2023; A.S. Erst , S.V. Smirnov, V.O. Tolmachev, I.A. Shestakov
12.	ARL	*A. racemosa*	Leaves	United Kingdom, London, Royal Botanic Gardens, Kew	24.09.2023 A.S. Erst
13.	ARuL	*A. rubra*	Leaves	United Kingdom, London, Royal Botanic Gardens, Kew	24.09.2023 A.S. Erst
14.	APL	*A. podocarpa*	Leaves	United Kingdom, London, Royal Botanic Gardens, Kew	24.09.2023 A.S. Erst
15.	AAL	*A. asiatica*	Leaves	United Kingdom, London, Royal Botanic Gardens, Kew	24.09.2023 A.S. Erst

Ethanol Extract Preparation

Approximately 0.5 g (precise weight was recorded) of raw material was placed into a 100 mL round-bottom flask with a ground-in stopper. The raw material was then covered with 30 mL of 70% ethanol, and the flask was attached to a reflux condenser and placed in a boiling water bath for 30-min incubation. The flask was shaken periodically to wash the raw material particles off the walls. After that, the flask with the extract was cooled, and the first portion of the extract was passed through a paper filter into a 100 mL conical flask with a ground-in stopper. Next, the raw material from the filter was then placed back into a round-bottom flask, covered again with 30 mL of 70% ethanol, and extracted for 30 min in the boiling water bath. The second portion of the extract was cooled and filtered into the first portion of the extract in the 100 mL flask. The procedure was repeated one more time. The three portions of the extract were then mixed, and the volume of the resulting combined extract was measured (Kalendar *et al.*, 2023).

Estimation of Antiradical Activity

Free-radical–scavenging capacity of the samples was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Kumarasamy *et al.*, 2007; Gawron-Gzella *et al.*, 2016), with modifications. For this purpose, a 2 mL aliquot of the extract (dissolved in 70% ethanol to concentrations in the range of 5–2500 µg/mL) was mixed with 3 mL of a DPPH solution (62 µg/mL in ethanol). After 30-minute incubation in darkness at room temperature, optical density (A) was measured at 517 nm against a blank sample.

Free-radical–scavenging activity was calculated as percent inhibition using the following formula:

$$I\% = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100,$$

where A_{blank} is the optical density of a control solution (containing all reagents except the tested extract), and A_{sample} is the optical density of the sample.

The results were expressed in IC_{50}, DPPH, defined as the concentration of an antioxidant that causes 50% DPPH loss during the DPPH radical–scavenging activity assay. Solutions of 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and ascorbic acid (AA) at concentrations of 2.5–50.0 µg/mL served as positive controls.

Analysis of Phenolic Contents

Quantification of phenolic compounds.

The total phenolic content was determined using the Folin–Ciocalteu reagent (Djeridane *et al.* 2006). An extract (0.5 mL) was placed in a 5 mL volumetric flask, then 2.5 mL of the Folin–Ciocalteu reagent (diluted at 1:10 with distilled water) and 2 mL of a 7.5% aqueous sodium carbonate solution were added and shaken well. The mixture was kept at 45 °C in the water bath for 15 min. Absorbance was measured at a wavelength of 765 nm using an SF-56 spectrophotometer (Lomo, St. Petersburg, Russia). A blank sample consisting of distilled water and reagents served as a control. Gallic acid (Sigma-Aldrich, Darmstadt, Germany, concentration of 0.002–0.010 mg/mL) was used as a reference.

Total phenolic acid content.

The analysis of total phenolic (hydroxycinnamic) acids was performed according to Katanić *et al.* (2015). First, 5 mL of water was added to 1 mL of the extract. Then, 1 mL of HCl (0.1 M), 1 mL of the Arnow reagent (10% [*w*/*v*] of sodium molybdate and 10% [*w*/*v*] of sodium nitrite), and 1 mL of NaOH (1 M) were added to the mixture to bring it to a total volume of 10 mL. Optical density was measured immediately at 490 nm. The conversion factor was calculated using caffeic acid (Sigma-Aldrich, Darmstadt, Germany, concentration of 0.02–0.10 mg/mL). The results were expressed in mg/g.

Determination of flavonoid contents.

This procedure was performed by a spectrophotometric method based on the complexation reaction between flavonoids and aluminum chloride (Brighente *et al.* 2007). To one test tube, 0.2 mL of a 2% ethanol solution of aluminum chloride was added, and 1–2 drops of 30% acetic acid were added to the other. The volume of each solution was then brought to the mark with 96% ethanol. Each solution was mixed, and after 40 min, the optical density of the solution containing aluminum chloride was measured using the SF-56 spectrophotometer (Lomo, St. Petersburg, Russia) at a wavelength of 415 nm in a cuvette with a 1 cm light path, using the acetic acid solution as a control. The amount of flavonoids in each sample was determined using a calibration curve for rutin (Chemapol, Mumbai, MH, India, concentration of 0.01–0.10 mg/mL).

Quantification of catechins.

The content of catechins was determined spectrophotometrically by established methodology based on their ability to produce a crimson color in a solution of vanillin in concentrated hydrochloric acid (Sun *et al.* 1998; Kukushkina *et al.* 2024). A 0.8 mL aliquot of the extract was placed into two test tubes. Next, 4 mL of a 1% solution of vanillin in concentrated hydrochloric acid was added to one tube, and the volumes in both tubes were adjusted to 5 mL with concentrated hydrochloric acid. The tube without vanillin served as a control. In the presence of catechins, the sample became pink, raspberry, or orange-red in hue. After 5 min, the color intensity was measured using the SF-56 spectrophotometer (Lomo, St. Petersburg, Russia) at 504 nm in a cuvette with a 1 cm light path. The standard curve was constructed with (±)-catechin (Sigma, St. Louis, MO, USA; concentration of 0.001–0.020 mg/mL).

Quantification of tannins.

The assay of tannins (hydrolyzable tannins) was performed by the method proposed by Fedoseeva (Fedoseeva, 2005). An extract (10 mL) was placed into a 100 mL volumetric flask, and 10 mL of a 2% aqueous solution of ammonium molybdate was added. The content was brought to the nominal volume with distilled water and incubated for 15 min. The intensity of the resulting color was measured using the SF-56 spectrophotometer (Lomo, St. Petersburg, Russia) at 420 nm in a cuvette with a 1 cm light path. A government standard sample of tannic acid (Sigma, St. Louis, MO, USA, concentration of 0.01–0.10 mg/mL) served as a standard.

Total content of coumarins.

For the quantification of coumarins, the method proposed by Amorim *et al.* (2012) was employed. In brief, 500 µL of the extract was transferred to a test tube, to which 2 mL of distilled water and 500 µL of a 5% lead acetate solution were added. Each sample was shaken, and then 7 mL of distilled water was introduced. Next, 2 mL of the resulting solution was transferred to a new test tube, and 8 mL of 0.1 M hydrochloric acid was added. The samples were then incubated at room temperature for 30 min. Measurements were carried out at 320 nm using the SF-56 spectrophotometer (Lomo, St. Petersburg, Russia) in a cuvette with a 1 cm light path. The amount of coumarins in each sample was determined using a calibration curve for coumarin (Sigma, St. Louis, MO, USA).

Quantifications of Other Biologically Active Substances

Quantification of carotenoids and chlorophylls a and b.

Concentrations of pigments (carotenoids and chlorophylls a and b) were determined in an acetone–ethanol extract (Ermakov *et al.*, 1987). A 0.1 g air-dried sample was ground in a mortar to a homogeneous mass with a successive addition of 0.1 g of calcium carbonate (to neutralize organic acids, as carotenoids are unstable in an acidic environment), 1 mL of dimethylformamide (for pigment stability), and 2 g of anhydrous sodium sulfate. Pigments were extracted first with acetone (20 mL: once, and 5 mL: two times), and the extraction was continued with 96% ethanol (5 mL: three times) to extract lycopene. Next, exhaustive extraction was continued with acetone until discoloration. The volume of the combined extract was measured. The carotenoid content was measured at 662 nm (for chlorophyll a), at 644 nm (for chlorophyll b), and at 440.5 nm (for carotenoids) using the SF-56 spectrophotometer (Lomo, St. Petersburg, Russia) in a cuvette with a 1 cm light path. The control was 96% ethanol. The concentration of pigments (mg/dm^3) was calculated using the formula:

$$C_a = 9.784 \times D_{662} - 0.99 \times D_{644},$$

where D is the optical density of the extract; C_a is the concentration of chlorophyll a, mg/L.

$$C_b = 21.426 \times D_{644} - 4.65 \times D_{662},$$

where D is the optical density of the extract; C_b is the concentration of chlorophyll b, mg/L.

$$C_a + C_b = 5.134 \times D_{662} + 20.436 \times D_{644},$$

where D is the optical density of the extract; $C_a + C_b$ is the concentration of the sum of chlorophylls a and b, mg/L.

$$C_{car} = 4.695 \times D_{440.5} - 0.268 \times (C_a + C_b),$$

that is

$$C_{car} = 4.695 \times D_{440.5} - 0.268 \times (5.134 \times D_{662} - 20.436 \times D_{644}),$$

where D is the optical density of the extract, and C_{car} is the concentration of carotenoids, mg/L. The concentration of pigments (mg/100 g) was calculated using the formula:

$$X = C \times V \times V_2 \times 100/(M \times V_1 \times 1000),$$

where C_{car} is the concentration of carotenoids and chlorophylls a and b, mg/L; V is the volume of the initial extract, mL; V_1 is the volume of the initial extract used for dilution, mL; V_2 is the volume of the diluted extract, mL; and M is the absolute dry weight of the raw material, g.

Determination of saponin contents.

For quantification of total saponins, a direct spectrophotometric method was employed based on the reaction between triterpene saponins and sulfuric acid (Pisarev *et al.*, 2009). A precisely weighed dry extract (0.5–1.0 g) was dissolved in 10 mL of a mixture of glacial acetic acid, hydrochloric acid, and purified water (3.5:1.0:5.5) for hydrolysis of glycosides via 2-h incubation in the boiling water bath. Then, the hydrolysate was cooled, diluted with distilled water in a 1:2 ratio, and filtered. The residue on the filter was rinsed with distilled water, put with the filter into a 25 mL volumetric flask, and dissolved in 25 mL of hot ethanol. To 1 mL of this ethanol solution, 4 mL of concentrated sulfuric acid was added, and absorbance was measured at 310 nm after 10 min using the SF-56 spectrophotometer (Lomo, St. Petersburg, Russia). Concentrated sulfuric acid served as a blank. To calculate saponin concentration, a calibration curve for oleanolic acid (Sigma-Aldrich, Saint Louis, MO, USA) was built in the range of 0.01–0.4 mg/mL. The results were expressed as milligrams of oleanolic acid equivalent per gram of dry extract.

Statistical Analysis

All experiments were conducted with two biological replicates and with three technical replicates per treatment. Multiple comparisons were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) test to evaluate the significance of differences between the means. The data were analyzed in Microsoft Excel 2010 (Microsoft Corp., Redmond, Washington, USA) and GraphPad Prism v.6.01 (GraphPad Software, San Diego, CA, USA). Spearman's correlation coefficients were calculated to analyze associations between the concentrations of biologically active substances and mean antiradical activity. The significance level was set at $p \leq 0.01$ and $p \leq 0.05$, and data are presented as means ± standard deviation.

RESULTS

At the first stage of our work, 15 extracts were prepared from the aboveground organs (stems, leaves, and inflorescences) and underground organs (roots and rhizomes) parts of six *Actaea* species (Table 1). For some species selected for this study, no data on the profile of their secondary metabolites are available, for some species, these data are fragmentary, and a few species have been well studied before. Nonetheless, data on the antioxidant activity of the analyzed species from Russia have been reported for the first time.

Extraction was carried out using 70% ethanol; this technique enables extraction of a wide range of phenolic compounds: simple phenols, phenolic acids, and flavonoid glycosides. It was found that the studied plant species (Table 2) exhibit different phenolic contents, with the concentrations ranging from 14 mg/g (the lowest) to 61 mg/g (the highest).

Table 2. Contents (mg/g of dry weight) of biologically active substances of a phenolic nature in extracts from organs of *Actaea* species.

ID	Phenolic compounds	Phenolic acids	Flavonoids	Catechins	Tannins	Coumarins
AEL	60.64 ± 2.41^{a}	72.81 ± 2.91^{b}	14.92 ± 0.45^{d}	4.34 ± 0.07^{a}	119.77 ± 1.02^{d}	86.77 ± 3.47^{b}
AEI	59.90 ± 2.38^{a}	$50.72\pm2.03^{d,e}$	47.41 ± 1.42^{b}	2.59 ± 0.04^{d}	105.41 ± 0.90^{f}	86.82 ± 3.47^{b}
AES	$18.00\pm0.71^{f,g}$	19.20 ± 0.77^{f}	5.70 ± 0.17^{h}	$1.09\pm0.02^{h,i}$	23.96 ± 0.20^{l}	$30.84\pm1.23^{j,k}$
AER	31.50 ± 1.25^{e}	44.22 ± 1.77^{e}	1.69 ± 0.05^{j}	$1.16\pm0.02^{g,h}$	55.58 ± 0.47^{j}	45.77 ± 1.83^{h}
ACL	$46.39\pm1.84^{b,c}$	60.44 ± 2.42^{c}	11.63 ± 0.35^{e}	3.79 ± 0.07^{b}	97.32 ± 0.83^{g}	$72.12\pm2.88^{d,e}$
ACI	58.59 ± 2.33^{a}	59.03 ± 2.36^{c}	29.29 ± 0.88^{c}	3.24 ± 0.06^{c}	91.09 ± 0.77^{h}	$83.00\pm3.32^{b,c}$
ACS	22.74 ± 0.90^{f}	24.34 ± 0.97^{f}	3.75 ± 0.11^{i}	2.01 ± 0.03^{e}	49.55 ± 0.42^{k}	35.85 ± 1.43^{i}
ACR	33.27 ± 1.32^{e}	46.40 ± 1.86^{e}	$3.55\pm0.11^{i,j}$	0.96 ± 0.02^{i}	71.17 ± 0.60^{i}	$50.69\pm2.03^{g,h}$
AC2L	$45.41\pm1.80^{b,c}$	$57.27\pm2.29^{c,d}$	13.77 ± 0.41^{d}	4.41 ± 0.08^{a}	96.45 ± 0.82^{g}	$76.10\pm3.04^{c,d}$
AC2I	59.43 ± 2.36^{a}	60.16 ± 2.41^{c}	50.33 ± 1.51^{a}	3.04 ± 0.05^{c}	109.58 ± 0.93^{e}	95.46 ± 3.82^{a}
AC2S	13.63 ± 0.54^{g}	11.21 ± 0.45^{g}	1.67 ± 0.05^{j}	0.96 ± 0.02^{i}	18.56 ± 0.16^{m}	23.08 ± 0.92^{k}
ARL	42.87 ± 1.70^{d}	68.36 ± 2.73^{b}	$7.79\pm0.23^{f,g}$	2.61 ± 0.04^{d}	130.16 ± 1.11^{c}	$64.35\pm2.57^{e,f}$
AruL	43.32 ± 1.72^{d}	74.01 ± 2.96^{b}	9.13 ± 0.27^{f}	1.29 ± 0.02^{g}	145.07 ± 1.23^{b}	$57.27\pm2.29^{f,g}$
APL	50.56 ± 2.01^{b}	93.03 ± 3.72^{a}	11.35 ± 0.34^{e}	1.74 ± 0.03^{f}	187.87 ± 1.60^{a}	$71.38\pm2.86^{d,e}$
AAL	$47.88\pm1.90^{b,c}$	91.12 ± 3.64^{a}	$7.20\pm0.22^{g,h}$	1.24 ± 0.02^{g}	146.62 ± 1.25^{b}	60.63 ± 2.43^{f}

Note: Means followed by different letters in the same column are significantly different ($p \leq 0.05$), according to Tukey's HSD test.

In terms of the concentration of phenolic compounds, the samples were clearly classified into four categories: extracts from stems (13 to 22 mg/g); extracts from roots and rhizomes (31 to 33 mg/g); extracts from leaves (42 to 50 mg/g); and extracts from inflorescences with the highest concentration of phenolic compounds (up to 59 mg/g). An exception was the leaf extract of *A. erythrocarpa*, with the concentration of phenolic compounds exceeding that of all the assayed extracts, which amounted to more than 60 mg/g. Values of the antiradical activity in the analyzed extracts are presented in Figure 1.

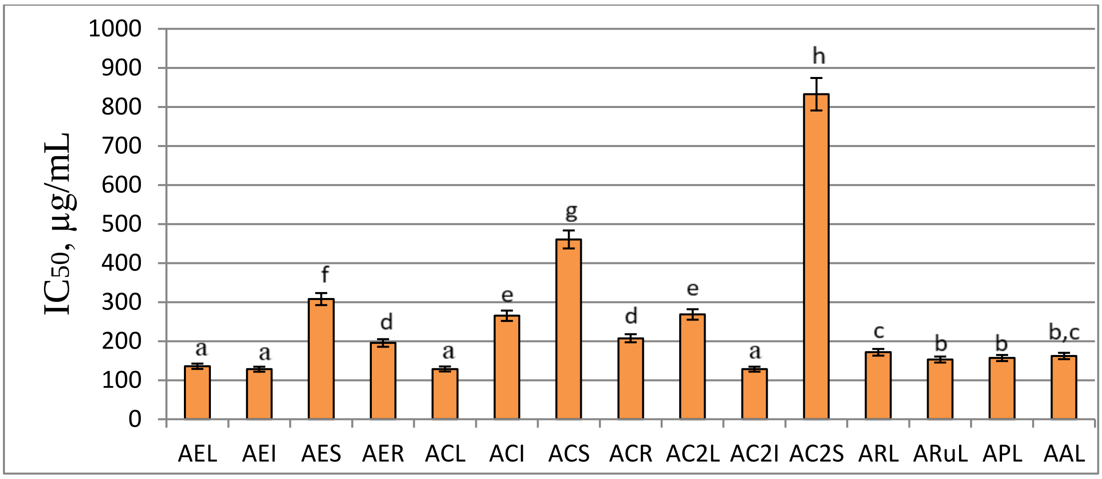

Figure 1. DPPH radical–scavenging activity of extracts of *Actaea* species. IC_{50}, µg/mL: the concentration of the extract that causes 50% DPPH loss in the DPPH radical–scavenging activity assay. Different letters indicate significant differences in this parameter ($p \leq 0.05$), according to Tukey's HSD test.

Most extracts manifested moderate activity against the DPPH radical, in the IC_{50} range of 152.85 to 268.3 µg/mL. Samples with a strong antiradical effect (extracts from the leaves and inflorescences of *A. erythrocarpa* and *A. cimicifuga*) yielded extracts with medium-to-high concentrations of soluble phenolic compounds. In this category, the IC_{50} range was 128.7 to 135.65 µg/mL. The lowest antioxidant activity was shown by stem extracts, which is also consistent with the observed low concentrations of phenolic compounds in these extracts. It should be noted that the antioxidant activity of standard substances—Trolox (IC_{50} = 7.74 µg/mL) and ascorbic acid (IC_{50} = 8.69 µg/mL)—was significantly higher than compared of extracts from any organs of *Actaea* species.

The extracts of the studied *Actaea* species were analyzed for the contents of phenolic acids, flavonoids, catechins, tannins, coumarins (Table 2), and other biologically active substances: plant pigments (carotenoids and chlorophylls a and b) and saponins (Table 3).

Table 3. Contents (dry weight) of carotenoids, chlorophylls a and b, and saponins in extracts of *Actaea* species.

ID	Carotenoids (mg/100 g)	Chlorophyll a (mg/100 g)	Chlorophyll b (mg/100 g)	Saponins (mg/g)
AEL	760.59 ± 7.45^{g}	346.54 ± 3.36^{f}	210.86 ± 7.38^{e}	173.99 ± 0.20^{f}
AEI	183.16 ± 1.79^{h}	$32.33 \pm 0.31^{k,l}$	$27.27 \pm 0.95^{h,i,j}$	248.29 ± 0.40^{b}
AES	$76.74 \pm 0.75^{j,k}$	$38.45 \pm 0.37^{j,k}$	$33.56 \pm 1.17^{h,i}$	$121.43 \pm 0.30^{h,i}$
AER	$68.47 \pm 0.67^{j,k}$	8.69 ± 0.08^{m}	$18.04 \pm 0.63^{i,j}$	31.95 ± 0.20^{l}
ACL	822.29 ± 8.06^{f}	316.38 ± 3.07^{g}	161.55 ± 5.65^{f}	176.12 ± 0.19^{e}
ACI	131.06 ± 1.28	$52.18 \pm 0.51^{h,i}$	66.97 ± 2.34^{g}	284.19 ± 9.91^{a}
ACS	95.31 ± 0.93	$46.21 \pm 0.45^{i,j}$	40.79 ± 1.43^{h}	95.21 ± 0.17^{k}
ACR	$70.21 \pm 0.69^{j,k}$	4.87 ± 0.05^{m}	10.71 ± 0.37^{j}	100.02 ± 0.29^{k}
AC2L	1369.58 ± 13.42^{c}	531.73 ± 5.16^{d}	235.65 ± 8.25^{d}	195.55 ± 0.77^{d}
AC2I	207.76 ± 2.04^{h}	57.33 ± 0.56^{h}	36.83 ± 1.29^{h}	228.24 ± 0.72^{c}
AC2S	59.93 ± 0.59^{k}	26.56 ± 0.26^{l}	$25.16 \pm 0.88^{h,i,j}$	110.92 ± 0.60^{j}
ARL	1447.11 ± 14.18^{c}	565.94 ± 5.49^{b}	265.81 ± 9.30^{b}	$117.85 \pm 0.07^{i,j}$
ARuL	1312.38 ± 12.86^{e}	469.09 ± 4.55^{e}	$235.93 \pm 8.26^{c,d}$	126.10 ± 0.08^{h}
APL	1705.75 ± 16.72^{b}	542.38 ± 5.26^{c}	$253.84 \pm 8.88^{b,c}$	143.05 ± 0.04^{g}
AAL	1786.05 ± 17.50^{a}	682.13 ± 6.62^{a}	335.35 ± 11.74^{a}	129.16 ± 0.03^{h}

Note: Means followed by different letters in the same column are significantly different ($p \leq 0.05$), according to Tukey's HSD test.

The highest concentrations of tannins (up to 146 mg/g), coumarins (up to 95 mg/g), phenolic acids (up to 93 mg/g), flavonoids (up to 50 mg/g), and catechins (up to 4 mg/g) were recorded in extracts from leaves and inflorescences. The lowest concentrations of these compounds were found in extracts from stems, rhizomes, and roots. Carotenoids, saponins, and chlorophylls a and b demonstrated a similar pattern of distribution among different plant organs.

The analysis of correlations between biological activity and chemical composition of the studied *Actaea* species revealed significant correlations with some of the assayed compounds (Figure 2).

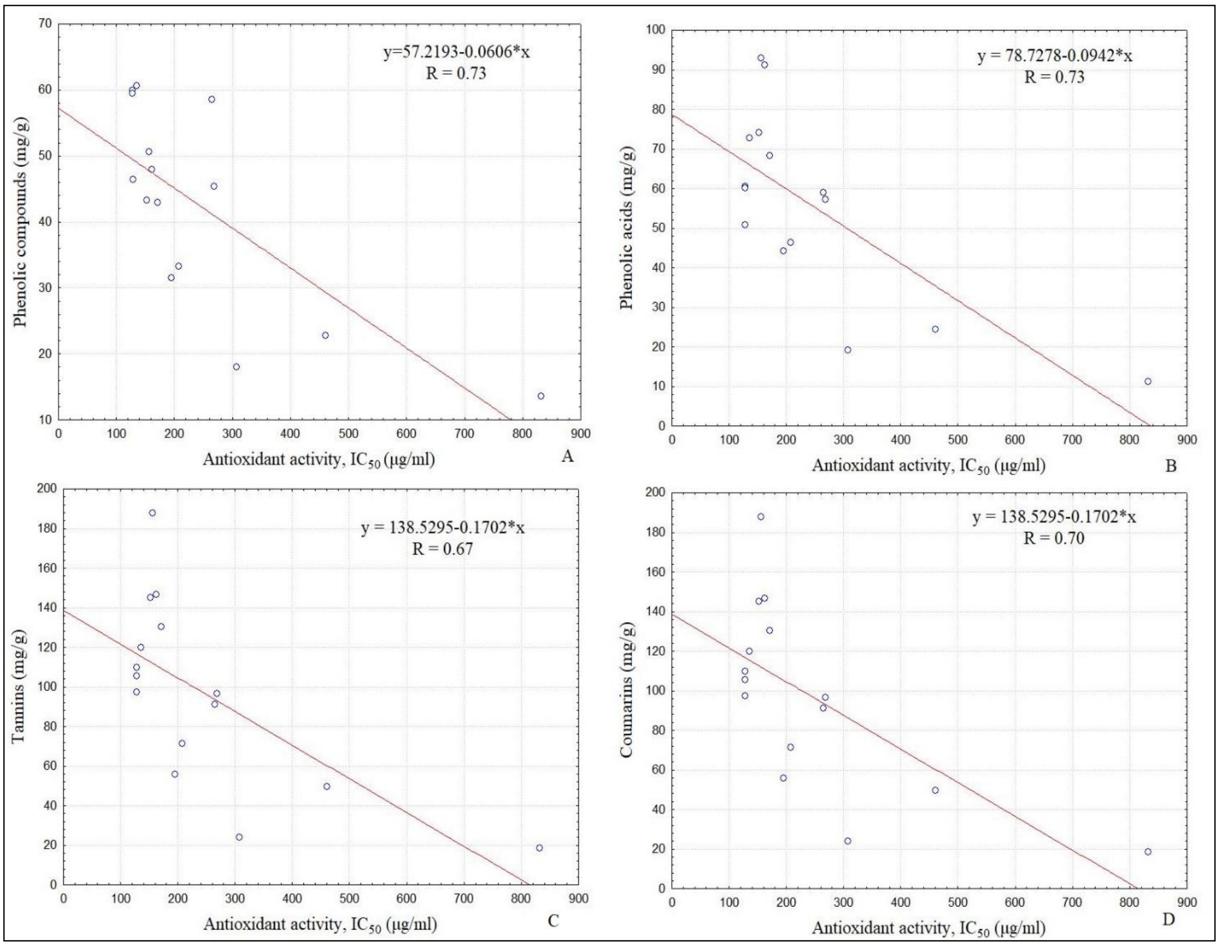

Figure 2. Correlations between antioxidant activity (IC_{50}, µg/mL) and contents of phenolic compounds, mg/g (A), phenolic acids, mg/g (B), tannins, mg/g (C), and coumarins, mg/g (D) in the extracts of the *Actaea* species. Equations of linear dependencies "y–x" are given, where x represents IC_{50} (µg/mL), and y denotes the content of phenolic compounds, mg/g (A), of phenolic acids, mg/g (B), of tannins, mg/g (C), or of coumarins, mg/g (D); correlation coefficients (R) are presented too.

The most pronounced correlation between antioxidant activity and chemical composition was found in pairs involving the total content of phenolic compounds (R = 0.72903, $p < 0.05$), phenolic acids (R = 0.72958, $p < 0.05$), tannins (R = 0.670487, $p < 0.05$), or coumarins (R = 0.694508, $p < 0.05$) (Figure 2). The other analyzed biologically active substances of the *Actaea* species did not significantly correlate with the antioxidant activity.

DISCUSSION and CONCLUSION

Of all studied extracts, the extracts from the leaves and inflorescences of *A. erythrocarpa* and *A. cimicifuga* exhibited the highest concentration of total phenolics and flavonoids, catechins, and coumarins. The elevated concentration of saponins was observed in inflorescence extracts of *A. cimicifuga*. High concentrations of phenolic acids and tannins were detected in leaf extracts of *A. podocarpa*. Overall, carotenoid concentrations in the assayed extracts were low, but the highest levels were observed in extracts of *A. podocarpa* and *A. asiatica*. Furthermore, leaf extracts of the latter exhibited elevated concentrations of chlorophylls a and b. Analysis of the distribution of the studied substances among plant organs revealed the lowest concentrations in stems and roots, and the highest concentrations in leaves and inflorescences. Thus, both the underground and aboveground parts of *Actaea* species should be included in a comprehensive study to enhance our understanding of their biology and ecology.

A considerable amount of data is available in the literature on the quantification of phenolic compounds in *Actaea* species (Sakakibara *et al.*, 2003; Jiang *et al.*, 2005). Specific analytical methods have been developed for this purpose (He *et al.*, 2006). Nevertheless, some studies refer to "*Actaea* rhizomes" without specifying the species (Li *et al.*, 2012). Most publications focus on biologically active compounds from the rhizomes and roots of black cohosh (*A. racemosa*), and the proposed methods generally cannot differentiate this species from other *Actaea* species. Nonetheless, some data on the content of phenolic compounds in extracts prepared from the rhizomes and roots of *Actaea* species (using solvents of varied polarity) are in the range of 12.5 to 65 mg/g (Li *et al.*, 2012), which is consistent with our findings.

The prepared extracts moderately reduced the concentration of free radical DPPH, whereas the strongest effect was recorded for extracts from the leaves and inflorescences of *A. erythrocarpa* and *A. cimicifuga*. By contrast, according to the literature, in other analyzed species (*A. racemosa*, *A. rubra*, *A. pachypoda*, and others), free radicals are actively neutralized by extracts from the underground parts (Burdette *et al.*, 2002; Nuntanakorn *et al.*, 2007; Da *et al.*, 2015). Such antiradical activity is likely due to both the low concentration of phenolic compounds and the presence of phenolic compounds associated, for example, with carbohydrates, which are weak antioxidants or significantly reduce the antiradical activity. In addition, the low activity may reflect limitations of the assay, as some compounds readily scavenge the DPPH radical, whereas other antioxidants react slowly or not at all with DPPH. To avoid subjectivity, simultaneous use of different assays of antioxidant activity is recommended to identify differences between the reactions of compounds extracted from the plant samples (Vasco *et al.*, 2008).

The analysis of biological activity and chemical composition of the investigated *Actaea* species revealed a significant correlation only with some of the studied compounds. These findings are consistent with data obtained in previous studies on *Actaea* plants. For example, Chinese researchers (Li *et al.*, 2012) reported a significant positive correlation between total phenolic content and antioxidant activity in extracts from *Actaea* rhizomes (mean $R = 0.56$). Yet, the concentrations of sugars and saponins did not manifest a positive correlation with the antioxidant activity (average R values were −0.20 and −0.26 for total sugars and total saponins, respectively). Among all phenolic compounds, three phenolic acids (caffeic, ferulic, and isoferulic) also showed positive correlations (average R values were 0.51, 0.50, and 0.51 for caffeic, ferulic, and isoferulic acids, respectively) (Li *et al.*, 2012). In some studies, concerning *Actaea* species, individual compounds were isolated and identified, and their antioxidant activities were tested. For example, the reported half-maximal inhibitory concentrations in terms of scavenging of reactive oxygen species were as follows: IC_{50} = 14.1 µM (fukinolic acid), IC_{50} = 27.7 µM (cimicifugic acid A), IC_{50} = 22.9 µM (cimicifugic acid B), and IC_{50} = 20.5 µM (cimicifugic acid F) (Burdette *et al.*, 2002). It was found that in the DPPH assay, the radical-scavenging activity of the phenolic acids identified in *Actaea* plants can be ranked in the following descending order: caffeic acid (IC_{50} 58.3 µM) > ferulic acid (IC_{50} 121.4 µM) > isoferulic acid (IC_{50} 289.1 µM) (Nuntanakorn *et al.*, 2007).

In this study, we quantified total phenolic content (including flavonols, catechins, phenolcarboxylic acids, coumarins, and tannins) and measured saponins, carotenoids, and chlorophylls a and b in 15 extracts prepared from the aboveground and underground parts of six *Actaea* species. The plants for the assays were collected from natural populations in Siberia (Russia) and in the Royal Botanic Gardens, Kew (England), including species that have not yet been studied in this regard. The results revealed a wide variation in the concentrations of the analyzed classes of substances among the tested plant samples; however, the highest concentrations were observed in leaves and inflorescences. Therefore, in addition to the traditionally utilized underground parts, the aboveground parts of *Actaea* species represent a promising source of secondary metabolites and exhibit significant biological activity. It was confirmed that the antioxidant activity of the extracts of *Actaea* species positively correlates with the content of phenolic compounds, including phenolic acids, tannins, and coumarins. The highest antiradical activity in the DPPH assay was recorded for extracts from the leaves and inflorescences of *A. erythrocarpa* and *A. cimicifuga*. These species warrant a separate, comprehensive investigation of their chemical composition, which will be a focus of our future work.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Data Availability Statement

Research data is available and can be sent with e-maill upon request.

Authorship Contribution Statement

Andrey S. Erst: Resources, Writing-original draft, and Writing-review & editing. **Natalia V. Petrova**: Formal Analysis, and Writing-original draft. **Tatiana V. Leonova**: Resources, and Visualization. **Tatiana M. Shaldaeva**: Investigation, and Methodology. **Anastasiia S. Gusar**: Visualization, and Software. **Sergey V. Smirnov**: Resources, and Software. **Kunli Xiang**: Software, and Writing-original draft. **Wei Wang**: Writing-review & editing. **Vera A. Kostikova**: Investigation, Methodology, and Formal Analysis, Writing-original draft, and Writing-review & editing.

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