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Datura innoxia and *Dipsacus laciniatus*: Biological activity and phenolic composition

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ABSTRACT

In the present study, we investigated the phenolic composition of the methanol extract from the aboveground of two plants of Turkish flora; *Datura innoxia* and *Dipsacus laciniatus*. In addition, the antioxidant (phosphomolybdenum, CUPRAC, FRAP, DPPH, ABTS) and enzyme inhibitory activities (α-amylase and tyrosinase), and total phenolic and flavonoids contents were determined in these plant extracts. The results showed high significant differences among plants ($p \leq 0.05$) for the antioxidant capacities measured with the different methods, the enzyme inhibitory activities, and the all identified phenolic compounds except for hyperoside. From a total of 23 identified phenolic compounds, 19 were found in both plants. The main flavonoids identified were (+)-catechin and (−)-epicatechin and hyperoside. (+)-Catechin and (−)-epicatechin showed very high concentrations in *D. innoxia* (12937.39 ± 108.86 and 24147.64 ± 2512.35 μg/g of dry plant, respectively) and in *D. laciniatus* (4947.99 ± 14.18 and 13171.30 ± 2410.76 μg/g of dry plant, respectively). Hyperoside had interesting contents with comparable values (115.11 ± 16.20 in *D. innoxia*, and 110.77 ± 16.32 μg/g of dry plant in *D. laciniatus*). The major phenolic acid was chlorogenic acid especially in *D. laciniatus* (12124.22 ± 598.13 μg/g of dry plant). These compounds were correlated to the high antioxidant and enzyme inhibitory activities for both species and confirm their medicinal traditional uses. Hence, further screening of bioactive compounds in different organs such as flowers and roots of these plants is crucial for the discovering of new source of natural antioxidants and enzyme inhibitors.

1. Introduction

Floristic analysis has shown that our planet has approximately 500,000 plant species, and about 120,000 plant species can be used to create biologically active products that could be used in treatment of many diseases (Kallassy, 2017). Several bioactive compounds found in medicinal plants showed high activities against many diseases (rheumatic, inflammatory, microbiologic origins … etc.) (Mukhtar et al., 2008; Prakash et al., 2007). In Turkey, it is estimated that the Turkish flora has more than 10,000 species of vascular plants (about 34% are endemic) (Kizilarslan and Özhatay, 2012). Ethnobotanical studies reported that a wide range of Turkish plants are used for medicinal purposes in the traditional medicine (Sadikoglu and Alpinar, 2004). Despite their high potential biological activities, a lot of plants are not well characterized and still under debate such as *Datura inoxia* and *Dipsacus laciniatus*.

Datura innoxia Mill. originates from Central America. It is an annual ornamental plant with attractive flowers and is alien of Turkey (Uludag et al., 2017) growing in the gardens for decorative purposes. It is present as both cultivated plant and as well as wild population. Despite *D. innoxia* is extremely toxic plant, there is evidence that in Serbian Vojvodina province it is cultivated as medicinal plant (Lakušić et al., 2017). However, little information is available on its phenolic composition and bioactive properties. In genus *Datura*, some phytochemicals were screened in *D. metel* such as alkaloid, phenols, flavonoids, tannins, saponins and sterols (Okwu and Igara, 2009).

Dipsacus laciniatus, native to Eurasia, is an invasive weed species (Sforza, 2004). In Turkey, it is very largely distributed in north Turkey (from Ankara to the Black Sea), but rare or absent in the rest of the country (Sforza, 2004). Plants appear to be a mixture of rapidly growing rosettes and rosettes with the development of reproductive structures (Bentivegna and Smeda, 2011). In the other side, this species

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is used in the processing of wool (Rector et al., 2006). Also, *D. laciniatus* is cultivated for its attractive purple flowers, its attractiveness to butterflies, and as natural enemies of crop pests (Judd, 1983). The benefits of this species for human health and their phytochemical composition are still unclear. In fact, plants of genus *Dipsacus* have been used as medicinal agents to treat several diseases (Alzheimer's disease, cancer, lime disease, fibromyalgia, etc.) (Zhao and Shi, 2011). Previous studies on plants of the genus *Dipsacus* has reported many biological activities such as antimicrobial activity, cytoprotective capacities, inhibition of HIV-1 reverse transcriptase, and antinociceptive effects, etc. (Zhao and Shi, 2011). *Dipsacus asper* herbs have been documented to have neuroprotective compounds for treating or preventing neurodegenerative diseases such as the phenolic acid 3,5-dicaffeoylquinic acid (Kim et al., 2005). The neuroprotective agents could have a supressor effect of neuroinflammation inducing the attenuation of neuronal damage (Mollica et al., 2012). To the best of our knowledge, there is very few information on the phytochemical composition, the benefits and the potential uses both *Datura innoxia* and *Dipsacus laciniatus*.

In this context, this study aims to screen the phenolic composition of the aerial parts of *D. innoxia* and *D. laciniatus*. Methanol was used to extract bioactive molecules from the plant material. The antioxidant (phosphomolybdenum, CUPRAC, FRAP, DPPH, ABTS) and enzyme inhibitory activities (α-amylase and tyrosinase), total phenolic and flavonoids, and the phenolic profiles of *D. innoxia* and *D. laciniatus* extracts were determined.

2. Materials and methods

2.1. Plant material

The aerial parts of *Datura innoxia* Mill. (Solanaceae) and *Dipsacus laciniatus* L. (Dipsacaceae) were collected from Senir town, Keciborlu, Isparta-Turkeyon 10 June 2016 (846 m, 37° 47′ 27.03″N 30° 13′ 28.01″E) and Karakent village, Burdur-Turkey on 10 July 2016 (925 m, 37° 41′ 42.89″N 30° 03′ 50.73″E), respectively. The plants were deposited and recognized by Prof. Dr. Hasan Ozcelik from the Department of Biology, Süleyman Demirel University, Isparta-Turkey.

2.2. Preparation of the extracts

Five grams of air-dried samples of the aerial parts of each plant was macerated with 100 ml of methanol for 24 h. This process was repeated once more. The methanol extracts were mixed, concentrated under reduced pressure and conserved at +4 °C for further analyzes. The yields of methanol extract were 5.07% and 2.00% (w/w) for *D. innoxia* and *D. laciniatus*, respectively.

2.3. Phenolic composition analysis by LC–ESI–MS/MS (Liquid Chromatography–Electrospray Tandem mass spectrometry)

Total phenolic and flavonoid contents in the extracts were firstly determined spectrophotometrically by calculating as gallic acid and quercetin equivalents, respectively (Zengin et al., 2015a, 2015b). Then phenolic composition in the extracts were detected by LC–ESI–MS/MS (Cittan and Çelik, 2018) using experimental conditions given in detail in the supplementary file.

2.4. Antioxidantand enzyme inhibition activities

Diverse assays such as free radical scavenging [on 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\cdot}$) radical and 2,2-azino-bis (3-ethylbenzothiazloine-6-sulphonic acid) radical cation (ABTS$^{\cdot+}$)] (Zengin et al., 2015b), phosphomolybdenum (Zengin et al., 2015a), ferrous ion chelating (Tepe et al., 2011), and reducing power [cupric ion reducing (CUPRAC), and ferric reducing antioxidant power (FRAP)] (Apak et al., 2006; Kocak et al., 2016) were used to screen the antioxidant activities of the two plant extractsusing experimental conditions given in detail in the supplementary file. The activity is expressed as both IC_{50} and EC_{50} value (the sample concentration providing 50% of radical scavenging/ferrous ion chelating, or 0.500 absorbance for the reducing power/phosphomolybdenum assays, respectively) and standard (Trolox or EDTA) equivalents (μg/g extract).

Enzyme inhibitory activities of the methanol extracts were measured toward α-amylase and tyrosinase using experimental conditions given in detail in the supplementary file (Zengin et al., 2015c). The enzyme inhibitory activity is expressed as both standard (kojic acid and acarbose) equivalents and IC_{50} values (the sample concentration providing 50% of enzyme inhibition).

2.5. Statistical analysis

All the experiments were repeated in triplicate and the results were given as the mean value and the standard deviation (mean ± SD). Statistical significance was analyzed by using ANOVA (one-way analysis of variance) and Tukey's honestly significant difference post hoc test and Student's t-test with $\alpha = 0.05$ for biological activity assays and phenolic composition (SPSS v. 22.0 software package).

3. Results and discussion

3.1. Antioxidant and enzyme inhibitionactivities

Due to their role against oxidative stress, the determination of the radical scavenging activity of plant extracts is one of the most popular topics in plant study (Fadda et al., 2018). Herein, for both plants, the antioxidant activity of the methanol extracts was determined as radical

Table 1
Radical scavenging and metal chelating activities of the methanol extracts from *D. innoxia* and *D. laciniatus*[x].

Samples	Inhibition concentration (IC_{50}: mg/mL)[y]			Activity (mg TEs/g extracts)[z]		Activity (mg EDTAEs/g extracts)[z]
	DPPH radical	ABTS radical cation	Ferrous ion chelating	DPPH radical	ABTS radical	Ferrous ion chelating
D. innoxia	0.61 ± 0.01[b]	0.74 ± 0.03[b]	6.88 ± 0.93[b]	534.55 ± 4.88[a]	638.87 ± 26.11[a]	11.69 ± 1.50[a]
D. laciniatus	1.11 ± 0.04[c]	1.69 ± 0.04[c]	13.31 ± 0.56[c]	292.01 ± 9.23[b]	281.04 ± 5.93[b]	6.25 ± 0.24[b]
Trolox	0.33 ± 0.01[a]	0.46 ± 0.01[a]	–	–	–	–
EDTA	–	–	0.074 ± 0.005[a]	–	–	–

[x] The mean values followed by the same superscripts within a column do not differ, according to the Tukey's honestly significant difference post hoc test at 5% significance level.
[y] IC_{50} (mg/mL), inhibition concentration at which 50% of the DPPH and ABTS radicals were scavenged and the ferrous ion-ferrozine complex were inhibited. EDTA, ethylenediaminetetraacetic acid (disodium salt). "- ", not determined.
[z] The mean values followed by the same superscripts within a column do not differ, according to the Student's t-test at 5% significance level. EDTAEs and TEs, ethylenediaminetetraacetic acid (disodium salt) and trolox equivalents, respectively.

Table 2
Reducing power and total antioxidant (by phosphomolybdenum assay) activities of the methanol extracts from *D. innoxia* and *D. laciniatus*[x].

Samples	Effective concentration (EC_{50}: mg/mL)[y]			Activity (mg TEs/g extracts)[z]		
	CUPRAC reducing	FRAP reducing	Phosphomolybdenum	CUPRAC reducing	FRAP reducing	Phosphomolybdenum
D. innoxia	0.61 ± 0.01[b]	0.44 ± 0.01[b]	3.01 ± 0.22[a]	830.64 ± 5.35[a]	446.38 ± 9.94[a]	435.52 ± 30.99[a]
D. laciniatus	1.09 ± 0.01[c]	0.69 ± 0.01[c]	5.68 ± 0.70[b]	460.30 ± 3.57[b]	287.58 ± 2.38[b]	240.16 ± 27.45[b]
Trolox	0.53 ± 0.01[a]	0.21 ± 0.01[a]	1.28 ± 0.02[a]	–	–	–

[x] The mean values followed by the same superscripts within a column do not differ, according to the Tukey's honestly significant difference post hoc test at 5% significance level.
[y] EC_{50} (mg/mL), effective concentration at which the absorbance was 0.5 for reducing power and phosphomolybdenum assays.
[z] The mean values followed by the same superscripts within a column do not differ, according to the Student's t-test at 5% significance level. TEs, trolox equivalents.

scavenging activity, metal chelating activities, reducing power, and total antioxidant (by phosphomolybdenum assay). High significant differences were observed between *D. Innoxia* and *D. laciniatus* methanol extracts for all antioxidant tests (Tables 1 and 2). The DPPH and ABTS methods were used to assay the radical scavenging activities and results were expressed as trolox equivalents (TEs) antioxidant capacity. Table 1 shows that the extract from *D. innoxia* has the highest radical scavenging activities using DPPH and ABTS radicals with respective values 534.55 ± 4.88 and 638.87 ± 26.11 mg TE/g extracts. However, extract of *D. laciniatus* shows a fold change decrease more than 0.5 when compared to *D. innoxia,* with respective values 292.01 ± 9.23 and 281.04 ± 5.93 mg TE/g extracts. In addition, this result was confirmed using the ferrous ion chelating activity which shows a significantly high activity (11.69 ± 1.50 mg EDTAEs/g extract) of *D. innoxia* compared to *D. laciniatus* (6.25 ± 0.24 mg EDTAEs/g extract) (Table 1). Similarly, CUPRAC and FRAP reducing powers and phosphomolybdenum assay, were highest in *D. innoxia* with respective values 830.64 ± 5.35, 446.38 ± 9.94 and 435.52 ± 30.99 mg TE/g of dry plant (Table 2). However, *D. laciniatus* extract showed lesser CUPRAC, FRAP and total antioxidant activities, with values 460.30 ± 3.57, 287.58 ± 2.38, and 240.16 ± 27.45, respectively. When assessing the antioxidant potential of plant extracts, both antioxidant activity and phenolic composition must be studied (Elfalleh et al., 2019).

In fact, the key enzyme inhibition is an important pathway for disease treatment, especially with the use of natural products derived from plants in order to avoid the side effects of synthetic drugs (Grochowski et al., 2017). Herein, *in vitro* enzyme inhibitory activities of the methanol extracts against α-amylase and tyrosinase have been investigated. Results are shown in Table 3. Both plant extracts were remarkably active against the two enzymes with significant difference between them. *D. innoxia* extract showed the strongest activity against α-amylase (356.35 ± 4.60 mg ACEs/g extracts) and tyrosinase (317.03 ± 2.00 mg KAEs/g extracts). However, the *D. laciniatus* extract were slightly lower than *D. innoxia* with values of 329.21 ± 2.27 mg ACEs/g extracts (against α-amylase) and 267.48 ± 6.58 mg KAEs/g extracts (against tyrosinase). These results confirm that aerial parts extracts of *D. innoxia* and *D. laciniatus* could be considered as new key enzyme inhibitors. They can be used for pharmaceutical industries, especially in the antidiabetic and skin care bio-products which usually assessed by α-amylase, tyrosinase key enzymes (Atay Balkan et al., 2018). These observed biological activities (antioxidant activity and enzyme inhibitory effects) of the two plants extracts can be attributed to the existence of phenolic compounds (Uysal et al., 2019).

3.2. Total phenolics and flavonoids

The contents of total flavonoids (in mg QE/g extract) and total phenolic (in mg GAE/g extract) of the extracts from the aerial parts of both species are shown in Table 4. Results show high significant variation among specie of these amounts. Total phenolic amount was higher in *D. inoxia* (73.80 ± 0.57 mg GAE/g extract). However, the highest Total flavonoids content was detected in *D. laciniatus* (39.20 ± 1.96 mg QE/g extract). This result confirmed that total phenolic is due to flavonoids and other phenolic compounds such as phenolic acids. In addition, possibly, the variation in total phenolic amounts might be explained by both genetic variation and geographic origins of plants (Bajalan et al., 2016). Since, phenolic compounds present a high variation of solubility following their structure guided polarity (Elfalleh et al., 2019), additional analysis on individual phenolic compounds is important.

3.3. Individual phenolic compounds

The methanolic extracts from both plants were measured for individual phenolic compounds concentration. Results, expressed as μg/g of dry plant, were shown in Table 5. The LC-ESI-MS/MS phytochemical analysis concerned thirty-one phenolic compounds. From them, 23 compounds were identified and quantified in methanolic extracts and only 19 compounds were detected in both species. Statistical tests showed significant differences ($p \leq 0.05$) among plants for all identified compounds except forhyperoside. Four major compounds were identified from the LC-ESI-MS/MS method (Fig. 1) in both plants. (+)-catechin and (−)-epicatechin showed very high amounts in *D.*

Table 3
Enzyme inhibitory activities of the methanol extracts from *D. innoxia* and *D. laciniatus*[x].

Samples	Inhibition concentration (IC50: mg/mL)[y]		Activity (mg ACEs/g extracts)[z]	Activity (mg KAEs/g extracts)[z]
	α-Amylase	Tyrosinase	α-Amylase	Tyrosinase
D. innoxia	2.94 ± 0.04[b]	1.02 ± 0.01[b]	356.35 ± 4.60[a]	317.03 ± 2.00[a]
D. laciniatus	3.18 ± 0.02[c]	1.21 ± 0.03[c]	329.21 ± 2.27[b]	267.48 ± 6.58[b]
Acarbose	1.05 ± 0.01[a]	–	–	–
Kojic acid	–	0.33 ± 0.01[a]	–	–

[x] The mean values followed by the same superscripts within a column do not differ, according to the Tukey's honestly significant difference post hoc test at 5% significance level.
[y] EC_{50} (mg/mL), inhibition concentration at which 50% of the α-amylase and tyrosinase activities were inhibited. "-", not determined.
[z] The mean values followed by the same superscripts within a column do not differ, according to the Student's t-test at 5% significance level. ACEs and KAEs, acarbose and kojic acid equivalents, respectively.

Table 4
Total phenolics and flavonoids of methanolic extracts from *D. innoxia* and *D. laciniatus*[x].

Extracts	Total flavonoids (mg QEs/g extract)	Total phenolics (mg GAEs/g extract)
D. innoxia	33.15 ± 2.82[a]	73.80 ± 0.57[a]
D. laciniatus	39.20 ± 1.96[a]	51.01 ± 0.25[b]

[x] The mean values followed by the same superscripts within a column do not differ, according to the Student's t-test at 5% significance level. QEs and GAEs quercetin and gallic acid equivalents.

innoxia (12937.39 ± 108.86 and 24147.64 ± 2512.35 µg/g of dry plant, respectively) and in *D. laciniatus* (4947.99 ± 14.18 and 13171.30 ± 2410.76 µg/g of dry plant, respectively). Also, chlorogenic acid was predominant especially in *D. laciniatus* with values of 12124.22 ± 598.13 µg/g of dry plant. However, hyperoside was high in both plant extracts with comparable values of 115.11 ± 16.20 (*D. innoxia*) and 110.77 ± 16.32 (*D. laciniatus*) µg/g of dry plant.

In fact, (+)-catechin and (−)-epicatechin are the most predominant flavonoids metabolites (Punyasiri et al., 2004). These compounds have different monomeric constituents and variable degrees of oligomerization (Takanashi et al., 2017). Previous results reported that the combination of (+)-catechin and (−)-epicatechin had an adipogenic activity of Labrador tea (Eid et al., 2016). In addition, these two flavonoids compounds have many important and beneficial activities. Shin et al. (2009) reported that (+)-catechin and (−)-epicatechin highly activated the differentiation of human bone marrow mesenchymal stem cells into adipocytes. Cruz-González et al. (2016) found that epicatechin and catechin decrease oxidative stress and neuro-inflammation. Also, they may prevent nervous cells from oxidative damage (Blount et al., 2012). The highest content of these compounds found in our study, brings further evidence for the therapeutic potential of *D. innoxia* and *D. laciniatus* which could be an important natural source of these flavonoid compounds.

Concerning the chlorogenic acid, it is phenolic acid and a predominant isomer among caffeoylquinic acid isomers (Elfalleh et al., 2019). It has antioxidant and DNA-protective activities (Crozier et al., 2012). In this study, we found highest content of chlorogenic acid in *D. laticianus* than in *D. inoxia*. This confirms the traditional use of plants of the genus *Dipsacus* as medicinal agents to treat several diseases (Alzheimer's disease, cancer, lime disease, fibromyalgia … etc.) and their important biological activities such as antimicrobial activity, cytoprotective capacities … etc. (Zhao and Shi, 2011). Also, other therapeutic uses of chlorogenic acid include hepatoprotective, free radical scavenger, antipyretic, cardioprotective, neuroprotective, anti-microbial, anti-obesity, anti-inflammatory, antiviral, anti-hypertensive, and central nervous system stimulator (Naveed et al., 2018).

Finally, hyperoside, also called quercetin-3-*O*-b-D-galactoside pyranose, was found predominant flavonoid compound in both plant extracts. It is well known with their biological effects, such as antioxidant, anti-inflammatory, and anticancer capacities (Middleton et al., 2000). Besides, it has noticeable cardiovascular benefits to improve heart functions, this compound is frequently used in clinic as a pain reliever (Liu et al., 2012). The high amount of hyperoside confirms the traditional use of aerial parts preparations of *D. laticianus* as an anti-inflammatory and to activate pulmonary and the cardiovascular functions (Zaurov et al., 2013). These results encourage carrying out more analyses in *D. innoxia* and *D. laticianus* in order to screen other plant organs such as flowers and roots and discover as many antioxidants compounds as possible.

Table 5
Concentration and analytical characteristics of selected phenolic compounds in the methanol extracts from *D.innoxia*and *D.laciniatus*[x].

R_t (min)	Compounds	Concentration (µg/g extract)		Analytical characteristics			
		D. innoxia	*D. laciniatus*	Linear equation	R^2	LOD (µg/L)	LOQ (µg/L)
8.891	Gallic acid	3.66 ± 0.41[b]	13.32 ± 0.39[a]	y = 4.82x − 26.48	0.9988	1.46	4.88
10.818	Protocatechuic acid	62.15 ± 0.72[b]	192.56 ± 0.73[a]	y = 5.65x − 9.99	0.9990	1.17	3.88
11.224	3,4-Dihydroxyphenylacetic acid	nd	2.68 ± 0.80	y = 5.13x − 12.39	0.9990	1.35	4.51
11.369	(+)-Catechin	12937.39 ± 108.86[a]	4947.99 ± 14.18[b]	y = 1.45x + 1.95	0.9974	3.96	13.20
11.506	Pyrocatechol	nd	nd	y = 0.11x − 0.52	0.9916	9.62	32.08
11.802	Chlorogenic acid	14.20 ± 0.44[b]	12124.22 ± 598.13[a]	y = 12.14x + 32.34	0.9995	0.55	1.82
12.412	2,5-Dihydroxybenzoic acid	nd	nd	y = 3.79x − 14.12	0.9980	2.12	7.08
12.439	4-Hydroxybenzoic acid	7.75 ± 0.12[b]	15.53 ± 1.19[a]	y = 7.62x + 22.79	0.9996	1.72	5.72
12.458	(−)-Epicatechin	24147.64 ± 2512.35[a]	13171.30 ± 2410.76[b]	y = 9.11x − 9.99	0.9971	1.85	6.18
12.841	Caffeic acid	17.58 ± 1.24[b]	220.19 ± 12.28[a]	y = 11.09x + 16.73	0.9997	3.15	10.50
12.843	Vanillic acid	86.17 ± 2.17[a]	33.92 ± 1.86[b]	y = 0.49x − 1.61	0.9968	2.56	8.54
12.963	Syringic acid	13.69 ± 0.15[a]	4.59 ± 0.16[b]	y = 0.74x − 1.54	0.9975	3.75	12.50
13.259	3-Hydroxybenzoic acid	nd	nd	y = 3.69x − 12.29	0.9991	1.86	6.20
13.397	Vanillin	12.27 ± 0.53[b]	20.78 ± 0.53[a]	y = 2.02x + 135.49	0.9926	15.23	50.77
13.589	Verbascoside	7.16 ± 0.58[a]	3.16 ± 0.58[b]	y = 8.59x − 28.05	0.9988	0.82	2.75
13.909	Taxifolin	6.14 ± 0.16	nd	y = 12.32x + 9.98	0.9993	1.82	6.05
13.992	Sinapic acid	2.93 ± 0.73[a]	2.36 ± 0.74[a]	y = 2.09x − 6.79	0.9974	2.64	8.78
14.022	p-Coumaric acid	28.52 ± 0.93[a]	13.48 ± 0.94[b]	y = 17.51x + 53.73	0.9997	1.93	6.44
14.120	Ferulic acid	79.04 ± 1.47[a]	24.81 ± 1.31[b]	y = 3.32x − 4.30	0.9992	1.43	4.76
14.266	Luteolin 7-glucoside	14.36 ± 0.68[b]	111.71 ± 6.89[a]	y = 45.25x + 156.48	0.9996	0.45	1.51
14.412	Hesperidin	2.15 ± 0.11[b]	109.16 ± 10.34[a]	y = 5.98x + 0.42	0.9993	1.73	5.77
14.506	Hyperoside	115.11 ± 16.20[a]	110.77 ± 16.32[a]	y = 16.32x − 1.26	0.9998	0.99	3.31
14.600	Rosmarinic acid	nd	nd	y = 9.82x − 17.98	0.9989	0.57	1.89
14.781	Apigenin 7-glucoside	nd	3.47 ± 0.11	y = 21.33x − 31.69	0.9983	0.41	1.35
15.031	2-Hydroxycinnamic acid	nd	nd	y = 16.72x − 26.94	0.9996	0.61	2.03
15.118	Pinoresinol	135.61 ± 4.39[a]	21.69 ± 0.43[b]	y = 0.80x − 2.69	0.9966	3.94	13.12
15.247	Eriodictyol	nd	nd	y = 14.24x − 0.50	0.9998	0.80	2.68
15.668	Quercetin	9.47 ± 0.14[a]	5.05 ± 0.14[b]	y = 14.68x − 18.25	0.9997	1.23	4.10
15.923	Luteolin	nd	3.37 ± 0.21	y = 8.96x + 26.80	0.9992	1.34	4.46
16.236	Kaempferol	nd	nd	y = 0.82x − 3.06	0.9959	3.30	10.99
16.382	Apigenin	nd	nd	y = 11.29x + 38.05	0.9987	0.96	3.20

[x] The mean values followed by the same superscripts within a row do not differ, according to the Student's t-test at 5% significance level. LOD and LOQ, limits of detection and quantification, respectively. R_t, retention time.

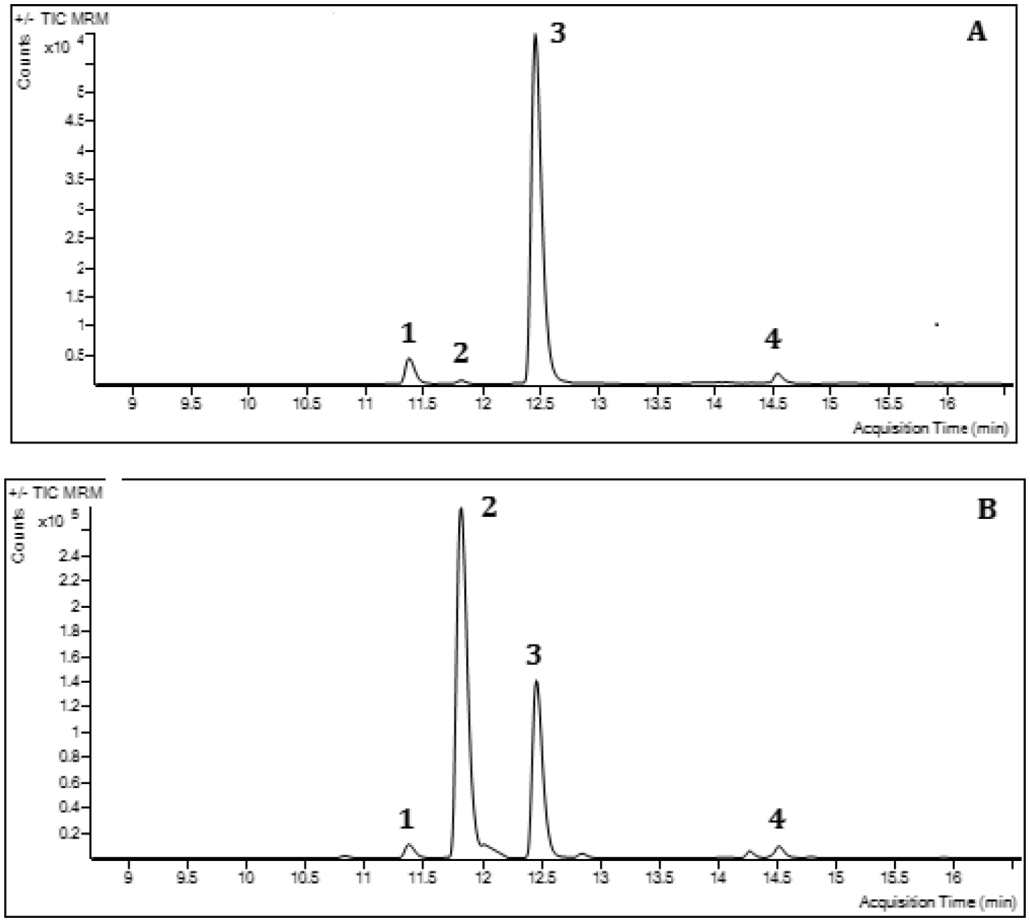

Fig. 1. LC–ESI–MS/MS chromatograms of the methanol extracts from *D. innoxia* (A) and *D. laciniatus* (B), respectively. 1: (+)-catechin, 2: chlorogenic acid, 3: (−)-epicatechin, and 4: hyperoside.

4. Conclusions

The antioxidant and enzyme inhibition activities with different methods, and phenolic composition by LC–ESI–MS/MS analyses of *D. innoxia* and *D. laticianus* were investigated. The methanol extract from *D. innoxia* had the highest activities for all antioxidant methods (DPPH, ABTS, CUPRAC and FRAP) and for the enzyme inhibition against α-amylase and tyrosinase. To the best of our knowledge, this is the first detailed report on phenolic compounds from extracts of both species of Turkish flora. The two plants are endowed with phenolic acids and flavonoids having significant antioxidant capacities. Twenty-three compounds were identified and quantified in methanolic extracts of the aerial parts and only 19 compounds were shared in both plants. The predominant compounds identified in both speciesarewell-known by their biological activities. They were (+)-catechin and (−)-epicatechin, chlorogenic acid, and hyperoside. These results supported the importance of the traditional uses of *D. innoxia* and *D. laticianus* as natural sources of bioactive molecules. Therefore, more investigations should be done for individual phenolic compounds on different organs such as flowers and roots of these plants for the isolation of natural antioxidant and enzyme inhibitor molecules.

Conflicts of interest

The authors confirm that there are no known conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101163.

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