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Phenolic profile and antioxidant activities of different vegetative parts from *Colchicum autumnale* L. and *Colchicum diampolis* Delip. et Češchm grown in Bulgaria

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The genus *Colchicum* L. is well known for its medicinal uses. The therapeutic potential of these plants was mainly associated with the presence of tropolone alkaloids, with colchicine being the best studied bioactive substance. The accompanying bioactive compounds are very poorly studied in the Bulgarian populations of the genus *Colchicum*. Thus, the purpose of the present study was to investigate the phenolic profile and antioxidant activity of different vegetative parts (flowers, leaves, corms, and seeds) of two wild species grown in Bulgaria, namely C*olchicum autumnale* L. and the endemic species C*olchicum diampolis* Delip. et Češchm. Gas chromatography-mass spectrometry (GC-MS) analyses of phenolic compounds revealed the presence of 11 phenolic acids (salicylic acid, 4-hydroxybenzoic acid, protocatechuic acid, α-resorcylic acid, γ-resorcylic acid, gentisic acid, (*E*)-p-coumaric acid, (*E*)-ferulic acid, vanillic acid, (*E*)-caffeic acid and syringic acid) with the domination of p-coumaric acid. The antioxidant potential was determined through four of the most used spectrophotometric methods – 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC). The highest antioxidant activity was detected in obtained extracts from flowers and leaves of all populations.

Keywords: *Colchicum autumnale* L., *Colchicum diampolis* Delip. et Češchm, phenolic acids, antioxidant activity

INTRODUCTION

In the past few years, there is a growing demand for phytochemicals with antioxidant activity. Antioxidants have the ability to neutralize free radicals and supply protection against damage owing to these free radicals [1]. Free radicals are generated by various metabolic processes and the uncontrolled or increased formation of free radicals in the body may lead to oxidative stress [2]. Oxidative stress has been known to contribute to various diseases such as cancer, atherosclerosis, diabetes, neurological disorders, and hypertension [3]. The most effective antioxidant compounds seem to be phenolic acids and flavonoids of many plant raw materials, especially in fruits, flowers, seeds and herbs [4].

The genus *Colchicum* (*Colchicaceae*) comprises approximately 90 geophytic species [5]. Its distribution extends from North America, Asia, North Africa, Europe, and Eurasia [6]. Generally, in Bulgarian flora, 10 species are documented [7], with five protected under the Biodiversity Act. These include both spring-flowering (*C. diampolis*, *C. doerfleri* Halascy, *C. davidovii* Stef.) and autumn-flowering species (*C. bivonae*, *C. autumnale*, *C. turcicum* Janka, *C. haynaldii* Heuff.). *Colchicum* species (*Colchicaceae* family) are one of the significant plants with medicinal properties associated with the presence of tropolone alkaloids,

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colchicinoids, mainly colchicine [8]. Ancient Greek physicians used the plant as a therapeutic agent for gout. In India and Africa, various preparations of colchicum are still used traditionally to treat gastroenterological, musculoskeletal, and cutaneous diseases [9]. Colchicine and its natural analogues are used clinically for the treatment of several disorders such as Familial Mediterranean Fever, amyloidosis, Behcet's disease, cirrhosis, psoriasis, and many other dermatological diseases. Pharmacological studies have shown that the *Colchicum* species possess antioxidant, antibacterial, acetylcholinesterase, anti-inflammatory, and antiarthritic properties [9].

Concerning *Colchicum* species from the Bulgarian flora, studies were focused mainly on the botanical characteristic and distribution of the species [10, 11]. Data about detailed phytochemical analyses and biological activity of the genus is insufficient. Recently, Dincheva *et al.* [12] described comprehensive GC-MS metabolite profiling of *C. autumnale* L. (Ribaritza), *C. bivonae* Guss. (Slivnitsa), and *C. diampolis* Delip. et Ceschm. (Iskra) and revealed the presence of 66 metabolites, including free amino, organic, phenolic, and fatty acids, sugars, and alkaloids. Phenolic acids possessed valuable biological and pharmacological properties (strong antioxidant, anti-inflammatory, anticancer, antimicrobial, antiallergic, antiviral, antithrombotic, hepatoprotective activity and many more) [13] that are of great importance for *Colchicum* species. In this context, the presented study aimed at finding and comparing the phenolic profile and antioxidant activity of different vegetative parts (flowers, leaves, corms, and seeds) of two wild grown in Bulgaria species of the genus *Colchicum,* namely C. *autumnale L.* and the endemic species *C. diampolis* Delip. et Ceschm.

MATERIALS AND METHODS

Plant material

The plant materials of *C. autumnale L.* and *C. diampolis* Delip. et Ceschm. (namely flowers, leaves, corms, and seeds), were collected from their natural habitats. Samples of *C. autumnale L.* were collected from Gela village, Smolyan municipality, Rhodope Mountains (41°39'53.9"N, 24°33'48.2"E) and from Iskrets village, municipality Svoge, Western Stara Planina (42°59'02.9"N, 23°17'27.6"E). Plants of *C. diampolis* were collected from Karnobat Municipality, Eastern Stara Planina (42°39'00"N, 26°58'05"E). The samples were collected under an official permit № 965/27.01.2023 from the Ministry of Environment and Water of Bulgaria. The samples were identified by Dr. I. Semerdjieva (Agricultural University, Plovdiv, Bulgaria/Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences). Voucher specimens of target species were deposited at the herbarium at the Agricultural University (SOA), Plovdiv, Bulgaria. The herbarium specimen numbers were as follows: *C. autumnale* – 063587 and 063588; *C. diampolis* - 062422. Approximately twenty plants were collected from the same population. The collected plant materials were thoroughly cleaned by rinsing with tap water and then with distilled water to remove soil and other contaminants. The plant parts, including flowers, leaves, seeds, and corms, were carefully separated and then lyophilized in a laboratory freeze dryer Alpha 1-2 LSC basic (Martin Christ GmbH, Osterodeam Harz, Germany). The dried plant materials were ground into a fine powder using a sample disruption/homogenizer system QIAGEN Tissue Lyser II (Retsch GmbH, Haan, Germany). Powdered materials were used for extract preparation.

Extraction methods

Dried ground plant parts were extracted with 40% ethanol in an ultrasonic bath SIEL UST 5.7-150 (Gabrovo, Bulgaria) at 35 kHz frequency and 240 W power, at 40 ℃ for 30 min. The residue of the plant material was removed through filter paper filtration, and the obtained water-ethanol extracts were used for analysis of total phenols, total flavonoids content and antioxidant activity.

The extraction procedure for GC-MS analysis of phenolic acids was described previously by Dincheva *et al*. [12].

Analysis of total polyphenol and total flavonoid content

The total polyphenol content was measured using Folin–Ciocalteu's assay, described by Vrancheva *et al*. [14]. Analysis of total flavonoids was determined spectrophotometrically following the method described by Vrancheva *et al.* [14].

Determination of antioxidant activity

- *DPPH scavenging assay.* The ability of extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by the method described by Kivrak *et al*. [15] and Andonova *et al.* [16].
- *$ABTS^{\bullet+}$ scavenging assay*. The radical scavenging activity of the extracts against 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

(ABTS) was estimated according to Thaipong *et al.* [17] and Andonova *et al.* [16].

- *Ferric reducing antioxidant power (FRAP) assay.* The FRAP assay was carried out according to the procedure of Benzie and Strain [18] and Andonova *et al.* [16].
- *Cupric reducing antioxidant capacity (CUPRAC) assay.* The CUPRAC assay was carried out according to the procedure of Apak *et al.* [19] and Andonova *et al.* [16]. The antioxidant activity determined by DPPH, ABTS, FRAP, and CUPRAC assays was expressed as mmol Trolox equivalents (TE) per g dry weight (dw) by using a calibration curve built in the range of 0.05–0.5 mmol Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Fluka), dissolved in methanol (Merck).
- *GC-MS analysis of phenolic acids.* The analysis was performed using a 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled with a 5975C mass-selective detector (Agilent Technologies, Santa Clara, CA, USA) according to method described by Dincheva *et al*. [12]. To calculate retention indices (RI), a mixture of aliphatic hydrocarbons (C_{10}–C_{40}) from Sigma was injected under the same temperature program. Compounds were identified by comparing the RIs and spectral data with references from a custom reference library, the Golm Metabolome Database (http://gmd.mpimp-golm.mpg.de/analysisinput.aspx, accessed 6 June 2024) [20] and the NIST'08 database (National Institute of Standards and Technology, Gaithersburg, MD, USA) [21]. The results were presented in mg/g dw.

Statistical analysis

Three independent extracts from each plant part were prepared and each extract was analyzed in triplicate for total phenolic and flavonoid contents, individual phenolic acids, and antioxidant activity. The presented values are means with standard deviations (± SD) calculated by Microsoft Office Excel® 2010.

RESULTS AND DISCUSSION

The total polyphenolic content varied between 6.17 ± 0.57 and 269.01 ± 3.44 mg GAE/g dw in the different plant parts, as the highest values were established in the extracts of flowers and leaves of all tested species (Table 1). Besides, flowers and leaves of both populations of *C. autumnale* contained higher amounts of total polyphenols than that of *C. diampolis.* In terms of total flavonoids, flowers and leaves were also determined to be richer than corms and seeds extracts (in the range of 0.18 ± 0.00 and 3.31 ± 0.18 mg QE/g dw). The established total flavonoid content in flowers and leaves of *C. autumnale* from Gela was about ten times lower than that in *C. autumnale* from Iskrets and *C. diampolis* population.

In accordance with our data, Suica-Bunghez *et al.* [22] found that the flower extract of *C. autumnale* contained a higher amount of total polyphenols and flavonoids than root (bulb) extract. Davoodi *et al*. [23] reported significantly lower total phenol (0.56 g gallic acid/100 g corm) and total flavonoid content (0.37 g quercetin/100 g corm) in 80 % water-methanol corms extract of *C. autumnale* than our data. It is interesting to note that Senizza *et al.* [24] found that different root extracts of *Colchicum triphyllum* contained higher total phenolic acids than flower and leaf extracts. The differences observed in total phenolic and total flavonoid content could be explained with different solvents and extraction methods used, interspecies differences, climatic and geographical differences, as well.

To obtain detailed information about the phenolic profile, GC-MS analysis was carried out. Eleven phenolic acids were identified (salicylic acid, 4-hydroxybenzoic acid, protocatechuic acid, α-resorcylic acid, γ-resorcylic acid, gentisic acid, (*E*)-p-coumaric acid, (*E*)-ferulic acid, vanillic acid, (*E*)-caffeic acid, and syringic acid) with the domination of p-coumaric acid (Table 2). Salicylic and 4-hydroxybenzoic acid were detected only in the leaf extracts of both populations of *C. autumnale* and in the leaf and seed extracts of *C. diampolis*. Protocatechuic acid and α-resorcylic acid were in the highest concentration in the leaves of all samples, as flowers contained the highest amount of γ-resorcylic acid. Ferulic acid was not detected in the corms of all samples and seeds of *C. autumnale* (Iskrets). Vanillic and caffeic acid were found only in flowers of *C. diampolis* and flowers, corms, and seeds of two *C. autumnale* populations. Flowers were the only plant parts with the presence of syringic acid.

The same phenolic acids were identified in the plant parts of other populations of *C. autumnale* (from Ribaritsa village) and *C. diampolis* (from Iskra village) grown in Bulgaria, but in different quantities [12].

The relatively high amounts of total polyphenols, total flavonoids and individual phenolic acids in the analyzed plant species is a prerequisite for their antioxidant activity. In order to investigate the antioxidant potential, four methods differing in conditions and mechanism of action were applied (DPPH, ABTS, FRAP and

CUPRAC, respectively). The highest ability to quench DPPH radical was detected in the extract of leaves of *C. autumnale* from Isktets (582.65 ± 0.47 mM TE/g dw), followed by the extract of flowers of *C. diampolis* (566.61 ± 0.17 mM TE/g dw), Table 3. Flower and leaf extract of all samples tested also showed the highest antioxidant potential according to ABTS method. Corm and seed extracts possessed the lowest antioxidant activity defined by DPPH and ABTS methods. Flower and leaf extracts also had the highest ability to reduce Fe^{3+} (FRAP) and Cu^{2+} (CUPRAC) in comparison with corm and seed extracts. The highest antioxidant activity of flowers and leaves determined by all methods could be explained with the highest quantity of total polyphenols and individual phenolic acids found in their extracts. Similarly to our finding Suica-Bunghez *et al.* [22] reported that flower extract of *C. autumnale* had a higher ability to scavenge DPPH radical than root extract (flowers – 52.81%, roots – 34.60 % inhibition of DPPH). Rocchetti *et al.* [9] also reported that leaf and flower extracts of *Colchicum szovitsii* possessed higher antioxidant activity (defined by DPPH, ABTS, FRAP and CUPRAC methods) than root extracts.

Table 1. Total polyphenol content (TPC) and total flavonoid content (TFC) of different vegetative parts from *C. autumnale* and *C. diampolis*.

	C. diampolis				*C. autumnale* (Gela)				*C. autumnale* (Iskrets)			
	*F	L	C	S	F	L	C	S	F	L	C	S
TPC, mg GAE/g dw	157.49 ± 3.96	96.73 ± 4.73	16.61 ± 0.90	24.46 ± 0.17	192.56 ±1.52	269.01 ± 3.44	6.17 ± 0.57	27.54 ± 0.45	174.99 ± 4.71	204.05 ± 5.79	19.80 ± 0.62	28.29 ± 1.25
TFC, mg QE/g dw	2.79 ± 0.02	3.31 ± 0,02	0.23 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.71 ± 0.01	0.23 ± 0.01	0.20 ± 0.01	2.60 ± 0.02	2.73 ± 0.02	0.18 ± 0.01	0.27 ± 0.01

*F – flowers; L – leaves; C – corms; S – seeds.

Table 2. GC-MS analysis of phenolic profile of different vegetative parts from *C. autumnale* and *C. diampolis*. The results are presented in mg/g dw.

		C. diampolis				*C. autumnal* (Gela)				*C. autumnale* (Iskrets)			
Phenolic acid	RI	*F	L	C	S	F	L	C	S	F	L	C	S
Salicylic acid	1505	**nd	1.07 ± 0.02	nd	0.89 ± 0.02	nd	0.93 ± 0.02	nd	nd	nd	0.82 ± 0.02	nd	nd
4-Hydroxy benzoic acid	1620	nd	0.29 ± 0.01	nd	0.38 ± 0.01	nd	1.27 ± 0.02	nd	nd	nd	1.10 ± 0.02	nd	nd
Proto catechuic acid	1820	0.44± 0.01	1.31 ± 0.03	0.46 ± 0.01	0.14 ± 0.01	0.38 ± 0.01	1.14 ± 0.03	0.64 ± 0.01	0.24 ± 0.01	0.34 ± 0.01	1.01 ± 0.02	0.93 ± 0.02	0.31 ± 0.01
α-Resorcylic acid	1762	0.34± 0.01	0.61 ± 0.01	0.26 ± 0.01	0.50 ± 0.01	0.29 ± 0.01	0.53 ± 0.01	0.13 ± 0.01	0.20 ± 0.01	0.26 ± 0.01	0.47 ± 0.01	0.43 ± 0.01	0.25 ± 0.01
γ-Resorcylic acid	1765	0.40± 0.01	0.14 ± 0.01	nd	0.12 ± 0.01	0.35 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	0.09 ± 0.01	0.31 ± 0.01	0.11 ± 0.01	0.10 ± 0.00	0.12 ± 0.01
Gentisic acid	1779	0.31± 0.01	0.50 ± 0.01	0.04 ± 0.00	0.43 ± 0.01	0.27 ± 0.01	0.24 ± 0.01	0.74 ± 0.02	0.26 ± 0.01	0.24 ± 0.01	0.39 ± 0.01	0.36 ± 0.01	0.33 ± 0.01
(*E*)-p-Coumaric acid	1942	0.73± 0.02	0.95 ± 0.02	0.56 ± 0.01	0.08 ± 0.00	0.63 ± 0.01	0.48 ± 0.01	0.86 ± 0.02	0.40 ± 0.01	0.56 ± 0.01	0.73 ± 0.02	0.41 ± 0.01	0.50 ± 0.02
(*E*)-Ferulic acid	2099	0.16± 0.01	0.07 ± 0.00	nd	0.06 ± 0.00	0.14 ± 0.01	0.06 ± 0.00	nd	0.12± 0.01	0.13 ± 0.01	0.05 ± 0.00	nd	nd
Vanillic acid	1773	0.29± 0.01	nd	nd	nd	0.25 ± 0.01	nd	0.40 ± 0.01	0.15± 0.01	0.22 ± 0.01	nd	0.25 ± 0.01	0.19 ± 0.01
(*E*)-Caffeic acid	2136	0.70± 0.01	nd	nd	nd	0.91 ± 0.02	nd	0.90 ± 0.02	0.57± 0.01	0.81 ± 0.02	nd	0.28 ± 0.01	0.71 ± 0.02
Syringic acid	1838	0.27± 0.01	nd	nd	nd	0.24 ± 0.01	nd	nd	nd	0.21 ± 0.01	nd	nd	nd

*F – flowers; L – leaves; C – corms; S – seeds; ** nd - not detected

Table 3. Antioxidant activity of different vegetative parts from *C. autumnale* and *C. diampolis*.

	Colchicum diampolis				*Colchicum autumnale* (Gela)				*Colchicum autumnale* (Iskrets)			
	*F	L	C	S	F	L	C	S	F	L	C	S
DPPH, mM TE/g dw	566.61 ± 0.17	45.69 ± 0.91	2.33 ± 0.01	6.72 ± 1.12	144.57 ± 2.97	110.44 ± 4.98	0.87± 0.21	18.31± 0.88	53.34 ± 0.04	582.65 ± 0.47	6.58 ± 0.17	16.96 ± 0.54
ABTS, mM TE/g dw	192.30± 0.67	118.50 ± 1.94	25.23 ± 0.21	31.89± 1.89	236.89± 0.84	243.56 ± 5.05	10.77± 0.13	32.58 ± 0.88	178.43 ± 3.68	235.89 ± 1.56	24.05± 0.14	31.34 ± 0.12
FRAP, mM TE/g dw	143.34± 1.40	92.14 ± 0.87	14.90 ± 0.11	23.00± 0.17	230.67± 1.89	219.58 ± 2.47	42.74± 1.06	25.83 ± 0.39	129.98 ± 1.33	181.99 ± 1.22	14.13 ± 0.13	24.52 ± 0.40
CUPRAC, mM TE/g dw	266.54± 6.88	189.29 ± 4.08	31.80 ± 0.54	57.69± 0.76	349.49± 10.98	446.66 ± 14.42	88.60± 3.94	58.00± 1.31	203.55 ± 9.62	247.67 ± 7.08	25.99 ± 0.24	76.50 ± 1.69

*F – flowers; L – leaves; C – corms; S – seeds.

CONCLUSION

To the best of our knowledge, this is the first report of detailed analyses on antioxidant activity of wild grown in Bulgaria populations of *C. autumnale* and *C. diampolis*. Flower and leaf extracts of all *Colchicum* populations showed the highest ability to quench DPPH and ABTS radical and the highest capability to reduce Fe^{3+} (FRAP) and Cu^{2+} (CUPRAC), as well. The highest antioxidant potential of flower and leaf samples could be explained with the highest quantity of total polyphenols and individual phenolic acids determined in their extracts. The present study showed that all plant parts of investigated populations of *Colchicum* species are a valuable source of phenolic acids, and that the observed antioxidant activity could provide the basis for their further inclusion in different systems for better health benefits.

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