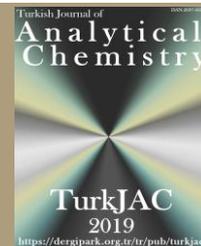




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Comparative analysis of phenolic compounds, volatile components, and antioxidant capacity in leaf and flower parts of *Aster caucasicus*

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Abstract

The study presents a comprehensive phytochemical analysis of *A. caucasicus*, examining both the leaf and flower parts of this plant. The researchers investigated the phenolic compound profiles using HPLC-DAD methodology, volatile compounds via SPME-GC-MS technique, and antioxidant properties through multiple assays (total phenolic content, total flavonoid content, FRAP, and CUPRAC).

Results revealed significant differences between plant organs. The flower parts contained higher total phenolic content (25.57 mg GAE/g) compared to leaves (17.81 mg GAE/g), with notably higher concentrations of compounds like gallic acid, caffeic acid, rosmarinic acid, and quercetin. Conversely, leaves demonstrated higher total flavonoid content (5.02 mg QE/g vs. 3.99 mg QE/g in flowers) and greater antioxidant capacity in both FRAP and CUPRAC assays.

Volatile compound analysis identified several bioactive components, including monoterpenes (beta-pinene, D-limonene, alpha-pinene), terpenoids, sesquiterpenes, and oxygenated heterocyclic compounds. Beta-pinene dominated in flowers (52.26%), while D-limonene was highest in leaves (28.68%).

This research fills a knowledge gap regarding *A. caucasicus*, suggesting its potential applications in pharmaceutical and cosmetic products. The flower parts show promise for pharmaceutical applications due to their rich phenolic content, while the leaf parts, with their high flavonoid content and antioxidant capacity, could be valuable in cosmetic products, particularly for UV protection.

Keywords: *A. caucasicus*, antioxidant capacity, phenolics, volatile compound

1. Introduction

Phenolic compounds are a group of phytochemical substances found as secondary metabolites in various plants. These compounds have received great interest in the scientific world due to their diverse biological activities and positive effects on human health. Natural polyphenols are considered important compounds due to their antioxidant properties, antimutagenic and/or anticarcinogenic effects, anti-inflammatory properties, and neuroprotective effects [1–4]. Scientific studies reveal that these compounds can both function as protective antioxidants against oxidative degradation and act as pro-oxidants that damage biomolecules, causing cellular death [5–6].

The medicinal properties of plant species are largely attributed to their secondary metabolites, particularly compounds like phenolic acids and flavonoids [7–8]. Recent scientific interest has grown around plant

polyphenols due to their antioxidant properties and various health benefits, as well as their applications in industry. Beyond therapeutic uses, medicinal plants serve nutritional purposes globally and are often classified as food products under regulatory frameworks in numerous countries [8–10]. The significant therapeutic potential of these plants makes the identification and characterization of their phenolic constituents a priority in analytical science research [11–12].

Many research efforts have documented the presence of polyphenols in plant materials and sought to elucidate their therapeutic applications, nutritional value, and antioxidant capabilities [13]. Consequently, developing analytical methodologies to identify and characterize these compound classes has become essential in phytomedicine research. Analytical approaches such as

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gas chromatography [14], high-performance liquid chromatography [7,15], and capillary electrophoresis [15,16] have been employed for phenolic compound characterization. However, due to the high melting points and thermal instability of phenolic acids and flavonoids above 200°C, liquid chromatography-based separation techniques are generally preferred.

High-Performance Liquid Chromatography (HPLC) is the most widely used separation technique applied to detect and quantify phytochemicals in plants [17–20]. Different detection modes are used, such as on-line diode array detection (DAD) [21–22], ultraviolet detectors [23], or electrochemical detectors [24].

As a more economical alternative, HPLC-DAD systems simplify the analytical methodology and significantly reduce associated costs, thereby making them suitable for routine analyses in laboratories with limited financial resources. In contrast, the integration of more sophisticated techniques such as electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), negative or positive ion mode coupled with MS-MS (utilizing triple quadrupole) or theoretical MS_n (employing ion trap technology) provides confirmatory evidence for peak identification, yet simultaneously increases both the financial investment required and the methodological complexity [25–30].

The technique of headspace solid-phase microextraction (HS-SPME) is extensively employed for isolation and pre-concentration of volatile compounds prior to gas chromatography-mass spectrometry (GC-MS) analysis [31–34]. Compared to alternative sample preparation methodologies such as solid-phase extraction or liquid-liquid extraction, SPME offers distinct benefits, including solvent-free operation, automation capability, and minimal sample volume requirements [35]. These advantageous characteristics render SPME particularly valuable for research necessitating the examination of large sample quantities, exemplified by studies evaluating plant populations utilized by plant breeders investigating genetic foundations of specific traits [36,37]. Researchers have documented the mapping of genes or quantitative trait loci (QTLs) governing volatile production, including those contributing to aroma, across numerous plant species such as tomato [32], melon [38,39], apple [40,41], and grape [42–44]. For plant volatile phenotyping, GC-MS methodology is typically implemented for both focused analysis of select volatiles [45,46] and comprehensive profiling of numerous targeted or untargeted volatile compounds (referred to as "metabolomics") [31,32,38,39,40,47].

The genus *Aster* within the Asteraceae family comprises approximately 600 species that have adapted

to diverse ecological environments and demonstrate widespread natural distribution. Although many species and interspecific hybrids serve ornamental purposes or contribute to the cut flower industry, these plants are predominantly valued for their medicinal properties, which have been recognized since antiquity. Traditional Chinese medicine has utilized *Aster* species for treating conditions including cough, fever, and tonsillitis. Contemporary scientific investigations have indicated that these plants exhibit diuretic, anti-tumor, antibacterial, antiviral, and anti-ulcer activities [48].

The therapeutic properties of *Aster* species are attributed to their high content of antioxidant compounds such as polyphenols and ascorbic acid, which exhibit antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic, and vasodilator effects, and are beneficial in the treatment and prevention of arteriosclerosis, cancer, diabetes, neurodegenerative diseases, arthritis, and other pathologies [49–50]. The variability of antioxidant content in plants results from the fact that the synthesis and accumulation of these compounds are a direct consequence of plant-environment interaction.

In the present study, the phenolic compound profile of the methanolic extract of *A. caucasicus* leaves and flowers, the composition of volatile compounds using solid-phase microextraction (SPME) technique, and their antioxidant potential were comparatively examined. Although there are individual content analyses on similar species in the literature, HPLC-DAD methodology for the simultaneous analysis of phytochemicals with a wide range of chemical structures, such as phenolic acids and flavonoids in these plant extracts, and SPME-GC-MS technique for the characterization of volatile compounds have not been reported. In the current literature, there are only studies comparing the antioxidant properties of *A. caucasicus* and *Aster sedifolis* species grown under cell culture conditions. However, there is no comprehensive research that comparatively evaluates both the phenolic and volatile compound profiles and antioxidant capacities of *A. caucasicus* leaves and flowers. The primary objective of this research is to determine the phenolic compound profile of *A. caucasicus* species, which lacks detailed characterization in the literature, to characterize its volatile compound composition, and to evaluate its antioxidant capacity. The polyphenol content determined in this study was evaluated by comparison with compounds reported in the literature to be effective in the treatment of pathologies such as arteriosclerosis, cancer, diabetes, neurodegenerative diseases, and arthritis. These comprehensive phytochemical characterization results will provide a foundation for future in vitro and in vivo bioactivity

studies investigating the antibacterial, antiviral, anti-inflammatory, anti-allergic, and antithrombotic activities of *A. caucasicus*.

2. Materials and methods

2.1. Plant material and methanolic extraction of leaf and flower parts of *A. caucasicus* species

The taxon names of *A. caucasicus*, the place of collection of the plant, and the receipt number are indicated as shown in Table 1. Also, the habit of *A. caucasicus* is shown in Fig. 1. Following collection, the plant material was promptly moved to a well-ventilated drying room with moderate ambient light, ensuring it remained protected from direct sunlight. The leaves and flowers of *A. caucasicus* were kept at -20°C for antioxidant capacity, some phenolic and flavonoid compound contents, and volatile compound content. All experiments and analyses were carried out in triplicate.

Table 1. Collection data of the examined *A. caucasicus*

Taxon	Locality	Voucher*
<i>Aster caucasicus</i>	(Aksu 399) specimens were collected on 26 July 2022 from the roadside habitat along the Heba Plateau road in Borçka (Artvin Province, NE Türkiye), at an altitude of approximately 1600 m, growing among Rhododendron shrubs along the roadside.	Aksu 399



Figure 1. Habitat of *A. caucasicus* plant

2.2. Preparation of samples

The extraction method was used by revising the methods [51,52]. Here, leaf and flower samples consisting of 20 grams of dry powder were treated separately with 200 ml of methanol to increase the extraction of target compounds, and the mixtures were subjected to ultrasonication for 30 minutes. After

ultrasonication, the samples were transferred to a shaker and kept at room temperature in the dark for 24 hours for optimal extraction efficiency. Following the incubation period, regular filter paper was used to remove large particles from the extracts, followed by a second filtration process with a 0.45 µm syringe filter to remove smaller particles. This comprehensive extraction procedure, including ultrasonication, long-term incubation, and double filtration stages, was designed to ensure maximum transfer of compounds from the dry powder samples to the extraction solution, and the resulting clear filtrates were prepared for analysis [51,52].

2.3. Antioxidant capacity of *A. caucasicus* leaves and flowers

2.3.1. Reagents and materials

For the analysis of polyphenols, flavonoids, and antioxidant capacity, various analytical reagents were sourced as follows: Methanol, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,4,6-tripyridyl-s-triazine (TPTZ), and Folin-Ciocalteu's phenol reagent were procured from Sigma Chemical Co. (St. Louis, MO, USA). Complementary reagents, including sodium carbonate, acetic acid, neocuproine (2,9-dimethyl-1,10-phenanthroline), aluminum nitrate nonahydrate, CuCl₂, FeSO₄·7H₂O and ammonium acetate were acquired from Merck Chemical Co. (Darmstadt, Germany). All chemical compounds utilized throughout the experimental procedures were of analytical grade purity.

2.3.2. Determination of total polyphenolic content

The quantification of total phenolic (TP) compounds was conducted using the Folin-Ciocalteu methodology [53]. A standard calibration curve was prepared using gallic acid at six different concentrations (1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/mL). The experimental protocol involved combining 20 µL of either standard solution or methanolic plant extract (1 mg/mL) with 400 µL of 0.5 N Folin-Ciocalteu reagent and 680 µL of distilled water. Following thorough mixing and a 3-minute reaction period, 400 µL of 10% Na₂CO₃ solution was introduced to the mixture. The reaction was allowed to develop for 2 hours at 25°C, after which absorbance measurements were recorded at 760 nm. The polyphenolic content was subsequently calculated and expressed as milligrams of gallic acid equivalents (GAE) per gram of plant dry weight.

2.3.3. Assessment of total flavonoid content

Total flavonoid (TF) (mg QE/g dry sample) quantification was performed according to a modified protocol from reference [54]. The analytical principle

exploits the characteristic of aluminum chloride to form coordination complexes with flavonoid compounds. Specifically, aluminum ions establish stable complexes with the C-4 keto group and either C-3 or C-5 hydroxyl groups present in flavones and flavonols, while forming fewer stable associations with ortho-dihydroxyl configurations in both A and B rings. For standardization purposes, quercetin solutions ranging from 0.03125 to 1.0 mg/mL were employed to generate a reference curve correlating absorbance measurements with known concentrations [54].

2.4. Ferric and copper reduction assays of antioxidant activity in *A. caucasicus* leaves and flowers

In the FRAP assay, total antioxidant potential was assessed through the conversion of a yellow Fe^{3+} -TPTZ (2, 4, 6-tripyridyl-s-triazine) complex into a blue Fe^{2+} -TPTZ complex when exposed to electron-donating compounds in acidic medium [55]. The protocol involved combining 3 mL of freshly prepared FRAP reagent with 100 μL of either experimental extract or control solvent in a test tube. Spectrophotometric measurements at 593 nm were recorded over a 4-minute interval at ambient temperature (25°C). The resulting absorbance values were quantified against an $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ calibration curve (100-1000 $\mu\text{mol/L}$), with final results expressed as micromoles of ferrous sulfate heptahydrate equivalents per gram of plant dry matter.

The CUPRAC methodology involves the interaction of antioxidant compounds with a reaction mixture containing Cu^{2+} ions, neocuproine (alcoholic solution), and ammonium acetate buffer (pH 7). Following a 60-minute incubation period, optical density was determined at 450 nm. The experimental procedure entailed combining equal volumes (1 mL each) of copper(II) chloride (10 mM), neocuproine (7.5 mM), and ammonium acetate (1 M) with sample extract (0.2 mL) and deionized water (0.9 mL) to achieve a final reaction volume of 4.1 mL. Absorbance readings were obtained after a 60-minute reaction period, and antioxidant capacity was calculated in terms of Trolox® equivalent antioxidant capacity (TEAC) [56].

2.5. HPLC-DAD analysis of phenolic compounds in *A. caucasicus* leaves and flowers

2.5.1. Chemical reagents

The acetonitrile HPLC gradient was acquired from Sigma-Aldrich Co. (St. Louis, MO, USA), while the methanol HPLC gradient was obtained from Merck KGaA (Darmstadt, Germany). All phenolic reference compounds utilized in the analysis were procured from Sigma-Aldrich, an established vendor recognized for supplying research-grade chemicals and analytical standards.

2.5.2. Instrumental parameters

Protocol A: Advanced chromatographic methodology for the quantification of compounds numbered 1, 2, 3, 4, 6, 7, 8, 9, 14, 15, 16, 17, 18, and 19 isolated from the plant, as shown in Fig. 2. Compound isolation was performed with an ACE 5 C18 stationary phase (250 \times 4.6 mm, 5 μm particle size). The binary mobile phase consisted of (A) acetonitrile and (B) dilute acetic acid (1.5% v/v). The elution profile commenced with 15% component A and 85% component B, transitioning to 40% component A and 60% component B at 29 minutes. The instrumental configuration incorporated a 1260 DAD WR spectrophotometric detector (monitoring at 250, 270, and 320 nm), a 1260 Quaternary Pump (maintaining 0.7 mL/min volumetric flow), a 1260 Vial Sampler (delivering 10 μL injection volume), and a G7116A thermostatic column compartment (maintained at 35°C). Protocol B: Advanced chromatographic methodology for the quantification of compounds numbered 5, 10, 11, 12, 13, 20, 21, 22, 23, and 24 isolated from the plant, as shown in Fig. 2: Compound isolation was performed with an ACE 5 C18 stationary phase (250 \times 4.6 mm, 5 μm particle size). The binary mobile phase consisted of (A) methanol and (B) dilute acetic acid (1.5% v/v). The elution profile commenced with 10% component A and 90% component B, transitioning to 40% component A and 60% component B at 29 minutes, followed by 60% component A and 40% component B through 40 minutes, concluding with 90% component A and 10% component B from 40 to 53 minutes.

No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
Compounds	Vitamin	Phenolics	Gallic acid	3-4 Hydroxybenzoic acid	Vanillic acid	Syringic acid	Cumaric acid	Caffeic acid	Ferulic acid	Rosmarinic acid	Progallol	Chlorogenic acid	Resveratrol	Oleuropein	Flavonoids	Catechin	Epicatechin	Rutin	Myricetin	Quercetin	Apigenin	Cyanidin chloride	Hesperidin	Kaempferol	Baicalin	Chrysin

Figure 2. It lists the standards separated by the HPLC-DAD method

Table 2. Phytochemical compounds of *A. caucasicus* leaf

No	RT (min)	RI	Name of the compound	Content [%]
1	4.11	705	Furan, 2-ethyl-	7.08
2	6.16	802	Hexanal	9.62
3	7.29	834	Butanal, 2-ethyl-3-methyl-	1.29
4	7.86	850	2-Hexenal, (E)-	4.66
5	8.12	857	6,6-Dimethylhepta-2,4-diene	0.99
6	11.17	932	α -Pinene	4.21
7	12.17	953	2-Heptanone,6-methyl-	1.51
8	12.39	958	Benzaldehyde	2.37
9	13.19	975	β -Pinene	19.34
10	13.33	978	Sabinene	0.76
11	13.95	991	Furan, 2-pentyl	3.01
12	15.63	1023	p-Cymene	0.52
13	15.84	1027	D-Limonene	28.68
14	16.90	1047	β -Ocimene	0.90
15	17.49	1058	Isophorone	2.94
16	24.90	1195	Myrtenal	0.97
17	31.68	1323	Silphiperfol-5-ene	5.02
18	34.47	1378	Modephene	4.66
19	34.81	1385	α -Isocomene	1.25

RT: Retention times on an HP-5MS UI column, **RI:** Experimentally determined retention indices on an HP-5MS UI column

The instrumental configuration incorporated a 1260 DAD WR spectrophotometric detector (monitoring at 280, 290, 320, 370 and 535 nm), a 1260 Quaternary Pump (maintaining 0.7 mL/min volumetric flow), a 1260 Vial Sampler (delivering 10 μ L injection volume) and a G7116A thermostatic column compartment (maintained at 35°C).

2.5.3. Preparation of reference standard solutions

Quantitative determination of phenolic constituents was accomplished through external calibration curves using six serial dilutions of each reference standard at precisely defined concentrations: 25, 50, 75, 100, 200, and 300 μ g/mL. Subsequent to preparation, these calibration solutions were subjected to HPLC-DAD analysis under identical instrumental conditions as the experimental samples.

2.6. SPME-GC-MS analysis of volatile compounds in leaves and flowers of *A. caucasicus*

2.6.1. SPME absorption of volatile compounds

The fiber was conditioned as recommended by the manufacturer before use. The plant was thoroughly fragmented in a laboratory-type grinder. From the powdered sample, enough to fill one-third of a 20 mL vial was placed in a 20 mL bottle sealed with PTFE/silicone septa (Supelco). Each sample was heated at 45°C for 15 minutes. Then, a syringe with an appropriate fiber tip was immersed in the bottle and absorbed for 40 minutes. The compounds exposed to the fiber tip were injected into the injection block of the GC unit and held for 20 minutes for absorption.

Table 3. Phytochemical compounds of *A. caucasicus* flower

No	RT (min)	RI	Name of the compound	Content [%]
1	6.14	800	Hexanal	9.83
2	9.30	890	2-Heptanone	1.23
3	11.16	932	α -Pinene	6.19
4	12.39	958	Benzaldehyde	2.47
5	13.19	975	β -Pinene	52.26
6	13.74	986	Sulcatone	1.36
7	13.94	991	Furan, 2-pentyl	3.19
8	15.62	1023	p-Cymene	1.28
9	15.84	1027	D-Limonene	7.29
10	16.90	1047	β -cis-Ocimene	0.87
11	24.89	1195	cis-Myrtenal	1.28
12	25.09	1198	Dodecane	1.58
13	31.67	1223	Silphiperfol-5-ene	2.83
14	34.46	1278	Modephene	1.09
15	34.81	1285	(-)-Isocomene	0.55
16	35.45	1298	Tetradecane	1.31
17	36.44	1318	Caryophyllene	5.19

RT: Retention times on an HP-5MS UI column, **RI:** Experimentally determined retention indices on an HP-5MS UI column

2.6.2. Instrument conditions

GC-MS analysis will be performed using an Agilent (Agilent Technologies, Santa Clara, CA, USA) gas chromatograph and HP-5MS ultra inert capillary column (30 m \times 0.25 mm \times 0.25 μ m). High-purity helium (>99.99%) will be used as the mobile phase at a flow rate of 1.0 mL/min. The injector temperature was set to 250°C. The GC program maintained an initial temperature of 50°C for 2 minutes. It will be raised to 150°C at a rate of 2.5°C/min and held constant there for 5 minutes. Finally, it will be increased to 250°C at a rate of 6.5°C/min and held constant there for 1 minute. The MS operating parameters include an ionization energy of 70 eV, with a scanned mass range of 35-500 m/z [57].

3. Results and discussion

3.1. GC-MS analysis and photochemical profile of *A. caucasicus*

Various phytochemical compounds characterized by retention times ranging from 4 to 36 minutes and different indices varying between 700 and 1385 were detected in samples taken from the leaf and flower parts of *A. caucasicus*. As shown in Table 2 and Table 3, compounds found in high amounts in the flower and leaf parts of the plant include monoterpenes, terpenoids, sesquiterpenes, oxygenated heterocyclic compounds, and volatile organic compounds, and the proportions of these compounds vary according to plant parts.

In the research, beta-pinene, D-limonene, and alpha-pinene were identified as monoterpenes; silphiperfol-5-ene as a terpenoid; modephene as a sesquiterpene; Furan, 2-ethyl and Furan, 2-pentyl, as oxygenated heterocyclic compounds; and hexanal as a volatile organic compound. The distribution and concentrations

of these compounds in plant tissues provide significant contributions to understanding the phytochemical profile of *A. caucasicus*.

In the leaf part of the plant, the monoterpenes β -pinene (19.34%), D-limonene (28.68%), and α -pinene (4.21%); silphiperfol-5-ene (5.02%) as a terpenoid; modephene (4.66%) as a sesquiterpene; Furan, 2-ethyl (7.08%), and Furan, 2-pentyl (3.01%) as oxygenated heterocyclic compounds; and hexanal (9.62%) as a volatile organic compound were determined. In the flower part, the monoterpenes β -pinene (52.26%), D-limonene (7.29%), and α -pinene (6.19%); silphiperfol-5-ene (2.83%) as a terpenoid; modephene (1.09%) as a sesquiterpene; Furan, 2-pentyl (3.19%) as an oxygenated heterocyclic compound; and hexanal (9.83%) as a volatile organic compound were detected.

When examining the phytochemical profile of the *A. caucasicus* plant, notable differences were observed between the leaf and flower parts. β -pinene (52.26%) stands out as the dominant compound in the flower part, while D-limonene (28.68%) was found in the highest proportion in the leaf part. This difference reflects the different physiological functions and ecological roles of plant organs. The high presence of monoterpenes in both plant parts is one of the characteristic features of the Aster genus, particularly containing compounds such as beta-pinene, which has antispasmodic, anti-inflammatory [59], hypotensive [60], antimicrobial [61], anti-depressant, and sedative [58] effects, and D-limonene, which has antibacterial, anti-inflammatory, antiviral, antinociceptive, and antidiabetic effects [62]. The approximately 2.7 times higher proportion of beta-pinene in the flower part compared to the leaf part can be associated with the flowers' pollination process of attracting insects and their protective functions. In contrast, the approximately 4 times higher proportion of D-limonene in leaves compared to flowers can be considered as part of the leaves' defense mechanism against herbivores.

The detection of terpenoids and sesquiterpenes such as silphiperfol-5-ene and modephene in both parts, but their presence in higher concentrations in the leaf part, indicates that these compounds are more actively synthesized in the vegetative tissues of the plant. It is particularly noteworthy that modephene is approximately 4.3 times higher in the leaf part (4.66%) compared to the flower part (1.09%). When the distribution of oxygenated heterocyclic compounds is

examined, the detection of Furan, 2-ethyl compound only in the leaf part (7.08%) indicates the presence of a biosynthesis pathway specific to leaf tissue. Volatile organic compounds such as hexanal were found in similar proportions (9.62-9.83%) in both tissues.

These differences in the chemical composition of the leaf and flower parts of *A. caucasicus* demonstrate that different tissues of the plant possess different physiological functions and ecological roles. These findings are expected to make significant contributions to the evaluation of the plant's potential pharmacological and bioactive properties.

3.2. Total phenolic content and antioxidant properties of *A. caucasicus* extract

In this study, total phenolic, total flavonoid, FRAP, and CUPRAC contents of *A. caucasicus* flower and leaf extracts were determined spectrophotometrically (Table 4).

When examining the analysis results, it was observed that the total phenolic content among the plant organs varied between 17.81 and 25.57 mg GAE/g dry weight. While the total phenolic content of the leaf part of the plant was 17.81±0.60 mg GAE/g, the flower part showed values of 25.57±5.40 mg GAE/g. These results indicate that although there is not a very significant difference between the values of the leaf and flower parts, the total phenolic content of the flower part is slightly higher compared to the leaf part. The leaf parts of plants are exposed to higher rates of sunlight compared to other parts due to their large surface areas. For this reason, flavonoids accumulate more abundantly in leaves compared to other parts as the plant's mechanism to protect itself from UV radiation and reduce oxidative stress [63-65]. As shown in Table 4, the total flavonoid content varied between 5.02 and 3.99 mg QE/g dry weight. It was observed that the total flavonoid content of the plant's leaf part (5.02±0.04 mg QE/g) was higher than the total flavonoid content of the flower part (3.99±0.11 mg QE/g). Unlike total phenolics (TP), which show higher concentrations in flowers, total flavonoid (TF) content is typically more abundant in leaves. This finding aligns with established research demonstrating that flavonoids primarily accumulate in leaf tissue as a protective mechanism against UV radiation and oxidative stress [66].

FRAP and CUPRAC assays were independently applied to the flower and leaf extracts of the plant.

Table 4. The antioxidant properties of *A. caucasicus* extracts were investigated, including the total phenolic content, total flavonoid content, FRAP, and CUPRAC analyses.

Taxon	Used part	Total phenolic content (mg GAE/g dry sample)*	Total flavonoid content (mg QE/g dry sample)*	FRAP (μ mol FeSO ₄ ·7H ₂ O/g sample)*	CUPRAC (mmol TEAC/g sample)*
<i>A. caucasicus</i>	Leaf	17.81 ± 0.60	5.02 ± 0.04	7.77 ± 0.18	0.32 ± 0.01
	Flower	25.57 ± 5.40	3.99 ± 0.11	7.53 ± 0.17	0.27 ± 0.01

According to these findings, FRAP results ranged between 7.77 and 7.53 $\mu\text{mol Fe}^{2+}/\text{g}$ dry weight, while CUPRAC values varied between 0.32 and 0.27 mmol TEAC/g dry weight. The FRAP activity in leaves ($7.77\pm 0.18 \mu\text{mol Fe}^{2+}/\text{g}$) was slightly higher than in flowers ($7.53\pm 0.17 \mu\text{mol Fe}^{2+}/\text{g}$). Similarly, the CUPRAC activity in leaves ($0.32\pm 0.01 \text{ mmol TEAC}/\text{g}$) exceeded that observed in flowers ($0.27\pm 0.01 \text{ mmol TEAC}/\text{g}$).

These results indicate that the higher total phenolic content in flowers compared to leaves demonstrates that flowers are richer in phenolic compounds, which is consistent with the overall findings. However, the lower total flavonoid content in flowers compared to leaves supports the premise that plant leaves accumulate flavonoids as a protective mechanism against UV radiation and to mitigate oxidative stress, resulting in a richer flavonoid profile than flowers.

Furthermore, the elevated FRAP and CUPRAC values observed in leaves suggest that flavonoids may contribute more significantly to total antioxidant capacity than other phenolic compounds present in flowers. This observation aligns with expectations given the powerful reducing properties characteristic of flavonoids.

When comparing our current research with similar studies in the literature, remarkably intriguing findings emerge. One study reported that the total phenolic content of 17 different species from the Asteraceae family, including *A. caucasicus*, varied between 2.65 and 13.34 mg GA/g [67]. In a 2021 investigation on *Cirsium englerianum*, the total flavonoid content of the methanolic extract was determined to be 5.88 ± 0.21 [68]. In comparison with these literature data, the total phenolic content of the plant in our study (17.81-25.57 mg GAE/g) is significantly higher than the values reported for other species in the Asteraceae family (2.65-13.34 mg GA/g). This suggests that our investigated plant is rich in phenolic compounds and may constitute a valuable resource for potential phytotherapeutic applications.

The total flavonoid content, meanwhile, is comparable to the value reported in the literature for *Cirsium englerianum*; particularly, the value in our leaf samples ($5.02\pm 0.04 \text{ mg QE}/\text{g}$) approximates that of *Cirsium englerianum* (5.88 ± 0.21). Another study examined the time-dependent variations of leaf extracts from *Leuzea carthamoides*, another member of the same family. In this investigation, FRAP values were observed to range between 1.2 and 60 $\mu\text{mol Fe}^{2+}/\text{g}$ [69]. Hence, it is evident that the FRAP values of the plant in our study ($7.53\text{-}7.77 \mu\text{mol Fe}^{2+}/\text{g}$) are situated in the lower-middle

range of the broad spectrum of values for *Leuzea carthamoides*.

In conclusion, the compositional differences among plant parts analyzed in our study distinctly reflect the characteristic physiological functions and ecological roles of different plant organs. While flowers demonstrate superiority in total phenolic content, leaves exhibit higher flavonoid content and robust antioxidant capacity. These findings indicate a noteworthy phytochemical richness when compared with other members of the Asteraceae family and suggest that different parts of the plant could be evaluated as valuable natural resources for various purposes in pharmacological and therapeutic applications. Particularly, the antioxidant potential exhibited by leaf extracts suggests that they could be utilized as promising components in the development of protective and therapeutic formulations against various diseases associated with oxidative stress.

3.3. Quantification of phenolic and flavonoid compounds via HPLC analysis of *A. caucasicus* extract

In this study, ascorbic acid as a vitamin, along with various phenolic compounds and flavonoids, was examined in the leaf and flower parts of the *A. caucasicus* plant. The standard compounds used included 12 phenolic compounds such as gallic acid, 3,4-hydroxy benzoic acid, vanillic acid, syringic acid, coumaric acid, caffeic acid, rosmarinic acid, pyrogallol, chlorogenic acid, oleuropein, and resveratrol; additionally, there were 11 flavonoids, including catechin, epicatechin, rutin, myricetin, quercetin, apigenin, cyanidin chloride, hesperetin, kaempferol, baicalein, and chrysin.

The study employed two distinct methodologies. Advanced chromatographic techniques were developed to quantify compounds isolated from the plant, as detailed in Table 5. Protocol A was used to determine compounds 1, 2, 3, 4, 6, 7, 8, 9, 14, 15, 16, 17, 18, and 19, whereas Protocol B was specifically optimized for compounds 5, 10, 11, 12, 13, 20, 21, 22, 23, and 24. Fig. 3 and Fig.4 demonstrate the clear separation achieved between the various phenolic compounds.

In the study, both identification and quantification of a total of 24 phenolic compounds in the leaf and flower parts of the *A. caucasicus* plant were carried out by applying Protocol A and Protocol B (Table 5). Upon examination of the chromatograms obtained from the phytochemical analysis of *A. caucasicus*, the

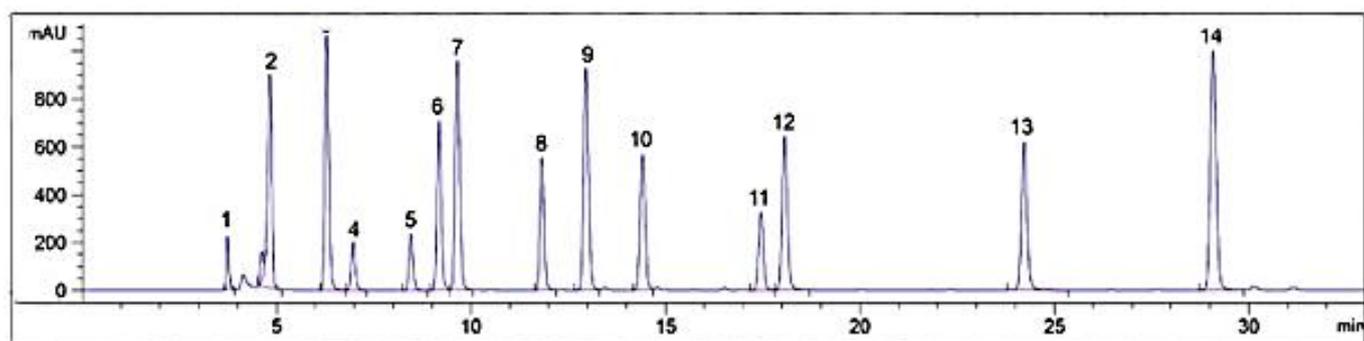


Figure 3. The HPLC Chromatograms of the Phenolic Standards (Protocol A). Their symbols and retention times are ascorbic acid (AsA) (1), gallic acid (2), 3-4 hydroxybenzoic acid (3), catechin (4), epicatechin (5), caffeic acid (6), vanillic acid (7), rutin (8), cumaric acid (9), ferrulic acid (10), rosmarinic acid (11), myricetin (12), quercetin (13), and apigenin (14)

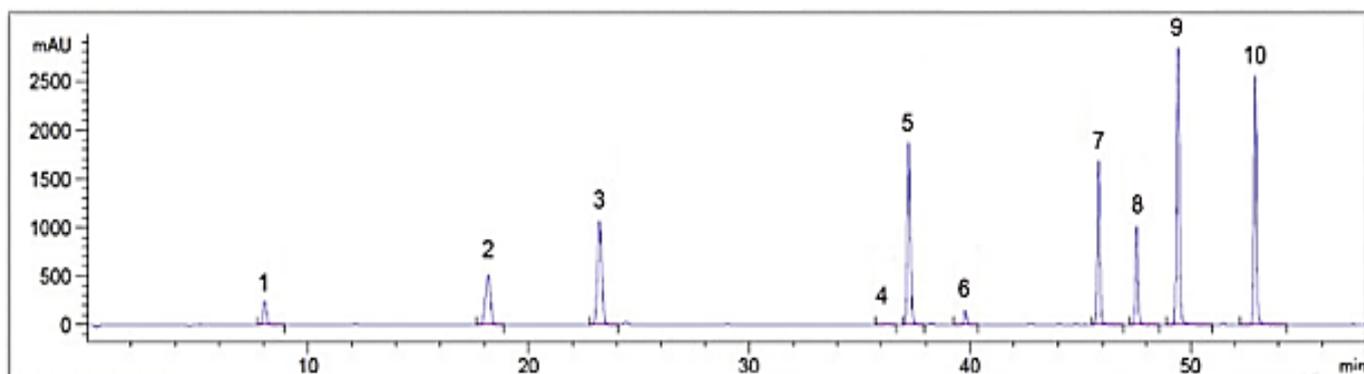


Figure 4. The HPLC Chromatograms of the Phenolic Standards (Protocol B.) Their symbols and retention times are pyrogallol (1), chlorogenic acid (2), syringic acid (3), cyanidin chloride (4), resveratrol (5), oleuropein (6), hesperitin (7), kaempferol (8), baicalein (9) and chrysin (10)

characteristic compounds of the flowers are presented in Fig. 5, while those of the leaves are presented in Fig. 6. Regarding ascorbic acid content, it was detected at a level of 178.3 mg/kg in the flower part but was not detected in the leaf part. This accumulation of ascorbic acid in flowers is attributed to increased metabolic activity and greater exposure to oxidative stress [70].

Phenolic compounds were predominantly concentrated in the flower part of the plant. Gallic acid (59.8 mg/kg) and 3,4-hydroxy benzoic acid (16.8 mg/kg) were detected in the flower part but not found in the leaf part. Caffeic acid was determined to be 15.5 mg/kg in flowers and 7.5 mg/kg in leaves. Although caffeic acid is present in both parts, it has approximately twice the concentration in the flower part compared to the leaf. Vanillic acid, however, is present in higher amounts in the leaf part (Table 5). These compounds are known for their properties to neutralize free radicals and protect against oxidative stress. The higher accumulation of these compounds in the flower part compared to the leaf part indicates that the flower part has a higher antioxidant potential than the leaf part.

Rosmarinic acid and *p*-coumaric acid, which have antioxidant and anti-inflammatory properties, were detected in both flower and leaf parts of the plant. The amounts of rosmarinic acid (131.6 mg/kg) and *p*-coumaric acid (141.9 mg/kg) in the flower part were higher than the amounts of rosmarinic acid (41.9 mg/kg) and *p*-coumaric

acid (119.2 mg/kg) in the leaf part. These results indicate that the flower part is more effective in terms of antioxidant activity.

Among flavonoids, Rutin, Quercetin, Apigenin, Hesperetin, Myricetin, and Chrysin were determined. Flavonoid compounds such as Apigenin (11.5 mg/kg), Hesperetin (321.5 mg/kg), Myricetin (4.7 mg/kg), and Chrysin (4.7 mg/kg) were detected in the flower part, while Quercetin was found to be 389.6 mg/kg in the flower part and 34.9 mg/kg in the leaf part. While Apigenin, Hesperetin, Myricetin, and Chrysin could not be detected at all in the leaf part, Quercetin was found in a much higher proportion in the flower part compared to the leaf part. This indicates that the immune-supporting, anti-inflammatory, and free radical scavenging properties [71] of flavonoid compounds are more prominent in the flower part compared to the leaf part. However, Rutin, which serves as a natural protection mechanism against harmful organisms and UV radiation, was found to be 1438.8 mg/kg in the leaf part and 315.2 mg/kg in the flower part, showing a significant difference in the leaf part of the plant.

Due to the limited studies on *A. caucasicus* in the literature, research on similar species has been examined. In one study [72], the phenolic content of 11 Aster species (*A. diplostephioides*, *A. souliei*, *A. himalaicus*, *A. flaccidus*, *A. farreri*, *A. sutschanensis*, *A. tongolensis*, *A.*

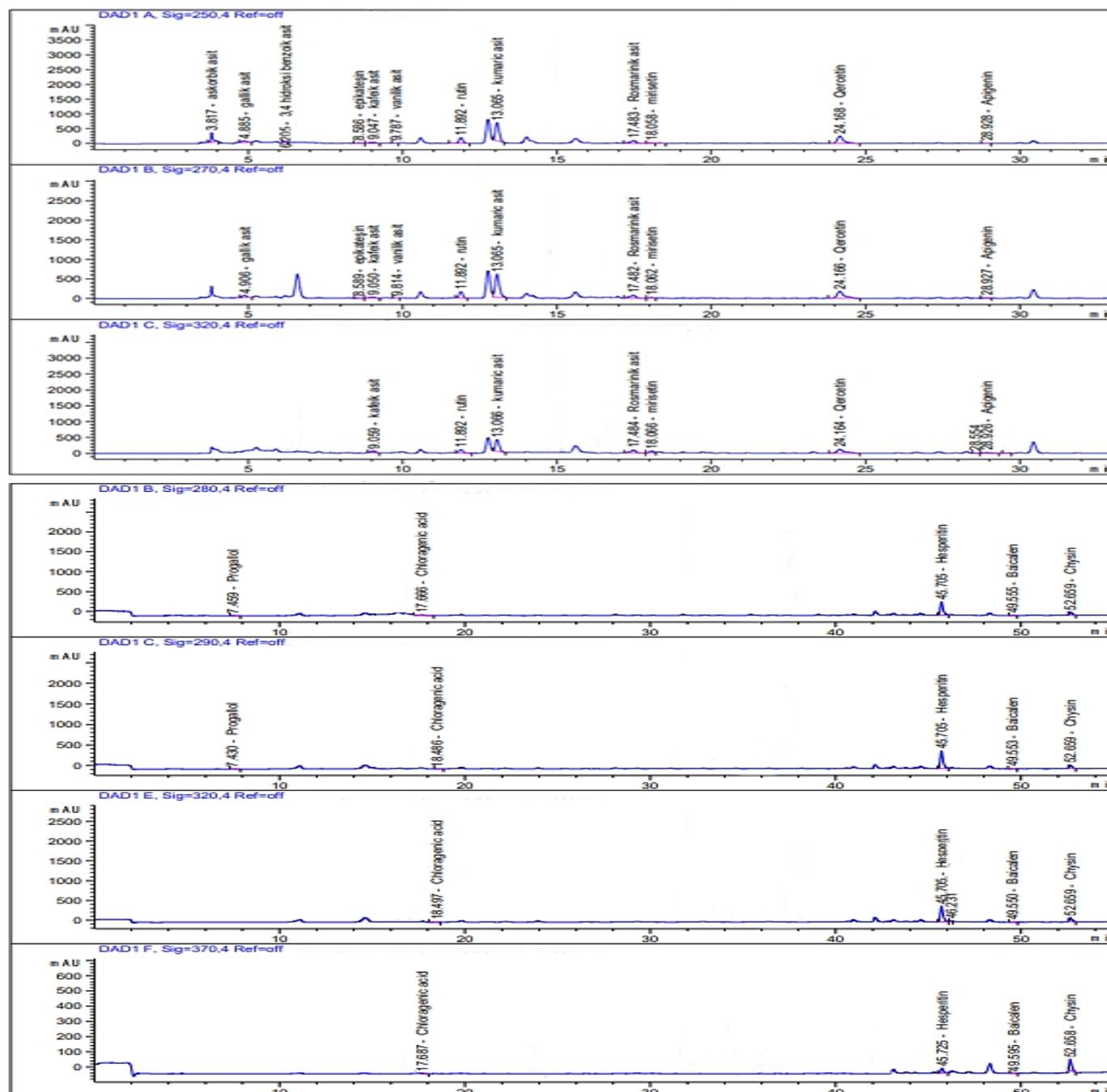


Figure 5. The chromatogram of the analysis results of *A. caucasicus* flowers

bietii, *A. yunnanensis*, *A. trinervius*, and *A. latibracteatus*) collected from different regions was compared. This study investigated the content of *chlorogenic acid* and its isomers, *rutin*, *isoquercitrin*, *3,5-dicaffeoylquinic acid*, *3,4-dicaffeoylquinic acid*, and *1,3-dicaffeoylquinic acid*. According to the results, *A. latibracteatus* and *A. trinervius* contained abundant amounts of *chlorogenic acid* and its isomers (23.5 ± 4.9 and 20.5 ± 5.8 mg/g, respectively), while these compounds were found in significantly lower quantities in samples of *A. latibracteatus* and *A. sutschanensis* (7.6 ± 0.55 and 9.5 ± 0.43 mg/g, respectively; $p < 0.01$). Several other *Aster* species (*A. souliei*, *A. bietii*, and *A. flaccidus*) also contained specific amounts of *chlorogenic acid* and its isomers (13.3 ± 5.1 , 14.3 ± 4.4 , and 15.2 ± 5.7 mg/g, respectively). In our current study, the

chlorogenic acid content in the leaves and flowers of *A. caucasicus* was determined to be approximately twice as high as the highest values reported in the literature, while the *rutin* content was approximately 10 times higher in leaves and more than 10 times higher in flowers. In another study from the literature [73], *quercetin* content was examined in the methanolic extract of *Aster spatulifolius* flowers but was not detected. In contrast, our current study identified high levels of *quercetin* in both the leaves and flowers of *A. caucasicus*. A subsequent study conducted in 2018 investigated the flavonoid content, including *apigenin*, *catechin*, *epicatechin*, *hesperidin*, *myricetin*, *quercetin*, and *rutin*, in the petals of various Chinese asters from the Asteraceae family (to which *A. caucasicus* also belongs), categorized

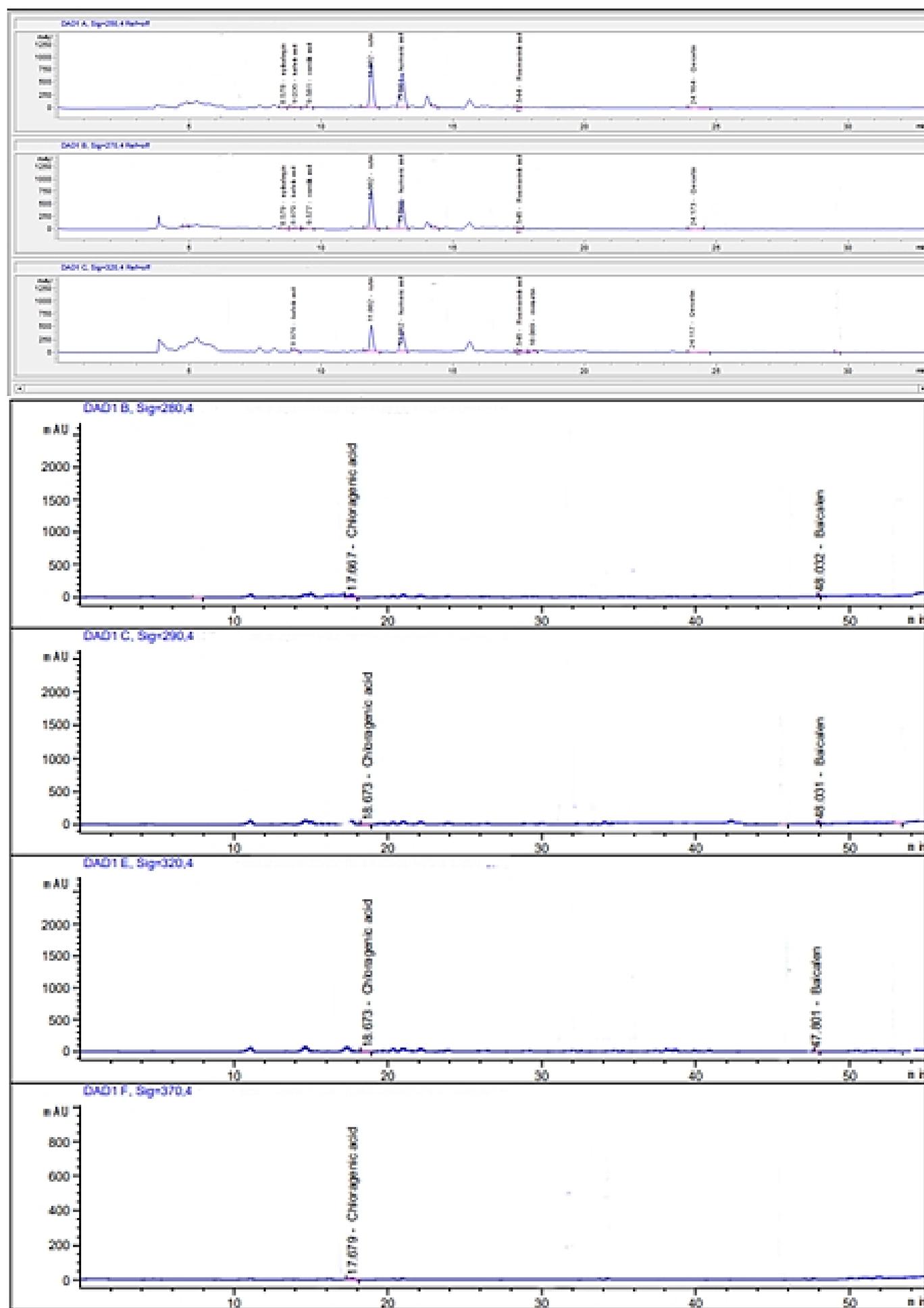


Figure 6. The chromatogram of the analysis results of *A. caucasicus* leaf

into seven groups based on flower colors [74]. According to this study, the highest apigenin value was reported as 266.1 ppm, while the lowest was 7.9 ppm; the highest catechin value was 284.3 ppm, while the lowest was 4 ppm. The *A. caucasicus* plant used in our current study demonstrated higher apigenin and catechin content in its flowers compared to most species examined. Furthermore, the content of other flavonoids such as epicatechin, hesperidin, quercetin, and rutin was found to be higher than all other species, while the myricetin content was higher than most species.

The results revealed that the diversity of phenolic compounds in the flower part was higher than in the leaf part. The presence of these compounds, which confer strong antioxidant and anti-inflammatory properties, in the flower part has shown that this part of the plant can be effectively included in pharmaceutical applications and can be a biological source for pharmaceutical products. Additionally, the very high amount of Rutin, which has natural protective properties against UV radiation, and Vanillic acid, which has antioxidant properties, in the leaf part compared to the other part has shown that the leaf parts can be included as an important biological source in cosmetic products.

4. Conclusions

In this study, a comprehensive analysis of *A. caucasicus*, a plant with limited and rare studies in the literature, was conducted. The phenolic and flavonoid contents, antioxidant capacities (total phenolic, total flavonoid, FRAP, and CUPRAC), and volatile compounds of the flower and leaf parts of the plant were examined in detail. This research has made a significant contribution to filling the knowledge gap in the literature regarding *A. caucasicus*.

The analysis results demonstrated that the leaf parts of the plant are rich in flavonoids to protect against UV radiation and reduce oxidative stress. The overall antioxidant capacity of the leaves was found to be higher compared to the flowers. On the other hand, flower parts were richer in phenolic compounds, and their antioxidant capacity was slightly lower than that of leaves.

In the chemical composition profile, beta-pinene (52.26%) stands out as the dominant compound in the flower part, while D-limonene (28.68%) was found in the highest proportion in the leaf part. This difference reflects the different physiological functions and ecological roles of plant organs. The high presence of monoterpenes in both plant parts is one of the characteristic features of the Aster genus.

The total phenolic content was higher in flower parts (25.57 mg GAE/g) compared to leaves (17.81 mg GAE/g),

Table 5. Contents of phenolic compounds in the leaves and flowers of *A. caucasicus*

No	Compounds	Flower (mg/L)	Leaf (mg/L)
Vitamin			
1	Ascorbic acid	178.3	N/D
Phenolics			
2	Gallic acid	59.8	N/D
3	3,4 hydroxy benzoic acid	16.8	N/D
4	Vanillic acid	1.0	20.5
5	Syringic acid	N/D	N/D
6	Coumaric Acid	141.9	119.2
7	Caffeic acid	15.5	7.5
8	Ferulic acid	N/D	N/D
9	Rosmarinic acid	131.6	41.9
10	Pyrogallol	46.7	84.6
11	Chlorogenic acid	51.2	54.5
12	Resveratrol	N/D	N/D
13	Oleuropein	N/D	N/D
Flavonoids			
14	Catechin	N/D	N/D
15	Epicatechin	54.8	39.4
16	Rutin	315.2	1438.8
17	Myricetin	4.7	N/D
18	Quercetin	389.6	34.9
19	Apigenin	11.5	N/D
20	Cyanidin chloride	N/D	N/D
21	Hesperitin	321.5	N/D
22	Kaempferol	N/D	N/D
23	Baicalein	10.5	11.6
24	Chrysin	4.7	N/D

N/D: Not Detected

while the leaves demonstrated higher total flavonoid content (5.02 mg QE/g) than flowers (3.99 mg QE/g). Furthermore, the leaves exhibited elevated FRAP (7.77 $\mu\text{mol Fe}^{2+}/\text{g}$) and CUPRAC (0.32 mmol TEAC/g) values, suggesting superior antioxidant capacity compared to the flower parts.

HPLC analyses revealed that phenolic compounds such as gallic acid (59.8 mg/kg), caffeic acid (15.5 mg/kg), rosmarinic acid (131.6 mg/kg), and quercetin (389.6 mg/kg) were present in higher concentrations in the flower parts compared to leaves, while rutin was detected at a significantly higher level in leaves (1438.8 mg/kg) than in flowers (315.2 mg/kg).

These findings highlight the potential of *A. caucasicus* flower and leaf parts in cosmetic and pharmaceutical applications and provide a solid foundation for future studies investigating the therapeutic applications of their bioactive properties. The flower parts, with their rich phenolic content, show promise for pharmaceutical applications, while the leaf parts, with their high flavonoid content and antioxidant capacity, could be valuable in cosmetic products, particularly for UV protection.

However, a significant limitation of the study is the lack of in vivo tests that would provide deeper insights into the bioavailability and therapeutic efficacy of these active compounds. Future research should address this

limitation by including in vivo studies to verify the current findings and further investigate the pharmacokinetics of the active components of the extract.

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