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Phenolic Diversity and Antioxidant Activity of *Artemisia abrotanum* L. and *Artemisia absinthium* L. during Vegetation Stages

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Abstract: Over the past ten years, particular attention has been paid to *Artemisia* L. genus plants and their chemical composition. *Artemisia abrotanum* L. and *Artemisia absinthium* L., which are traditional medicinal, aromatic plants with a strong digestive activity that is determined by the various phytochemicals. The research aim was to determine the variation in polyphenols in the samples of different vegetation stages of two *Artemisia* species and to evaluate the antioxidant activity of methanolic extracts in vitro. Phytochemical analysis of the *Artemisia* samples was carried out using spectrophotometric methods and HPLC-PDA techniques, while antioxidant activity was determined using DPPH, ABTS, FRAP, and CUPRAC assays. Significant differences in the diversity of phenolic compounds were found among *Artemisia* species during vegetation stages. Chlorogenic acid, 3,5-dicaffeoylquinic acid, and rutin were predominant among the phenolic compounds. The study provides valuable insights into the composition of phenolic compounds and flavonoids in *Artemisia* plants at different growth stages, shedding light on their potential pharmacological effects and antioxidant activities. These findings contribute to the knowledge of the quality and safety of herbal materials derived from *Artemisia* species.

Keywords: *Artemisia* L.; vegetation stages; phenolic compounds; antioxidant activity

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1. Introduction

The genus *Artemisia* L. (tribe *Anthemideae*, section *Dracunculus*), belonging to the family *Asteraceae*, includes about 500 species [1]. In Lithuania, three distinct species, including *Artemisia absinthium* L. and *Artemisia vulgaris* L., are found in the natural habitats [2,3]. *Artemisia* species are widespread and economically relevant medicinal plants in the mild temperature areas of Asia, Australia, Europe, North America, and South Africa [4,5].

In recent decades, medicinal and aromatic plants that accumulate considerable amounts of bioactive substances have gained more attention. *Artemisia abrotanum* L. and *Artemisia absinthium* L. are one such highly promising species [6,7]. The chemical profile of *Artemisia* plants is responsible for remedying human health; in vitro and in vivo assays been proven to exhibit comprehensive pharmacological activities (antibacterial, anthelmintic, antimalarial, antioxidant, antidiabetic, anticancer, anti-inflammatory, antitumor, and a decreased risk of cardiovascular diseases) [6–9]. *Artemisia* medicinal raw

material is an excellent source of biologically active compounds. The aerial parts of different *Artemisia* plants contain high levels of volatile [10] and non-volatile bioactive substances such as phenolics [11,12], fatty acids [13,14], organic acids [15], amino acids [13], coumarins [16], lignans [17], minerals [18,19], alkaloids [20], sterols [21], tannins [22], and carotenoids [23]. *Artemisia* extracts have attracted more attention in the last decades and various investigations of polyphenols, and their relation to biological activities have been conducted [24–27]. The main phenolic compounds found in *Artemisia* extracts are phenolic acids and flavonoids [28,29]. Polyphenols are important due to their various functions that help plants adjust to environmental changes (growth regulation and survival, disease resistance, UV protection) and have extensive pharmacological activities [30–32].

The plant growth phases are one of the main factors influencing the synthesis and accumulation of bioactive substances in plants' herbal material [33]. Despite the multiple studies that have highlighted the phenolic profile and antioxidant power of *Artemisia* extracts, as far as we know, studies of the different vegetation stages of *Artemisia* L. species are few or nonexistent. This study aimed to investigate the phenolic profile and the antioxidant activity during the vegetation period of *A. abrotanum* and *A. absinthium* introduced in Central Lithuania.

2. Materials and Methods

2.1. Plant Materials

A. abrotanum (collection no. XX-0-KAUN-1980-AR0025) has been introduced in the Medicinal plant collection at Botanical Garden of Vytautas Magnus University, Lithuania, since 1980, and *A. absinthium* (collection no. XX-0-KAUN-1951-AR0026) since 1951. Soil samples of the collecting location were analyzed in the Agrochemical Research Laboratory (Lithuanian Research Centre for Agriculture and Forestry). The neutral reaction (pH) of the tested soil samples was 7.4; P_2O_5—306 mg/kg; and K_2O—121 mg/kg. The average monthly temperature (+12.1 °C), precipitation (47 mm), and humidity (65%) were obtained from the archive of the Lithuanian Hydrometeorological Service under the Ministry of Environment of the Republic of Lithuania.

The herbal samples of *Artemisia* species were collected during the vegetation period (March–November) in 2019. Various vegetation stages have been separated: intensive growth (A3), butonization (B), the beginning of flowering (Z1), intensive flowering (Z2), and the end of flowering (Z3). The herbal material was dried at +25 °C temperature in a well-ventilated loft and protected from direct sunlight and moisture. The loss on drying was evaluated according to European Pharmacopoeia [34].

2.2. Chemicals and Solvents

Ethanol 96% (*v*/*v*) was obtained from AB Vilniaus Degtinė (Vilnius, Lithuania). The Folin–Ciocalteu reagent, calcium carbonate, acetonitrile, methanol 99.9% (*v*/*v*), aluminum chloride hexahydrate, DPPH (2,2-diphenyl-1-picrylhydrazyl), hydrochloric acid, copper (II) chloride dihydrate, and TPTZ (2,4,6-tripyridyl-s-triazine) were purchased from Sigma-Aldrich (Steinheim, Germany). The following reagents were used: acetic acid from Lachner (Neratovice, Czech Republic), trifluoroacetic acid, sodium carbonate from Carl Roth GmbH (Karlsruhe, Germany), ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), potassium persulfate, sodium acetate, Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromano-2-carboxylic acid), ammonium acetate, and iron (III) chloride hexahydrate from Vaseline-Fabrik Rhenania (Bonn, Germany).

Chlorogenic acid, neochlorogenic acid, caffeic acid 3,4-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, 4-O-caffeoylquinic acid, 3,5-dicaffeoylquinic acid, isorhamnetin-3-rutinoside, luteolin-7-rutinoside, luteolin-7-glucoside, and rutin were obtained from Sigma-Aldrich GmbH (Steinheim, Germany). Distilled water was produced using the Milli-Q® 180 (Millipore, Bedford, MA, USA) water purification system.

2.3. Sample Preparation

Chemical analysis was performed at the Laboratory of Biopharmaceutical Research at Lithuanian University of Health Science in 2019–2020. Extraction procedures were performed in triplicate (n = 3). The *Artemisia* methanolic extracts were prepared using 0.25 g of dried and crushed herbal material and 20 mL of 70% methanol. The samples were extracted in an ultrasonic bath for 30 min at 37 kHz frequency and 480 W power. The obtained extracts were filtered through a 0.45 µm pore size filter.

2.4. Spectrophotometric Analysis

2.4.1. Determination of Total Phenolic and Flavonoid Content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu assay proposed by Riahi et al. [35], calculated from a rutin calibration curve (y = 11.131x − 0.0224; R^2 = 0.9995) and expressed as mg RE/g DW. The total content of flavonoids (TFC) was determined using the described methodology by Moacă et al. [36], calculated from a rutin calibration curve (y = 0.9255x − 0.0705; R^2 = 0.9999) and expressed as mg RE/g DW.

2.4.2. Determination of Antioxidant Activity

The antioxidant activity of the *Artemisia* extracts was analyzed using spectrophotometric ABTS, DPPH, FRAP (Ferric Reducing Antioxidant Power), and CUPRAC (Cupric Reducing Antioxidant Capacity) assays. The antioxidant activity was expressed as µmol of the Trolox equivalent (TE) per gram of dry weight (DW). TE was calculated according to the following formula: TE = (c × V)/m; c—the concentration of Trolox established from the calibration curve (µmol); V—the volume of the extract (in L); m—the weight (exact) of the herbal powder (in grams); and expressed as µmol TE/g DW.

The ABTS assay was performed using the methodology reported by Sussela et al. [37]. The ABTS working solution (3 mL) was mixed with 10 µL of each test sample. The mixture samples were stored in the dark at room temperature for 1 h. A decrease in absorbance was established at 734 nm.

The DPPH assay was performed using Messaili et al.' methodology [38]. The DPPH working solution (120 µL) was mixed with 30 µL of each test sample. The mixture samples were stored in the dark at room temperature for 30 min. A decrease in absorbance was established at 515 nm.

The FRAP assay was performed using the methodology Mumivand et al. reported [39]. The FRAP working reagent was prepared by mixing reagents TPTZ (0.01 M, dissolved in 0.04 M HCl), $FeCl_3 \cdot 6H_2O$ (0.02 M in water), and an acetate buffer (0.3 M, pH 3.6) (1:1:10). The test solution was prepared by mixing 3 mL of a freshly prepared FRAP reagent with 10 mL of each test sample. The mixture samples were stored in the dark at room temperature for 30 min. A decrease in absorbance was established at 593 nm.

The CUPRAC assay was performed using the methodology reported by Koyncu [40]. The CUPRAC working reagent was prepared by mixing reagents copper (II) chloride (0.01 M in water), an ammonium acetate buffer solution (0.001 M, pH 7), and neocuproine (0.0075 M in ethanol) (ratio 1:1:1). The test solution was prepared by mixing 3 mL of a freshly prepared CUPRAC reagent with 10 mL of each test extract. A decrease in absorbance was established at 450 nm.

2.5. Chromatographic Studies

HPLC (high-performance liquid chromatography) analysis was performed using a "Waters e2695 Alliance system" chromatograph with a "Waters 2998" photodiode array detector according to the HPLC method reported by Raudone et al. [41]. Polyphenols were analyzed using a YMC-Pack ODS-A (5 µm, C18, 250 × 4.6 mm i.d.) column. The column was operated at a constant temperature of 25 °C. The volume of the analyzed extract was 10 µL, and the flow rate was 1 mL/min. The mobile phase consisted of 2% (v/v) acetic acid (solvent A) and acetonitrile (solvent B). Gradient variation: 0–30 min, 3–15% B; 30–45 min, 15–25% B; 45–50 min, 25–50% B; and 50–55 min, 50–95% B. The cali-

bration curves were obtained by injecting the known concentrations of different standard reference compounds. The identified polyphenols were quantified at a λ = 210–400 nm wavelength. Chromatographic peaks of polyphenols were identified by the retention time of the reference compound and the analyte, as well as the UV absorption spectra [41].

2.6. Statistical Analysis

The experiments were performed in triplicate. The mean values and standard deviations were calculated with SPSS Statistics 27.0 (IBM, Armonk, NY, USA) and Excel 2016 (Microsoft, Redmond, WA, USA). The coefficient of variation (CV) was determined. The dispersion analysis of one way ANOVA was applied. PCA with eigenvalues higher than 1 were used. The differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1. Determination of Total Phenolic and Flavonoid Content

The estimation of the herbal samples of different vegetation stages of *Artemisia* plants introduced in Central Lithuania showed that the total amount of phenolic compounds ranged from 155.19 to 298.07 mg RE/g DW ($p < 0.05$) (Figure 1). The highest content of phenolic compounds 298.07 ± 2.69 mg RE/g DW were found in *Absinthii herba* samples at the beginning of the flowering stage, and they significantly differed from the quantities found in the remaining vegetation stages. The lowest amount of phenolic compounds (155.19 ± 4.97 mg RE/g DW) were found in *Absinthii herba* samples in the intensive growth stage, which did not differ significantly from the amounts found in *Artemisiae abrotani herba* samples of the same stage ($p < 0.05$). The coefficient of variation was 4.16%.

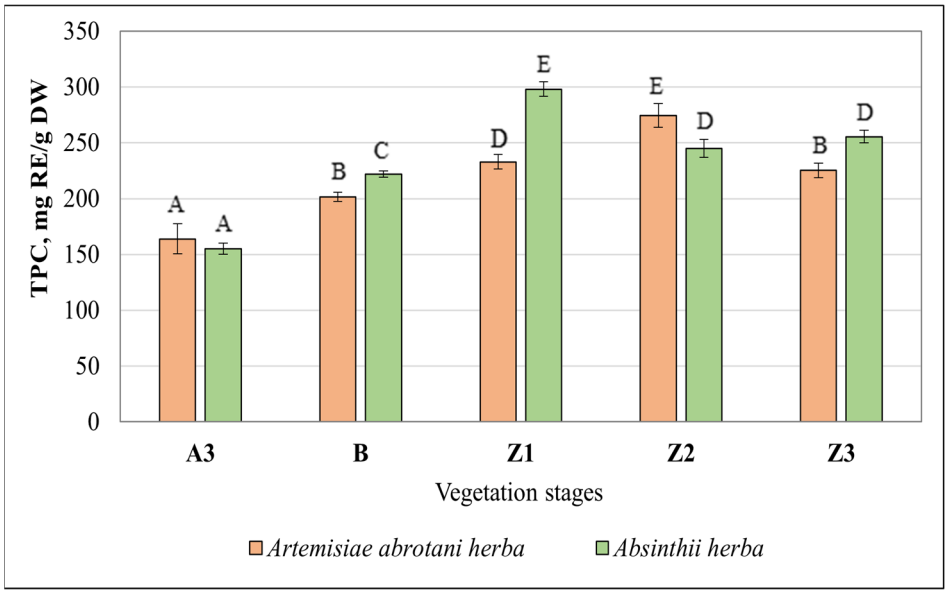


Figure 1. Variation in total phenolic compounds (TPC) content (mg RE/g DW) of *Artemisia* samples. Vegetation stages: intensive growth (A3), butonization (B), the beginning of flowering (Z1), intensive flowering (Z2), and the end of flowering (Z3). Different letters indicate significantly differences between vegetation stages ($p < 0.05$).

Recent studies focus to explain the relationship between polyphenol content and the plant growth stages. An increase in the content of polyphenols of the *Artemisia* species to their flowering, their maximum accumulation before flowering, and their decreasing amount at the end of flowering indicate an expense of these compounds on the plant physiological and adaptation processes [27].

Bordean et al. determined the amount of phenolics in the raw material extracts of *A. annua* and *A. absinthium.* The total amount of phenolics found in the flowering vegetation

stage ranged from 51.73 to 518.09 mg GAE/g DW ($p < 0.05$) [42]. Msaada et al. evaluated that in the flowering raw material extracts of *A. absinthium*, the total amount of phenolics varied from 49.39 to 99.89 mg GAE/g DW ($p < 0.05$) [43]. In a research study by Bhat et al., the amounts of phenolic compounds in the samples of *A. absinthium* ranged from 0.43 to 9.29 mg GAE/g DW ($p < 0.05$) [44]. Minda et al. evaluated the total amount of phenolics in the herbal extracts of *A. annua*, *A. dracunculus*, and *A. absinthium*. The total amount of phenolic compounds ranged from 129.28 to 193.61 mg GAE/g DW ($p < 0.05$) [45]. The differences between the quantitative composition of phenolic compounds of *Artemisia* extracts may have been due to genetic differences, climate conditions, and raw material preparation time [46].

Flavonoids are a huge class of non-volatile compounds with positive pharmacological effects on human health [45–47]. Determining the variability in the qualitative and quantitative flavonoid composition in the studied *Artemisia* extracts during the vegetation period is a critical step for evaluating the high quality and safety of herbal material. The obtained results showed that the total content of flavonoids ranged from 3.37 to 8.61 mg RE/g DW ($p < 0.05$) (Figure 2). The highest total flavonoid content (8.61 ± 0.21 mg RE/g DW) was found in *Artemisiae abrotani herba* extracts of the butonization stage, while the lowest (3.37 ± 0.07 mg RE/g DW) quantity was determined in *Artemisiae abrotani herba* extracts of the intensive growth stage, which did not differ significantly from the amounts found in *Absinthii herba* extracts on the same vegetation stage ($p < 0.05$). The coefficient of variation was high—11.25%.

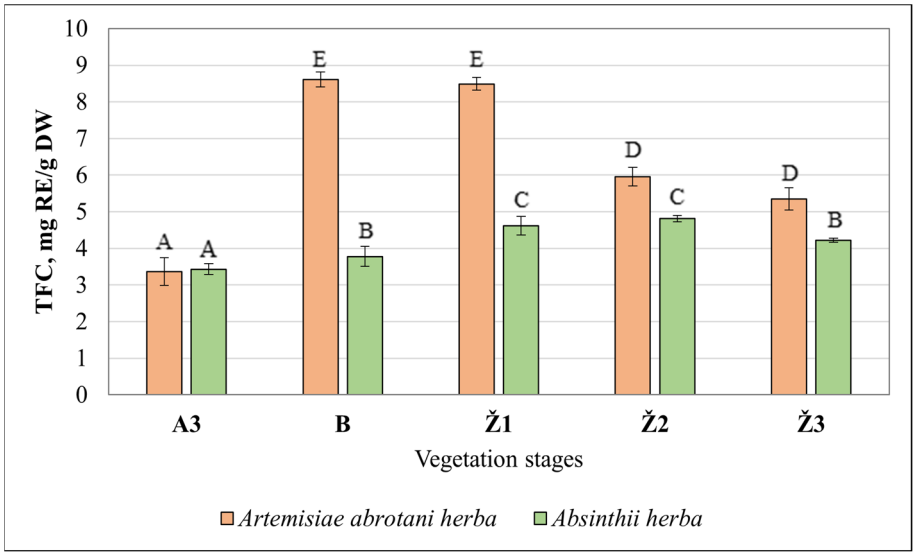


Figure 2. Variation in total flavonoids (TFC) content (mg RE/g DW) of *Artemisia* samples. Vegetation stages: intensive growth (A3), butonization (B), the beginning of flowering (Z1), intensive flowering (Z2), and the end of flowering (Z3). Different letters indicate significantly differences between vegetation stages ($p < 0.05$).

Carvalho et al. studied the variability in the total flavonoid levels in the leaf extracts of *A. annua*, *A. arborescens*, *A. ludoviciana*, *A. oleandica*, *A. priceps*, and *A. stelleriana*. The total flavonoid amount ranged from 0.03 to 0.19 mg QE/100 g DW ($p < 0.05$) [48]. Trifan et al. determined that the total flavonoid content in roots and the aerial parts of the *A. absinthium* samples ranged from 0.37 to 28.74 mg RE/g [49]. Sharma and Adhikari evaluated the phytochemical composition and biological effects of the roots and leaf extracts of *A. vulgaris*. The researchers found that the total flavonoid content in the extracts of *A. vulgaris* ranged from 31.54 to 71.15 mg QE/g [50]. In our study, the total flavonoid amount found in the herbal material of *Artemisia* was lower than that found by Carvalho et al., Trifan et al. and Sharma and Adhikari.

3.2. Quantitative and Qualitative Composition of Phenolic Compounds

Eleven phenolic compounds were detected in *Artemisia* L. extracts, according to standards, retention times, mass spectrometry, and the literature data (Table 1). Identified phenolic compounds fall into the phenolic acids group (caffeoylquinic and hydroxycinnamic acids) and flavonoids (flavonols, flavones), respectively. Identified compounds were 4,5-dicaffeoylquinic acid, 4-O-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, caffeic acid, chlorogenic acid, neochlorogenic acid, isorhamnetin-3-rutinoside, luteolin-7-glycoside, luteolin-7-rutinoside, and rutin. The most common compounds identified in all extracts, consistent with their concentration, were chlorogenic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and rutin. Chlorogenic acid was the predominant compound in *Artemisia* extracts, with a relatively low coefficient of variation. Meanwhile, 4,5-dicaffeoylquinic acid, 4-O-caffeoylquinic acid, caffeic acid, neochlorogenic acid, isorhamnetin-3-rutinoside, luteolin-7-glycoside, and luteolin-7-rutinoside were identified in the lowest concentrations. The total amount of phenolic acids in *Artemisia* extracts during vegetation phases ranged from 0.35 to 217.39 mg/g ($p < 0.05$). Of all the compounds of the phenolic acid group in the extracts of the studied *Artemisia* species, caffeic acid demonstrated the highest variability (coefficient of variation—5.54%), while the variability in the amount of 4,5-dicaffeoylquinic acid was the lowest (coefficient of variation—0.26%). Previous studies confirmed that *Artemisia* materials are rich in phenolic acids and flavonoids [42]. Baiceanu et al. in *A. abrotanum* raw material extracts indicated high levels of sinapic acid [51]. Minda et al. evaluated the chemical composition and biological effects of the extracts of *A. annua*, *A. dracunculus*, and *A. absinthium* species [45]. Moacă et al. compared the polyphenol content in ethanolic extracts from *A. absinthium* leaves and stems. The obtained results showed the high presence of chlorogenic acid (2.03 µg/mg DW) and rutin (0.55 µg/mg DW) in *A. absinthium* stem extracts [36]. Studies confirmed that phenolic acids and flavonoids have a strong effect in the cure of mental illness [52,53].

Flavonols are a class of polyphenols that are commonly found in *Artemisia* species, and they possess multiple pharmacological effects [28]. Therefore, it is important to determine the variation in the quality and quantity composition of flavonols in herbal materials. The flavonols, namely isorhamnetin-3-rutinoside and rutin, were identified. The largest amount of isorhamnetin-3-rutinoside (8.71 $\pm$ 0.05 mg/g) was found in the extracts of *A. abrotanum* in the intense flowering stage, which was different from the quantities found in other vegetation stages ($p < 0.05$). The lowest amount of isorhamnetin-3-rutinoside (0.80 $\pm$ 0.02 mg/g) was found in the extracts of *A. absinthium* at the end of the flowering vegetation stage ($p < 0.05$). The largest content of rutin (165.37 $\pm$ 0.11 mg/g) was detected in the extracts of *A. abrotanum* in the intensive flowering stage, and the lowest amount (0.96 $\pm$ 0.02 mg/g) was found in the samples of *A. absinthium* at the end of flowering stage, which was different from the quantities found in other vegetation stages ($p < 0.05$) (Table 1). Of all the compounds of the flavonols group in the herbal extracts of the studied *Artemisia* species, rutin demonstrated the highest variability (coefficient of variation—15.15%), while the variability in the amount of isorhamnetin-3-rutinoside was the lowest (coefficient of variation—0.22%). It has been reported that rutin and its derivatives appear to constitute the main flavonoid glycoside in *F. esculentum* and *F. tataricum* [54]. Concerning flavonoids, rutin, quercitrin, isoquercitrin, quercetin, and kaempferol are among the most abundant flavonoids found in the extracts of different *Artemisia* species [43,52,55–57].

Table 1. Diversification of the quantitative composition of phenolic compounds in *Artemisia* extracts during vegetation period (mg/g).

Raw Material	Compound	Vegetation Stages					The Coefficient of Variation (CV)
		Intensive Growth	Butonization	The Beginning of Flowering	Intensive Flowering	The End of Flowering	
Artemisiae abrotani herba	4,5-Dicaffeoylquinic acid	56.28 ± 0.34 ^{e}	46.45 ± 0.22 ^{d}	32.16 ± 0.01 ^{b}	35.08 ± 0.05 ^{c}	27.85 ± 0.02 ^{a}	0.26
Absinthii herba		NDE	NDE	NDE	NDE	NDE	-
Artemisiae abrotani herba	4-O-Caffeoylquinic acid	16.42 ± 0.42 ^{c}	11.88 ± 0.60 ^{b}	4.98 ± 0.24 ^{a}	4.92 ± 0.07 ^{a}	4.54 ± 0.49 ^{a}	4.43
Absinthii herba		1.07 ± 0.06 ^{a}	4.54 ± 0.06 ^{e}	1.89 ± 0.04 ^{c}	1.89 ± 0.04 ^{c}	1.16 ± 0.05 ^{b}	3.06
Artemisiae abrotani herba	3,5-Dicaffeoylquinic acid	136.39 ± 1.16 ^{e}	132.69 ± 0.14 ^{d}	86.63 ± 1.38 ^{b}	97.86 ± 0.34 ^{c}	67.84 ± 0.62 ^{a}	0.76
Absinthii herba		13.57 ± 0.58 ^{a}	36.48 ± 1.12 ^{b}	37.95 ± 1.48 ^{c}	100.33 ± 1.59 ^{e}	47.55 ± 0.01 ^{d}	4.28
Artemisiae abrotani herba	3,4-Dicaffeoylquinic acid	102.71 ± 0.05 ^{d}	109.13 ± 0.52 ^{e}	49.15 ± 0.36 ^{b}	85.71 ± 1.02 ^{c}	36.51 ± 0.05 ^{a}	0.54
Absinthii herba		6.78 ± 0.07 ^{a}	21.08 ± 0.23 ^{c}	24.05 ± 0.16 ^{d}	28.72 ± 0.19 ^{e}	12.15 ± 0.01 ^{b}	1.20
Artemisiae abrotani herba	Caffeic acid	3.62 ± 0.05 ^{d}	4.19 ± 0.05 ^{e}	1.54 ± 0.03 ^{a}	3.04 ± 0.02 ^{c}	1.65 ± 0.03 ^{b}	1.27
Absinthii herba		0.48 ± 0.02 ^{c}	1.16 ± 0.09 ^{e}	0.61 ± 0.03 ^{d}	0.84 ± 0.02 ^{a}	0.35 ± 0.06 ^{b}	5.54
Artemisiae abrotani herba	Chlorogenic acid	203.37 ± 1.63 ^{d}	217.39 ± 0.36 ^{e}	96.53 ± 2.54 ^{b}	169.95 ± 0.79 ^{c}	86.12 ± 1.16 ^{a}	1.08
Absinthii herba		11.26 ± 0.01 ^{a}	118.66 ± 0.52 ^{e}	20.31 ± 0.58 ^{b}	54.30 ± 0.06 ^{d}	28.84 ± 0.11 ^{c}	0.76
Artemisiae abrotani herba	Neochlorogenic acid	18.02 ± 2.23 ^{d}	14.78 ± 0.11 ^{c}	5.52 ± 0.35 ^{a}	9.32 ± 0.14 ^{b}	5.66 ± 0.02 ^{a}	4.23
Absinthii herba		4.06 ± 0.12 ^{c}	8.51 ± 0.03 ^{e}	6.62 ± 0.27 ^{d}	3.15 ± 0.14 ^{b}	1.59 ± 0.01 ^{a}	0.71
Artemisiae abrotani herba	Isorhamnetin-3-rutinoside	1.36 ± 0.01 ^{a}	5.58 ± 0.11 ^{c}	3.69 ± 0.01 ^{b}	8.71 ± 0.05 ^{d}	3.90 ± 0.31 ^{b}	2.23
Absinthii herba		1.27 ± 0.04 ^{b}	2.91 ± 0.11 ^{d}	3.68 ± 0.19 ^{e}	2.19 ± 0.08 ^{c}	0.80 ± 0.02 ^{a}	0.22
Artemisiae abrotani herba	Luteolin-7-glycoside	3.69 ± 0.14 ^{e}	2.64 ± 0.08 ^{c}	2.41 ± 0.05 ^{b}	1.36 ± 0.06 ^{a}	2.80 ± 0.06 ^{d}	2.03
Absinthii herba		2.55 ± 0.06 ^{a}	4.16 ± 0.04 ^{c}	3.68 ± 0.02 ^{b}	1.77 ± 0.10 ^{e}	0.65 ± 0.11 ^{d}	0.44
Artemisiae abrotani herba	Luteolin-7-rutinoside	3.87 ± 0.03 ^{d}	5.43 ± 0.26 ^{e}	1.98 ± 0.06 ^{b}	2.45 ± 0.03 ^{c}	1.37 ± 0.12 ^{a}	2.01
Absinthii herba		1.02 ± 0.03 ^{c}	1.56 ± 0.06 ^{d}	2.01 ± 0.02 ^{e}	0.43 ± 0.01 ^{b}	0.22 ± 0.03 ^{a}	0.17
Artemisiae abrotani herba	Rutin	60.79 ± 0.40 ^{a}	121.72 ± 90.20 ^{abc}	75.52 ± 0.47 ^{a}	165.37 ± 0.11 ^{c}	81.44 ± 0.23 ^{a}	15.15
Absinthii herba		1.31 ± 0.04 ^{b}	2.32 ± 0.01 ^{d}	2.99 ± 0.08 ^{e}	2.05 ± 0.06 ^{c}	0.96 ± 0.02 ^{a}	1.93

NDE is not detected. Different letters indicate significantly differences between different vegetation stages ($p < 0.05$).

Two compounds of the flavones (luteolin-7-glucoside and luteolin-7-rutinoside) were identified in the extracts of *Artemisia* species. The greatest amount of luteolin-7-rutinoside (5.43 ± 0.26 mg/g) was detected in the extracts of *A. abrotanum* butonization stage, and the lowest amount (0.22 ± 0.03 mg/g) was found in the extracts of *A. absinthium* at the end of flowering vegetation stage, which was different from the quantities found in other vegetation stages ($p < 0.05$). The greatest amount of luteolin-7-glucoside (4.16 ± 0.04 mg/g) was detected in the extracts of *A. absinthium* in the butonization stage, which was different from quantities found in other vegetation stages ($p < 0.05$). The smallest amount of luteolin-7-glucoside (0.65 ± 0.11 mg/g) was found in the extracts of *A. absinthium* at the end of the flowering stage, which was different from the quantities found in other vegetation stages ($p < 0.05$) (Table 1). The amount of luteolin-7-glycoside determined in the studied extracts varied widely, with the coefficient of variation being 2.03%, while the variation in the content of luteolin-7-rutinoside was lower (coefficient of variation—0.17%). Various studies confirm that luteolin derivatives inhibit the proliferation of human liver cancer cells and have a high antidiabetic activity [58,59].

3.3. Antioxidant Activity of Artemisia Extracts

The estimation using the DPPH assay showed that the strongest scavenging activity (14.23 ± 1.53 µmol TE/g DW) was observed in *Artemisiae abrotani herba* extracts of the intense flowering stage (Figure 3). The weakest antiradical activity (9.01 ± 0.02 µmol TE/g DW) was determined in *Absinthii herba* extracts during intensive growth vegetation stage ($p < 0.05$). The variability of scavenging activity estimated by this method between *Artemisia* extracts during different vegetation stages was low (coefficient of variation—5.92%).

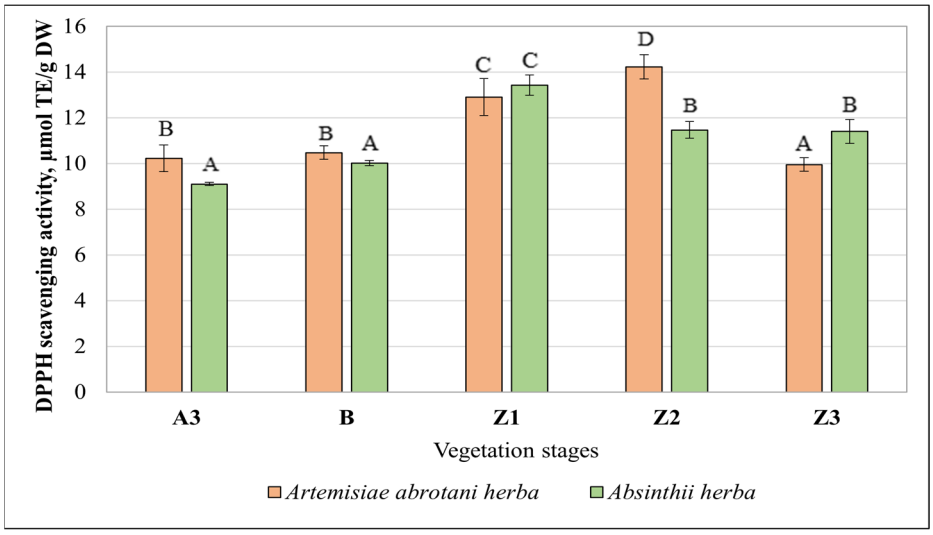


Figure 3. Variability of the antiradical activity (µmol TE/g DW, DPPH assay) of *Artemisia* extracts in vitro. Vegetation stages: intensive growth (A3), butonization (B), the beginning of flowering (Z1), intensive flowering (Z2), and the end of flowering (Z3). Different letters indicate significantly differences of antiradical potency between different vegetation stages ($p < 0.05$).

Bordean et al. investigated the scavenging activity of the different aboveground samples of *A. annua* and *A. absinthium*. The scientists reported that the strongest antiradical activity (735.77 ± 0.02 µmol TE/g DW), after using the DPPH method, was determined in *A. absinthium* leaf extracts [42]. Lithuanian researchers applied the DPPH assay and found that the strongest scavenging activity (140.0 ± 0.00 µmol TE/g DW) was observed in synthesized nanoparticles *A. absinthium*/AgNPs and *A. vulgaris*/AgNPs extracts [60].

The estimation of the scavenging activity of *Artemisia* extracts via the ABTS assay showed that this activity varied from 43.56 to 103.68 µmol TE/g DW ($p < 0.05$) (Figure 4). The butonization extracts of *Absinthii herba* have the strongest scavenging

activity (103.68 ± 2.59 µmol TE/g DW). The lowest scavenging activity (43.56 ± 2.59 µmol TE/g DW) was found in the intensive growth extracts of *Absinthii herba*. The variation in the scavenging activity determined by this assay between *Artemisia* extracts during different vegetation phases was the coefficient of variation at 5.92%. Trifan et al. determined that the scavenging activity in vitro of the *A. absinthium* extracts varied from 7.54 to 95.95 µmol TE/g. Our study revealed a stronger scavenging activity of *A. absinthium* extracts compared to the activity found by Trifan et al. [49]. Skowyra et al. indicated that high temperature can increase the strength of antioxidant activity [61,62]. Popov et al. determined that the antiradical activity in vitro of *A. vulgaris* extracts varied from 33.59 to 45.37 µmol TE/g [63].

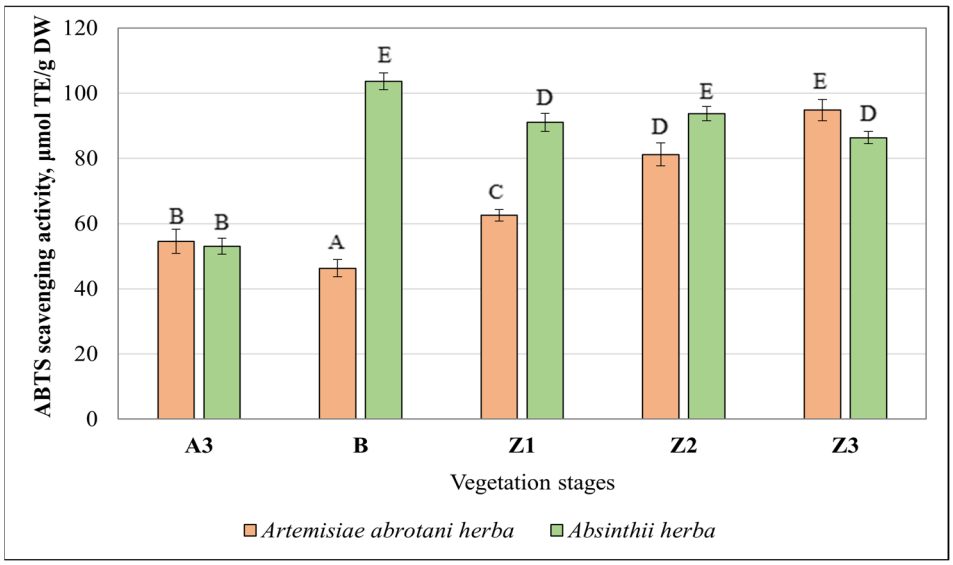


Figure 4. Variability of the antiradical activity (µmol TE/g DW, ABTS assay) of *Artemisia* extracts in vitro. Vegetation stages: intensive growth (A3), butonization (B), the beginning of flowering (Z1), intensive flowering (Z2), and the end of flowering (Z3). Different letters indicate significantly differences of antiradical potency between different vegetation stages ($p < 0.05$).

The estimation using the FRAP assay demonstrated that the strongest reducing activity (76.56 ± 2.12 µmol TE/g DW) was determined at the beginning of flowering in the extracts of *Absinthii herba* (Figure 5). The weakest reducing activity (25.21 ± 0.38 µmol TE/g DW) was found in the growth and leaf production extracts of *Artemisiae abrotani herba*, which significantly differs from the amounts found in *Absinthii herba* extracts on the same vegetation stage ($p < 0.05$). The coefficient of variation was 7.54%. Taherkhani et al. evaluated the reducing activity of *A. absinthium* leaf extracts using the FRAP method and found that the mean of the reducing activity was 10.67 mg GAE/g [64].

The strongest reducing activity using the CUPRAC assay was determined in the butonization (200.79 ± 0.57 µmol TE/g) and at the beginning of flowering (200.89 ± 4.01 µmol TE/g) stages of *Absinthii herba* extracts, while the lowest reducing activity (105.04 ± 2.03 µmol TE/g DW) was found in *Artemisiae abrotani herba* extracts of the butonization stage ($p < 0.05$) (Figure 6). The variation in the reducing activity between *Artemisia* extracts during different vegetation stages was high (coefficient of variation—18.02%). Trifan et al. applied the CUPRAC method when extracting five *Artemisia* species (*A. absinthium*, *A. annua*, *A. austriaca*, *A. pontica*, and *A. vulgaris*) roots and aerial parts samples with different extractants. The strongest reducing activity (498.32 ± 4.02 µmol TE/g) was found in the *A. vulgaris* aerial parts of methanolic extracts [49]. Our study revealed a stronger reducing activity of *A. absinthium* extracts compared to the reducing activity found by Trifan et al.

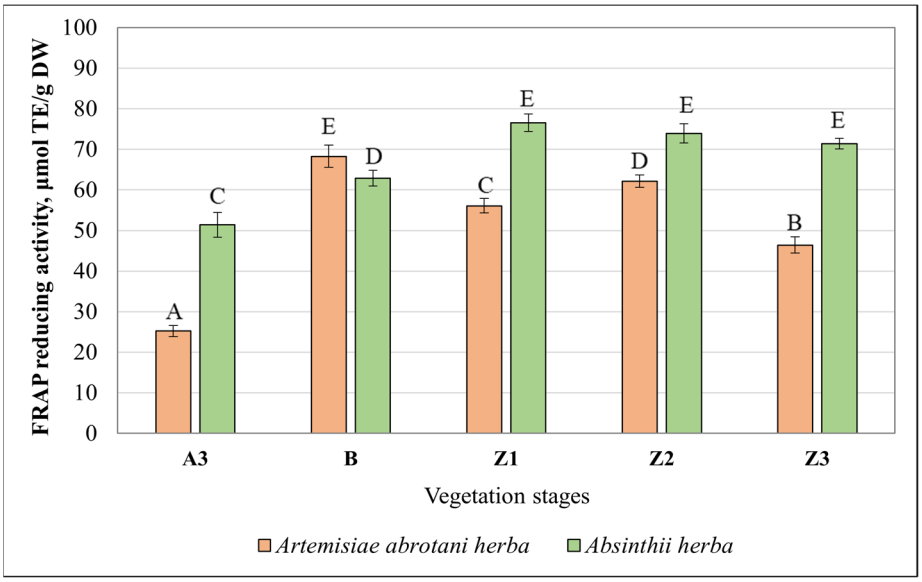


Figure 5. Variability of the reducing activity (µmol TE/g DW, FRAP assay) of *Artemisia* extracts in vitro. Vegetation stages: intensive growth (A3), butonization (B), the beginning of flowering (Z1), intensive flowering (Z2), and the end of flowering (Z3). Different letters indicate significantly differences of reducing potency between different vegetation stages ($p < 0.05$).

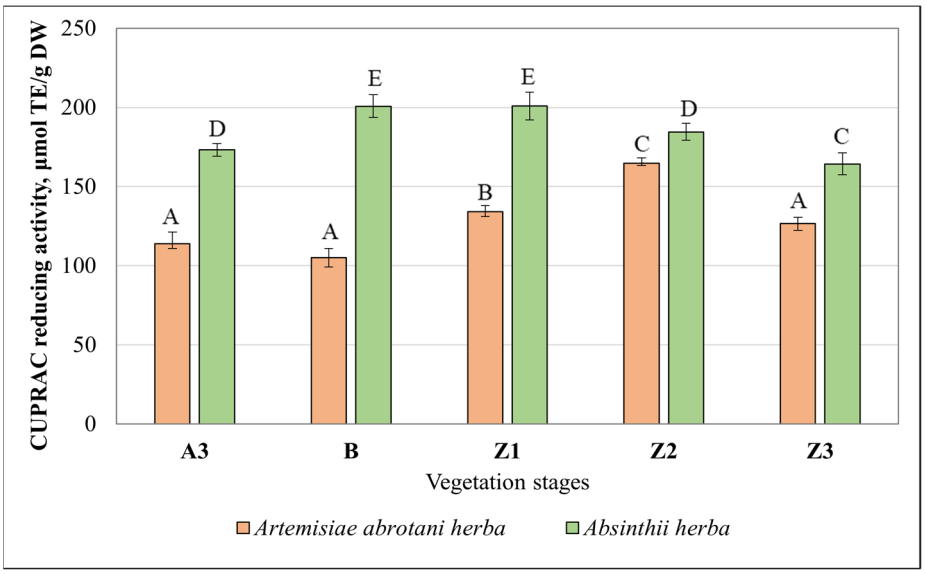
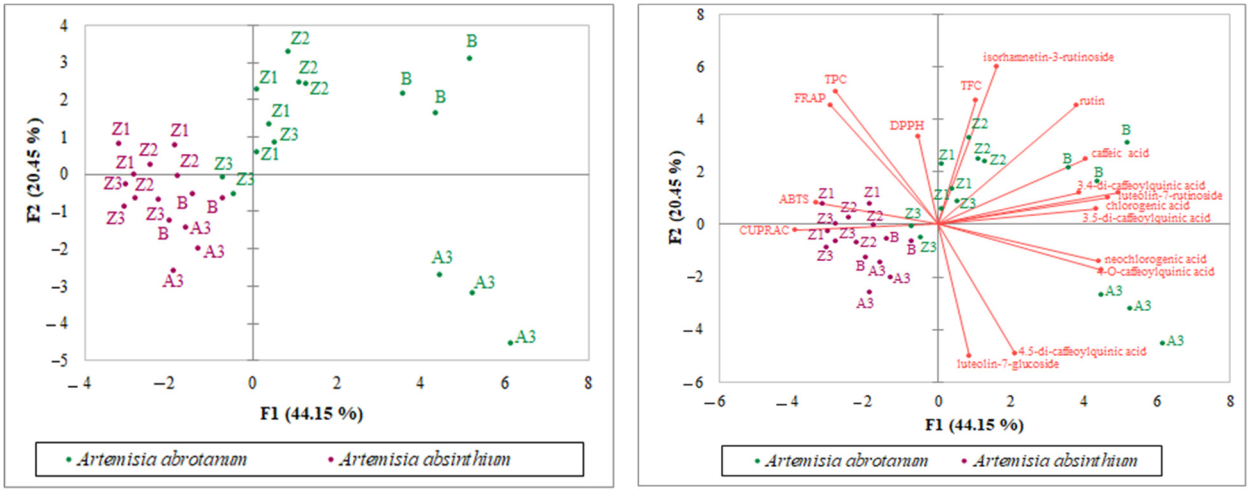


Figure 6. Variability of the reducing activity (µmol TE/g DW, CUPRAC assay) of *Artemisia* extracts in vitro. Vegetation stages: intensive growth (A3), butonization (B), the beginning of flowering (Z1), intensive flowering (Z2), and the end of flowering (Z3). Different letters indicate significantly differences of reducing potency between different vegetation stages ($p < 0.05$).

Systematized research data on the qualitative and quantitative amount of polyphenols and antioxidant activity in *Artemisia* herbal samples during different vegetation stages were obtained. Principal component analysis (PCA) was performed to select similarities and differences between the analyzed *A. abrotanum* and *A. absinthium* samples according to vegetation stages (Figure 7a) and the qualitative and quantitative amount of polyphenols and antioxidant activity (Figure 7b). The score plot models for the herbal samples have shown relatively good separation between the vegetation stages (Figure 7a). Figure 3 summarizes the PCA results based on the correlation matrix with PC1 and PC2, which

explains an 84.20% total variability in the data collections of *Artemisia* herbal samples during the vegetation period.



(**a**) (**b**)

Figure 7. Diversification of the total content of phenolics, flavonoids, and antioxidant activity in *Artemisia* medicinal raw material extracts during vegetation period: intensive growth (A3), butonization (B), the beginning of flowering (Z1), intensive flowering (Z2), and the end of flowering (Z3). (**a**) principal component analysis of *A. abrotanum* and *A. absinthium* vegetation stages; (**b**) PCA of TPC, TFC, DPPH, ABTS, FRAP, CUPRAC, and phenolic acids and flavonoids in *Artemisia* herbal samples during different vegetation stages.

In the PCA loading plot, PC1 described 44.15% of the total variation in the data and was highly correlated with the positive loadings of the scavenging activity DPPH (0.891) and the reducing activity FRAP (0.884). PC2 accounted for 42.04% of the total variation and was characterized by the positive loadings of the quantitative composition of 4-O-caffeoylquinic acid (0.832), 3,5-dicaffeoylquinic acid (0.832), 3,4-dicaffeoylquinic acid (0.951), neochlorogenic acid (0.848), and chlorogenic acid (0.896) (Figure 7b).

4. Conclusions

This study demonstrated that two selected *Artemisia* species, namely *A. abrotanum* and *A. absinthium*, accumulate reasonable amounts of phenolic compounds. Significant fluctuations were determined in the total phenolic compound content across different stages of vegetation. The highest total amount of phenolic compounds was found in *Absinthii herba* extracts at the beginning of the flowering stage (298.07 ± 2.69 mg RE/g DW, $p < 0.05$). The highest total amount of flavonoids was evaluated in *Artemisiae abrotani herba* extracts of the butonization vegetation stage (8.61 ± 0.21 mg RE/g DW, $p < 0.05$), suggesting potential health advantages linked to this growth stage. In studied *Artemisia* extracts chlorogenic acid, 3,5-dicaffeoylquinic acid and rutin predominated among the identified phenolic compounds, while the amounts of other compounds were significantly lower. Raw material samples of the *Absinthii herba* stood out due to their unique chemical composition and strong antioxidant activity. Understanding the fluctuations in chemical composition at different growth stages can be used in selecting the optimal time of plant harvesting and, ultimately, the efficacy and quality of herbal products and pharmaceuticals. The study findings are important for developing herbal formulations, pharmaceuticals, and medicinal products with optimized therapeutic effects.

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