www.biodicon.com Biological Diversity and Conservation

SSN 1308-8084 Online; ISSN 1308-5301 Print 10/1 (2017) 115-123

Research article/Araştırma makalesi

Contents of phenolic and flavonoid compounds in *Isatis demiriziana* Mısırdalı: an endemic to the Southeast Anatolia, Turkey

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Abstract

Isatis demiriziana Mısırdalı plant contains a number of compounds which has anticarcinogenic, antioxidant and other preventive effects. In this study, the flavonoid and phenolic contents in the plant samples harvested in the vegetative (leaf and root) and full flowering season (flower, leaf and root) were determined by LC-MS/MS. Among the 27 compounds studied, malic acid was found to be the most abundant compound in the methanolic extracts of samples and the amount of malic acid of vegetative root extracts were the highest (30124,37 µg g^{-1} dry-extract). Moreover, it was also determined considerable amount of salicylic acids and p-coumaric in the root extracts. This study is the first detailed study on the phenolic and flavonoid compounds of *I. demiriziana*. Based on the findings of this study, in further researches might be refered as an additional source in production of phenolic and flavonoid compounds.

Key words: Isatis, LC–MS/MS, phenolics, flavonoids, endemic

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Türkiye'de Güneydoğu Anadolu Bölgesi'ne endemik *Isatis demiriziana* Mısırdalı'ndaki fenolik ve flavonoid bileşiklerinin içerikleri

Özet

Isatis demiriziana Mısırdalı bitkisi antikanserojen, antioksidan ve başka koruyucu özelliklere sahip olan çok sayıda bileşik içermektedir. Bu çalışmada, *I. demiriziana*'nın vejetatif (yaprak ve kök) ve tam çiçeklenme (çiçek, yaprak ve kök) dönemlerinde hasat edilen bitki örneklerinin flavonoid ve fenolik içerikleri LC-MS/MS ile tespit edildi. Çalışılan 27 bileşik arasında, örneklerin metanolik ekstraktlarında malik asit miktarı en fazla düzeyde bulundu ve vejetatif köklerdeki malik asit miktarı (30124,37 µg g^{-1} kuru ekstrakt) en yüksek orana sahipti. Ayrıca kök ekstraktlarında büyük miktarda salisilik asit ve p-kumarik asit tespit edildi. Bu çalışma, *I. demiriziana*'nın fenolik ve flavonoid içerikleri üzerine yapılmış ilk detaylı çalışmadır. Bu çalışmanın sonuçlarına dayanılarak, ileriki çalışmalarda fenolik ve flavonoid bileşiklerin üretiminde bir ek kaynak olarak başvurulabilir.

Anahtar Kelimeler: *Isatis*, LC–MS/MS, fenolikler, flavonoidler, endemik

1. Introduction

Brassicaceae (Cruciferea) has about 350 genera and 3000 species, growing mostly in the North Temperate Zone and Mediterranean Region (Mabberley, 1987). *Isatis* genus belongs to Brassicaceae family and this genus is represented by 40 taxa which 24 of these are endemic to Turkey (Davis, 1988). Moreover, this genus has 31 species and 14 subspecies in Eastern and South-Eastern Anatolia (Mısırdalı, 1985). The chemical constitutients extracted from the roots and leaves of *Isatis* species have antiviral, anticancer, antibacterial, astringent, febrifuge and antirheumatic effects (Radwan et al., 2008; Vang, 1994; Kirtikau and Basu, 1983; Bown, 1995). They are also employed for different

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disorders such as encephalitis, meningitis, erysipelas, influenza, heat rash, mumps in the traditional medicine. It is reported that uses of *Isatis* species ethnobotanical in Eastern Anatolia (Polat et. al., 2012). Use of natural antioxidants including phenolic acids and flavonoids as preventive and therapeutic drug have been attracting considerable attention because of their antioxidant properties and anticarcinogenic potential. These compounds have several health promoting influences (Costa et al., 2012; Erdogan-Orhan et al., 2014; Prakash et al., 2007). Phenolic compounds act as antioxidants (Maillard et al., 1996). Phenolic compounds play a significant role in total antioxidant capacity of vegetables, fruits and grains (Jacobo-Velazquez and Cisneros-Zevallos, 2009). The benefits of flavonoids including reducing the risk of atherosclerosis developing have been clearly shown (Knekt et al., 1996). Flavonoids perform some important functions such as cell cycle inhibition, nitrogen fixation and filtration of UV rays (Kumar and Pandey, 2013). Several studies suggested that flavonoids have protective impacts against degenerative diseases such as cardiovascular diseases, cancers and other age-related diseases (Pandey, 2007; Kumar et al., 2013). Phenolic acids include two primary groups: Cinnamic and benzoic acid (Tarnawski et. al., 2006). The chlorogenic acid is the most significant cinnamic acid which is a combination of quinic and caffeic acids. Hydroxybenzoic acid derivatives are firstly present as vanillic acids but p-hydroxybenzoic and protocatechuic acids are more extensive (Kvasnicka et. al., 2008).

Study on several certain phenolic acids such as gallic acid, malic acid, p-coumaric acid, vanillic acid and syringic acid reveals that they have numerous advantage for human health. Malic acid plays a vital role in reversing muscle fatigue and mental clarity. Moreover, these actions can make it a beneficial treatment for sufferers of fibromyalgia (Russell et al., 1995). Vanillic acid and p-coumaric acid have hydroxide scavenging activity (Kang et al., 2006). Moreover, p-coumaric acid is a potent inhibitor of 5-S-cysteinyl dopamine induced neurotoxicity and this compound is used in treatment of Parkinson's disease (Vauzour et al., 2010). It was shown that the Gallic acid acted as an important agent in protection of renal damage causing death of tumor cells (Canbek et al., 2013). Salicylic acid plays a significant role in conservation against virus infection by inhibiting catalase resulting in the accumulation of H_2O_2 in plant cells (Chen et al., 1993).

Different parts of plants vary in terms of contents of phenolics and flavonoids, so each plant part needs to be analyzed to identify its potential advantage as a health product. It was reported that the chemical constituents in the leaves of some *Isatis* species possess antibacterial, antiviral, anticancer, febrifuge and astringent features (Karakoca et al., 2013) but there is no report on the phenolic and flavonoid contents of *I. demiriziana*. In this research, we aimed to determine the phenolic and flavonoid contents of different parts of *I. demiriziana* plants collected in the vegetative season (leaf and root) and full flowering season (flower, leaf and root). Liquid chromatography–tandem mass spectrometry (LC– MS/MS) technique was used to analyze the phenolic and flavonoid contents of *I. demiriziana*.

2. Materials and methods

2.1. Plant Material

Five different samples for *I. demiriziana* were collected from a height of 1300 meters from Ergani county of the Diyarbakır province and at vegetative (leaf and root) and full flowering season (flower, leaf and root). Voucher specimens were deposited at the Herbarium of Dicle University, Faculty of Science (voucher no. DUF-6050). Specimens were identified by Prof. Dr. Ömer SAYA, from the same institution. The samples were air-dried at root temperature and then the samples were pulverized with a laboratory mill and stored at 4^0C until the chemical tests were conducted. Quantitation and identification of phenolic and flavonoid compounds

2.2.1. Plant extract preparation for LC–MS/MS

The collected specimens were dried by air at room temperature, which were then powdered. The samples (100 g) were extracted three times with 300 mL of methanol for 24 h. Then, a rotary evaporator was used for removal of the solvent at 30°C. The remaining solid (Yield: 15.6%) was used to prepare a 1000 mg L^{-1} solution, which was then filtrated with a 0.2 μm microfiber filter to use for LC–MS/MS analysis.

2.2.2. Instruments and chromatographic conditions for LC–MS/MS

The phenolics were analyzed quantitatively by LC-MS/MS (Shimadzu LC/MS 8040 model). The liquid chromatograph has DGU-20A3R degasser, LC-30AD binary pumps, SIL-30AC autosampler and CTO-10ASvp column oven. The samples were separated chromatographically on a C18 reversed-phase Inertsil ODS-4 (150 mm × 4.6 mm, 3 μm) analytical column (40°C). The elution gradient comprised of mobile phase A:water, 0.1% formic acid and 5 mM ammonium formate and mobile phase B: methanol, 0.1% formic acid and 5 mM ammonium formate. The gradient program with the following ratios of solvent B was carried out t (min), B%: (0, 40), (20, 90), (23.99, 90), (24, 40), (29, 40). The solvent flow percentage was continued at 0.5 mL/min and injection volume was adjusted as 4 μL.

2.2.3. MS instrumentation

The samples were analyzed by MS employing a Shimadzu LC/MS 8040 model triple quadrupole mass spectrometer equipped (ESI source operating in both negative and positive ionization modes). Data obtained from LC–MS/MS were evaluated by Lab Solutions software (Shimadzu, Kyoto, Japan). The analyses were quantified by the multiple reaction monitoring (MRM) mode: the studied compounds were assayed following two or three transitions for each compound, the first one for quantitative uses and the second and/or the third one for checking of the finding. The optimum ESI conditions were determined as interface temperature; 350°C, DL temperature; 250°C, heat block temperature; 400°C, nebulizing gas flow (nitrogen); 3 L/min and drying gas flow (nitrogen); 15 L/min.

2.2.4. Analytical parameters for the validated LC–MS/MS method

Table 1 shows rectilinear regression equations and the linearity ranges of the studied standard chemicals. Correlation coefficients were higher than 0.99. Table 1 also displays the limit of detection (LOD) and limit of quantitation (LOQ) of the analytical method. LOD values of the compounds are between 0.05 and 25.8 g/L and LOQ values are between 0.17 and 85.9 g/L (the recoveries of the phenolics are between 96.9% and 106.2%).

Table 1. Analytical parameters of UHPLC-ESI-MS/MS method

No	Analytes	RT[a]	Parent ion (m/z)[b]	Ionization Mode	R^{2}[c]	RSD%[d]	Linearity Range (mg/L)	LOD/LOQ (µg/L)[e]	Recovery (%)	U[f]
1	Quinic acid	3.32	190.95	Neg	0.9927	0.0388	250-10000	22.3 / 74.5	103.3	4.8
2	Malic acid	3.54	133.05	Neg	0.9975	0.1214	250-10000	19.2 / 64.1	101.4	5.3
3	tr-Aconitic acid	4.13	172.85	Neg	0.9933	0.3908	250-10000	15.6 / 51.9	102.8	4.9
4	Gallic acid	4.29	169.05	Neg	0.9901	0.4734	25-1000	4.8 / 15.9	102.3	5.1
5	Chlorogenic acid	5.43	353	Neg	0.9932	0.1882	250-10000	7.3 / 24.3	99.7	4.9
6	Protocatechuic acid	5.63	152.95	Neg	0.9991	0.5958	100-4000	25.8 / 85.9	100.2	5.1
7	Tannic acid	6.46	182.95	Neg	0.9955	0.9075	100-4000	10.2 / 34.2	97.8	5.1
8	tr- caffeic acid	7.37	178.95	Neg	0.9942	1.0080	25-1000	4.4 / 14.7	98.6	5.2
9	Vanillin	8.77	151.05	Neg	0.9995	0.4094	250-10000	10.1 / 33.7	99.2	4.9
10	p-Coumaric acid	9.53	162.95	Neg	0.9909	1.1358	100-4000	15.2 / 50.8	98.4	5.1
11	Rosmarinic acid	9.57	358.9	Neg	0.9992	0.5220	250-10000	10.4 / 34.8	101.7	4.9
12	Rutin	10.18	609.1	Neg	0.9971	0.8146	250-10000	17.0 / 56.6	102.2	5.0
13	Hesperidin	9.69	611.1	Poz	0.9973	0.1363	250-10000	21.6 / 71.9	100.2	4.9
14	Hyperoside	10.43	463.1	Neg	0.9549	0.2135	100-4000	12.4 / 41.4	98.5	4.9
15	4-OH Benzoic acid	11.72	136.95	Neg	0.9925	1.4013	25-1000	3.0 / 10.0	106.2	5.2
16	Salicylic acid	11.72	136.95	Neg	0.9904	0.6619	25-1000	4 / 13.3	106.2	5.0
17	Myricetin	11.94	317	Neg	0.9991	2.8247	100-4000	9.9 / 32.9	106.0	5.9
18	Fisetin	12.61	284.95	Neg	0.9988	2.4262	100-4000	10.7 / 35.6	96.9	5.5
19	Coumarin	12.52	146.95	Poz	0.9924	0.4203	100-4000	9.1 / 30.4	104.4	4.9
20	Quercetin	14.48	300.9	Neg	0.9995	4.3149	25-1000	2.0 / 6.8	98.9	7.1
21	Naringenin	14.66	270.95	Neg	0.9956	2.0200	25-1000	2.6 / 8.8	97.0	5.5
22	Hesperetin	15.29	300.95	Neg	0.9961	1.0164	25-1000	3.3/ 11.0	102.4	5.3
23	Luteolin	15.43	284.95	Neg	0.9992	3.9487	25-1000	5.8 / 19.4	105.4	6.9
24	Kaempferol	15.43	284.95	Neg	0.9917	0.5885	25-1000	2.0 / 6.6	99.1	5.2
25	Apigenin	17.31	268.95	Neg	0.9954	0.6782	25-1000	0.1 / 0.3	98.9	5.3
26	Rhamnetin	18.94	314.95	Neg	0.9994	2.5678	25-1000	0.2 / 0.7	100.8	6.1
27	Chrysin	21.18	253	Neg	0.9965	1.5530	25-1000	0.05 / 0.17	102.2	5.3

RT: Retention time
[b]Parent ion (*m/z):* Molecular ions of the standard compounds (mass to charge ratio)
[c]R^2: coefficient of determination
[d]RSD: relative standard deviation
[e]LOD/LOQ (µg/L): Limit of detection/Limit of quantification
[f] U (%): Percent relative uncertainty at 95% confidence level (k=2).
[g] Values in µg g^{-1} (w/w) of plant methanol extract
[h]N.D: not detected.

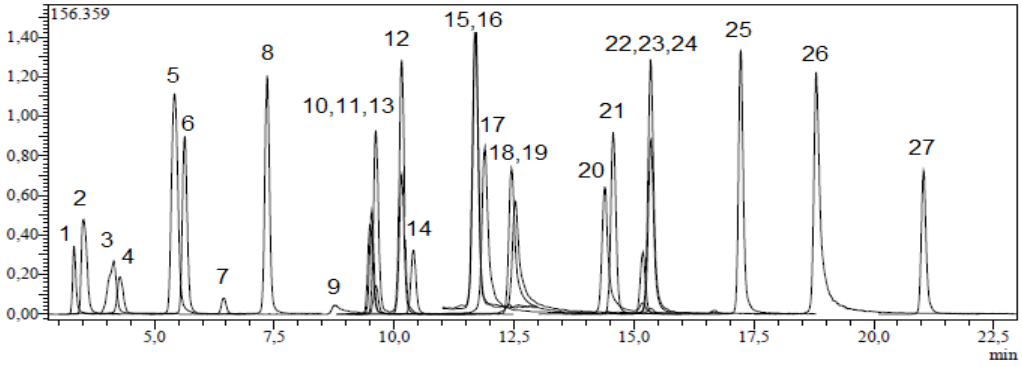

Figure 1. LC-MS/MS chromatograms of 250 ppb standard mix. quinic acid: 1, malic acid: 2, tr-aconitic acid:3, gallic acid:4, chlorogenic acid:5, protocatechuic acid:6, tannic acid:7, tr-caffeicacid:8, vanillin:9, *p*-coumaric acid:10, rosmarinic acid:11, rutin:12, hesperidin:13, hyperoside:14, 4-OH benzoic acid:15, salicylic acid:16, myricetin:17, fisetin:18, coumarin:19, quercetin:20, naringenin:21, hesperetin:22, luteolin:23, kaempferol:24, apigenin:25, rhamnetin:26, chrysin:27.

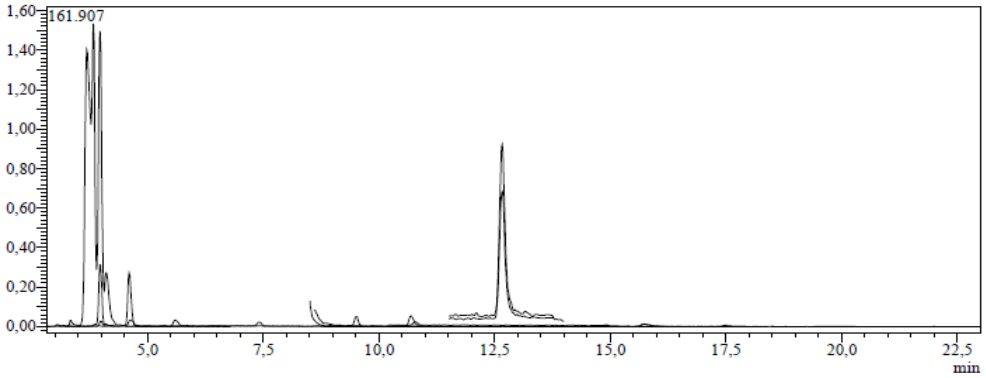

Figure 2. LC-MS/MS chromatogram of leaf in vegetative season.

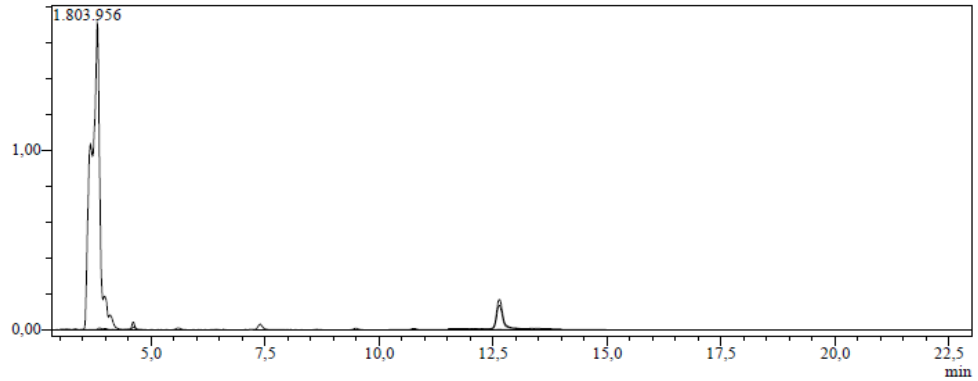

Figure 3. LC-MS/MS chromatogram of root in vegetative season

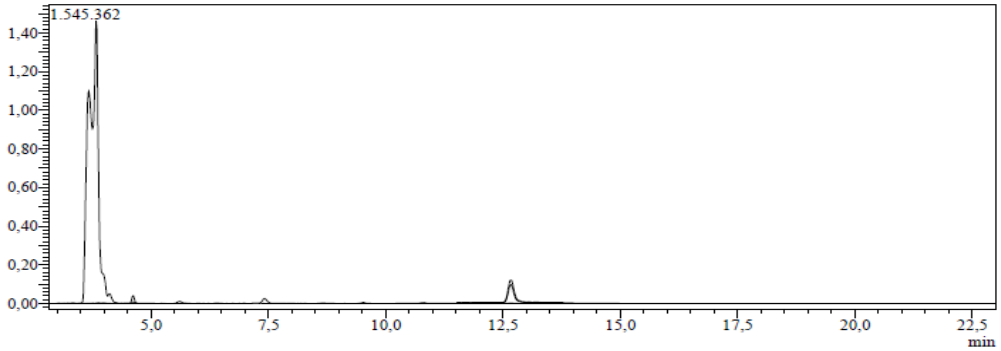

Figure 4. LC-MS/MS chromatogram of root in full flowering season

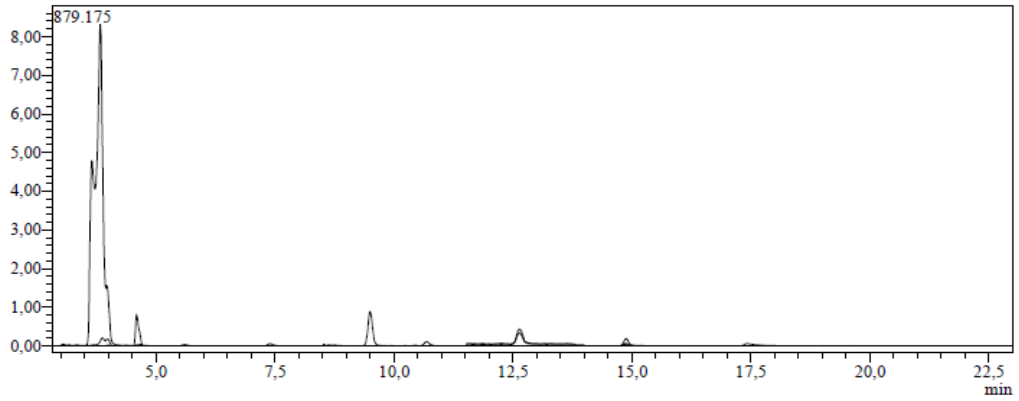

Figure 5. LC-MS/MS chromatogram of flower in full flowering season

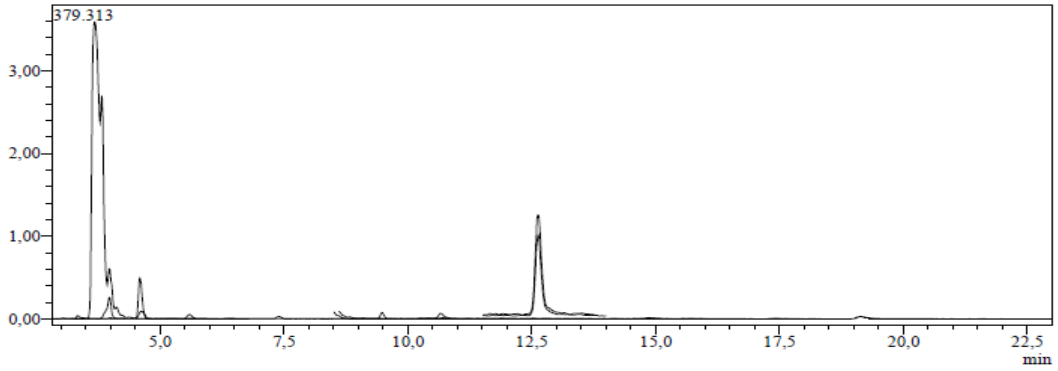

Figure 6. LC-MS/MS chromatogram of leaf in full flowering season

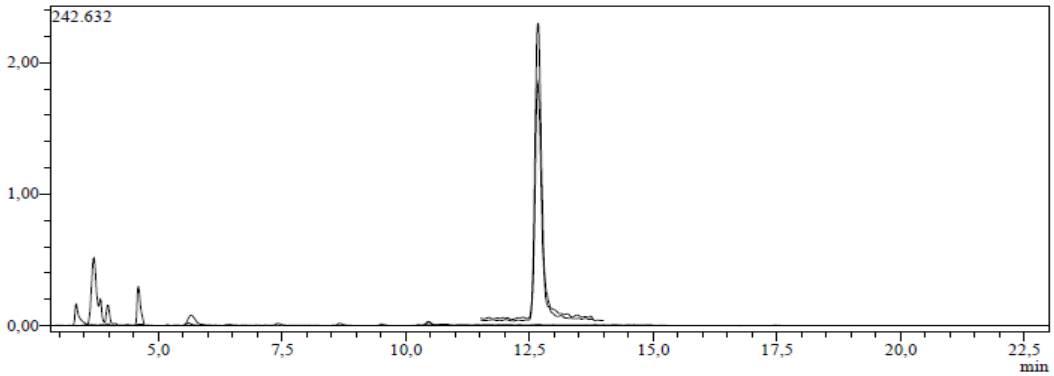

Figure 7. LC-MS/MS chromatogram of fruit

3. Results

3.1. Quantitative analysis of phenolics, flavonoids compounds by UHPLC-ESI-MS/MS

Results of phenolic and flavonoid content in the five samples of *I. demiriziana* have been presented in Table 2. Significant differences in the phenolic and flavonoid constituents of the different extracts of *I. demiriziana* were observed. LC–MS/MS analysis obviously display that the methanol extracts of *I. demiriziana* contain many phenolic and non-phenolic compounds Figure 2-7. Phenolic acids exist in most plants, and each plant can be adequately specific for the availability of various phenolic acids and their derivatives together with the other groups (Ziakova et. al., 2003).

It was determined that the main components of all plant samples were malic acid, quinic acid, tr-aconitic acid, vanillin, p-coumaric acid, 4-OH benzoic acid, salicylic acid, protocatechuic acid, tannic acid and tr-caffeic acid compounds. Out of non-phenolic compounds, *I. demiriziana* extracts include high amounts of quinic acid (221.18-1538.49 µg g^{-1}), tr-Aconitic acid (105.2 -298.62 µg g^{-1}), salicylic acid (35.94-216.27 µg g^{-1}), p-coumaric acid(19.13-1457.45 µg g^{-1}) and 4-OH Benzoic acid (27.99-176.57µg g^{-1}) and lower amounts of gallic acid (0.58-2.05 µg g^{-1}) (Table 2). Malic acid (MA) was single dominant compound among all samples studied. Among them, the vegetative root gave

the highest amount of malic acid with a 30124.37 µg g^{-1} extract (Table 2, Figure 3). This was followed by the flowering root, flower, the flowering leaf, the vegetative leaf and fruit stage with the amounts of 27733.72, 14.438.42, 6879.07, 3745.49 and 691.3 µg g^{-1} extract, respectively. Malic acid contents of the five samples of *I. demiriziana* were so different. This variation may be due to different organ or different growing stages of the samples studied and to gain more insight how growing stages and different organs influence phenolic content of this plant more studies are required. In prompting plant defense responses, present report declares that the metabolic levels of MA compounds play an important role (Huckelhoven, 2007). A corresponding induced defense response beginning intraplant signaling between roots and leaves was implicated in herbivory (Rasmann et al., 2005).

Quinic acid is a metabolite that responsible for metabolic response (inducible defense) to biotic stress (Murthy et al., 2009). It was the second highest phenolic acid determined as 994.56, 550.74, 1087.19, 221.18, 1538.49 and 487.62 µg g^{-1} extract in the vegetative leaf, vegetative root, flowering leaf, flowering root, flower and fruit samples, respectively. Besides, the amount of quinic acid that obtained from leaf extracts (vegetative leaf 994.56 µg g-1 and flowering leaf 1087.19 µg g-1) were greater amounts from root extracts (vegetative root 550.74µg g^{-1} and flowering root 221.18 µg g^{-1}). Similiarly, it is reported that the quinic acid and quercitol are present in high concentrations in wounded leaves of genus *Quercus* plants (Gargallo-Garriga et al., 2010). It is known that the *trans*-aconitic acid has antirheumatic and diuretic properties (Schnitzler, 2007) althought the distribition of this compound is rare (Nierhaus and Kinzel, 1971). The highest amount of *trans*-aconitic acid was obtained from flower stage with 298.62 µg g^{-1} (Figure 5). Salicylic acid (SA) is believed to be a plant signal molecule playing an important role in plant, development, growth and defense responses, and functioning in the commencement of systemic attained resistance (SAR) (Hahlbrock and Scheel, 1989; Ding et al., 2002). The vegetative and flowering root extracts contained significant amount of vanillin (124.09 and 311.94 µg g^{-1}), protocatechuic acid (59.4 - 66.91 µg g^{-1}) , tannic acid (40. 98- 25. 92 µg g^{-1}) and tr-caffeic acid (34. 09- 29. 41µg g^{-1}) respectively (Table 2, Figure 3,4). Vanillin is the main constituent of natural vanilla, a well-known food and cosmetic additive and has antioxidant and antimutagenic properties (Davidson and Naidu, 2000). Their collection is extremely sensitive to environmental situations such as water, light and nutrient availability, and pathogen infection (Harvell and Bosland, 1997). PCA is a natural phenolic acid and exist in several plants including mushrooms and microorganisms (Williams et al., 2012; Nguyen et al., 2013). It is known that the PCA has antiinflammatory and antioxidant (Liu et al., 2002; Syafni et al., 2012) and antibiotic activities (Nguyen et al., 2015). Tannic acid has antioxidant (Andrade et al., 2005), antimutagenic (Ferguson, 2001) and anticarcinogenic properties (Nepka et al., 1999). It is reported that the tannic acid induced by Rhizobia in rice, which is resistant to *Rhizoctonia (*Mishra et al., 2006*).*

Phenolic acid and flavonoids in plants have various functions such as protein synthesis, nutrient uptake, photosynthesis, allelopathy, enzyme activity, and structural components (Hung, 2016). Flavonoids are the largest group of phenolics having antimicrobial and antioxidant impacts (Lorenc et al., 2005). Along with their roles in plants, these compounds in human diet might introduce a number of benefits connected with reduced risk of chronic diseases including anti-inflammatory, anti-atherogenic, antiallergenic, antioxidant, anti-thrombotic, anti-microbial, vasodilatory and cardioprotective influences (Manach et al., 2004). In a study, Nahak et al. (2014) indicated that the phenolic constituent of a plant is usually a good sign of its antioxidant potential. It is found that the flower extracts of *I. demiriziana* have highest levels of flavonoids quercetin (7.98 µg g^{-1}), naringenin (22.96 µg g^{-1}), rhamnetin (72.74 µg g^{-1}), and hyperoside (49.19 µg g^{-1}) and non phenolics tr-aconitic acid (298.62 µg g^{-1}), and p-coumaric acid (1457.45 µg g^{-1}). Recently, Chang et al., (2016) stated that the acid hydrolysis extract of *I. indigotica* contained 61.02 mg/100g of p-coumaric acid, and 23.13 mg/100g of gallic acid. Similar propensity was also viewed for the flavonols, quercetin and hyperoside. The rutin mostly gathered at the fruiting and flowering phases (9.25- 29.67 µg g^{-1} respectively). Because they move as attractive to pollinators and/or to protect the reproductive structures against UV radiations and herbivores, probably, this accumulation pattern of quercetin, hyperoside and rutin may be associated with their biological roles (Gronquist et al., 2001; Kreft et al., 2003). Kaempferol (1.13- 0.13 µg g^{-1}), hesperetin (0-0.21 µg g^{-1}), luteolin (0-0.42 µg g^{-1}) and apigenin(0-0.35 µg g^{-1}) were the most abundant flavonoids in the exracts of *I. demiriziana* (Table 2).

According to our result, the highest amounts of hesperidin (27.61 µg g^{-1}), chlorogenic acid (110.4 µg g^{-1}), rutin (29.67 µg g^{-1}), 4-OH benzoic acid (176.57 µg g-1) and salicylic acid (216. 27 µg g^{-1}) were obtained from the fruit stage (Table 2, Figure 7). Hesperidin (Hsd) and hesperetin (Hst) have several biological activity such as antioxidant, anti-inflammatory and anti-cancer impacts. To struggle with different pathogens, these compounds play an important role in plant defense systems (Soares et al., 2015). Chlorogenic acid is widely employed in industries and medicine including food industries and the consumer chemicals (Kweon et al., 2001). It is a natural antioxidant and anticancer agent and has antiviral and antibacterial properties (Jiang et al., 2001). Karakoca et al., (2013) determined that the chlorogenic acid content was 1980.20 µg g^{-1} on the methanolic root extract of *I. floribunda.* Among twenty-seven references used; rosmarinic acid, myricetin, coumarin, fisetin and chrysin were not detected in *I. demiriziana* extracts employed in this study.

Table 2. Quantitative analysis of phenolic and flavonoid by LC-MS/MS in *I. demiriziana* (µg analyte/g extract)

Compounds	Vegetative-leaf	Vegetative-root	Flowering-leaf	Flowering-root	Flower	Fruit
Hesperidin	1.65	0.71	8.72	0.6	9.73	27.61
Coumarin	0	0	0	0	0	0
Quinic acid	994.56	550.74	1087.19	221.18	1538.49	487.62
Malic acid	3745.49	30124.37	6879.07	27733.72	14438.42	691.3
tr-Aconitic acid	105.15	129.5	187.85	114.53	298.62	105.2
Gallic acid	1.85	1.92	1.72	1.79	2.05	0.58
Chlorogenic acid	0.58	0.3	10.59	0.4	0.24	110.4
Protocatechuic acid	21	59.4	35.12	66.91	21.53	11.08
Tannic acid	4.53	40.98	25.68	25.92	4.53	4.63
tr-caffeic acid	2.35	34.09	2.83	29.41	7.08	1.86
Vanillin	26.31	124.09	24.64	311.94	6.39	133.67
Rosmarinic acid	0	0	0	0	0	0
p-Coumaric acid	77.77	129.35	98.44	85.92	1457.45	19.13
Rutin	0.46	0.69	5.41	0.20	9.25	29.67
Hyperoside	25.54	0.17	30.02	0.51	49.19	3.79
Myricetin	0	0	0	0	0	0
Fisetin	0	0	0	0	0	0
4-OH Benzoic acid	62.68	132.03	96.75	87.03	27.99	176.57
Salicylic acid	77.24	155.74	111.49	102.02	35.94	216.27
Quercetin	2.12	0.15	5.93	0	7.98	0.33
Kaempferol	1.13	0	0.13	0	0	0
Naringenin	1.26	0.04	1.5	0.04	22.96	0.16
Hesperetin	0.06	0.08	0.14	0	0.21	0.04
Luteolin	0.42	0	0.37	0.34	0	0.32
Apigenin	1.04	0.17	0.35	0.02	0	0.09
Rhamnetin	2.13	0	7.44	0	72.74	3.53
Chrysin	0	0	0	0	0	0

4. Conclusions and discussion

The present study can be deduced as the phenolic contents of different organs of *I. demiriziana* compared favorably with other plants. According to the results of this study, further investigations on *I. demiriziana* may be carried out to identify factors that will affect phenolic and flavonoids level in plant tissues, thus they might be grown and harvested under optimum circumstances to increase pleasing qualities chemical levels.

Acknowledgements

The authors would like to thank Dicle University research fund (DUBAP, project no:06-FF-141).

References

Andrade, R.G., Dalvi, L.T., Silva, J.M.C., Lopes, G.K.B., Alonso, A., Hermes-Lima, M. 2005. The antioxidant effect of tannic acid on the in vitro copper-mediated formation of free radicals. Archive of Biochemistry and Biophysics. 437. 1.

Bown, D. 1995. Encyclopaedia of Herbs and their Uses. Dorling Kindersley, London.

Canbek, M., Bayramoglu, G., Senturk, H., Oztopcu, V. A., Uyanoglu, M., Ceyhan, E., Kanbak, G. 2013. The examination of protective effects of gallic acid against damage of oxidative stress during induced-experimental renal ischemia-reperfusion in experiment. Bratislavske Lekarske Listy. 115. 557-562.

Chang A.C., Riskowsky G.L., Chang Y.C. and Chan W.K. 2016. Contents of Important Phenolic Compounds in Indigowoad (*Isatis indigotica* Fort.) and Plains Wild Indigo (*Baptisia bracteata*) Roots. Research Journal of Medicinal Plants. 10/2.167-174.

Chen, Z., Silva, H., Klessig, D. F. 1993. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science-AAAS-Weekly Paper Edition-including Guide to Scientific Information. 262/5141. 1883-1885.

Costa, P., Goncalves, S., Valentao, P., Andrade, P.B., Coelho, N., Romano, A. 2012. Thymus lotacephalus wild plants and in vitro cultures produce different profiles of phenolic compounds with antioxidant activity. Food Chemistry. 135. 1253–1260.

Davidson, P.M., Naidu, A.S., 2000. Phyto-phenols. In: Naidu, A.S. (Ed.), Natural Food Antimicrobial Systems. CRC Press LLC, Boca Raton, London, New York, Washington, DC. 265–294.

Davis, PH. 1988. Flora of Turkey and the East Aegean Islands. *Isatis*. Vol. 10. Edinburgh, UK: Edinburgh Univ. Press.

Ding, C.K., Wang, C.Y., Gross, K.C. 2002. Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to chilling injury in tomato fruit. Planta. 214. 895–901.

Erdogan-Orhan, I., Atasu, E., Sezer-Senol, F., Ozturk, N., Demirci, B., Das, K., Sekeroglu, N. 2014. Comparative studies on Turkish and Indian Centella asiatica (L.) Urban (gotu kola) samples for their enzyme inhibitory and antioxidant effects and phytochemical characterization. Industrial Crops Products. 47. 316–322.

Ferguson, L.R. 2001. Role of plant polyphenols in genomic stability Mutation Research. 75. 89.

Gargallo-Garriga, A., Sardans, J.V., Pérez-Trujillo, M., Parella, T., Seco, R., Filella, I., Peñuelas, J. 2010. Metabolomic responses of Quercus ilex seedlings to wounding simulating herbivory. SMASH Conference Santiago de Compostela Spain.

Gronquist, M., Bezzerides, A., Attygalle, A., Meinwald, J., Eisner, M., Eisner, T. 2001. Attractive and defensive functions of the ultraviolet pigments of a flower (*Hypericum calycinum*). PNAS. 98. 13745–13750.

Hahlbrock, K., Scheel, D. 1989. Physiology and molecular biology of phenylpropanoid metabolism. Annue Review of Plant Physiology, Plant Molecular Biology. 40. 347–469.

Harvell, K.P., Bosland, P.W. 1997. The environment produces a significant effect on pungency of chiles. Hort Science. 32. 1292–1297.

Huckelhoven, R. 2007. Cell wall-associated mechanisms of disease resistance and susceptibility. Annue Review Phytopathology. 45. 101–127

Hung, P. V. 2016. Phenolic Compounds of Cereals and Their Antioxidant Capacity. Critical Reviews in Food Science and Nutrition. 56/1. 25-35.

Jacobo Velazquez, D.A., L. Cisneros Zevallos. 2009. Correlations of antioxidant activity against phenolic content revisited: A new approach in data analysis for food and medicinal plants. Journal of Food Science. 74. R107-R113.

Jiang, Y., Satoh, K., Watanabe, S. 2001. Inhibition of chlorogenic acid-induced cytotoxicity by CoC12. Anticancer Research. 21. 3349-53.

Kang, H., Hahn, M., Fortin, D. R., Hyun, Y. J., & Eom, Y. 2006. Effects of Perceived Behavioral Control on the Consumer Usage Intention of E-coupons. Psychology & Marketing, 23. 841-864.

Karakoca, K., Ozusaglam, M. A., Cakmak, Y. S., Erkul, S. K. 2013b. Antioxidative, antimicrobial and cytotoxic properties of *Isatis floribunda* Boiss. ex Bornm. extracts. EXCLI journal. 12. 150.

Kirtikau, K.R., Basu, L. 1983.Indian medicinal plants, 2nd ed. Dehra Dun, India: Bishen Singh Mahendra pal singh, 1-V, Malik RS, Anand IJ, Srinvasachar S. Effect of glucosinolates in relation to aphid (lipaphiserysimi Kalt.) fecundity in crucifers. Indian Journal of Tropical Agriculture.1. 273-8.

Knekt, P., Jarvinen, R., Reunanen, A., Maatela, J. 1996. Flavonoid intake and coronary mortality in Finland: A cohort study. British Medicinal Journal. 312. 478-481.

Kreft, I., Fabjani, N., Germ, M. 2003. Rutin in buckwheat – protection of plants and its importance for the production of functional food. Fagopyrum 20. 7–11.

Kumar, S., Pandey, A. K. 2013. Chemistry and biological activities of flavonoids: an overview. The Scientific World Journal.

Kvasnicka, F., Copikova , J., Sevcık, R., Kratka , J., Syntytsia, A., Voldrich, M. 2008. Determination of phenolic acids by capillary zone electrophoresis and HPLC. Central European Journal of Chemistry. 6. 410–418.

Kweon, M.H., Hwang, H.J., Sung, H.C. 2001. Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllo stachys edulis*). Journal of Agricultural and Food Chemistry. 49. 4646-55.

Liu, C.L., Wang, J.M., Chu, C.Y., Cheng, M.T. 2002. In vivo protective effect of protocatechuic acid on tert-butyl hydroperoxide-induced rat hepatotoxicity. Food and Chemical Toxicology. 40. 635–641.

Lorenc-Kukula, K., Jafra, S., Oszmıánskı, I., Szopa, J. 2005. Ectopic Expression of Anthocyanin 5-O-Glucosyltransferase in Potato Tubers Causes Increased Resistance to Bacteria. Journal of Agricultural and Food Chemistry. 5. 272-281.

Mabberley, D.I. 1987. The plant book. Cambridge: Cambridge Univ. Press.

Maillard, M.N., Soum, M.H., Boivin, P., Berset, C., 1996. Antioxidant Activity of Barley and Malt: Relationship with Phenolic Content. LWT - Food Science and Technology. 29. 238-244.

Manach, C., Scalbert, A., Morand, C., Remesy, C., Jimenez, L. 2004. Polyphenols: Food sources and bioavailability; The American Journal of Clinical Nutrition. 79. 727–747.

Mishra, R.P.N., Singh, R.K., Jaiswal, H.K., Kumar, V., Maurya, S. 2006. *Rhizobium*-Mediated Induction of Phenolics and Plant Growth Promotion in Rice (*Oryza sativa* L.). Current Microbiology. 52. 383-9.

Mısırdalı, H. 1985. Taxonomic and cytological investigations on the species of *Isatis* L., grown in the Eastern and South Eastern Anatolia and over the regions of Eastern Mediterranean. TUBITAK Project No:TBAG-535, Eskişehir, Turkey.

Murthy, P.S. 2009. Physico-chemical, antioxidant and antimicrobial properties of Indian monsooned coffee. Europen Food Research and Technology. 229- 645.

Nahak, G., Share, M., Sahu, R.K. 2014. Antioxidant potential and nutritional values of vegetables: A review. Research Journal of Medicinal Plant. 8. 50-81.

Nepka, C., Sivridis, E., Antonoglou, O., Kortsaris, A., Georgellis, A., Taitzoglou, I., Hytiroglou, P., Papadimitrou, C., Zintzaras, I., Kouretas, D. 1999. Chemopreventive activity of very low dose dietary tannic acid administration in hepatoma bearing C3H male mice. Cancer Letters. 141. 57.

Nguyen, D.M.C., Seo, D.J., Kim, K., Yv Park, R.D. 2013. Nematicidal activity of 34-dihydroxybenzoic acid purified from Terminalia nigrovenulosa bark against Meloidogyne incognita. Microbial Pathogenesis. 59. 52–59.

Nguyen, X.H., Naing, K.W., Lee, Y.S., Moon, J.H., Lee, J.H., Kim, K.Y. 2015. Isolation and characteristics of protocatechuic acid from Paenibacillus elgii HOA73 against Botrytis cinerea on strawberry fruits. Journal of Basic Microbiology. 55. 625-634.

Nierhaus, D., Kinzel, H. 1971. Comparative investigations on organic acids in leaves of higher plants. Z Pflanzenphysiol. 64. 107-123.

Polat, R., Cakilcioglu, U., Ertug, F.,Satil, F. 2012. An evaluation of ethnobotanical studies in Eastern Anatolia. Biological Diversity and Conservation. *5*/2. 23-40.

Prakash, D., Suri. S., Upadhyay, G., Singh, B.N. 2007. Total phenols, antioxidant and free radical scavenging activities of some medicinal plants. International Journal of Food Science and Nutrition. 58. 18-28.

Radwan, H.M., Shams, K.A., Tawfik, W.A., Soliman, A.M. 2008. Investigation of the glucosinolates and lipids constituents of *Cakile maritime* (Scope) growing in Egypt and their biological activity. Research Journal of Medical Science. 3. 182-187.

Rasmann, S., Kollner, T.G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., Gershenzon, J., Turlings, T.C.J. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature. 434. 732–737

Russell, I. J., Michalek, J. E., Flechas, J. D., Abraham, G. E. 1995. Treatment of fibromyalgia syndrome with Super Malic: a randomized, double blind, placebo controlled, crossover pilot study. The Journal of Rheumatology. *22*/5. 953-958.

Schnitzler, M., Petereit, F., Nahrstedt, A. 2007. Trans-Aconitic acid glucosylflavones and hydroxy cinnamoyl tartaric acids from the leaves of Echinodorus grandiflorus ssp. aureus a Brazilian medicinal plant. Revista Brasileira de Farmacognosia. 17. 149-154.

Soares, M.S., da Silva, D.F., Forim, M.R., Fernandes, J.B., Vieira, P.C., Silva, D.B., Machado, M.A. 2015. Quantification and localization of hesperidin and rutin in Citrus sinensis grafted on C. limonia after Xylella fastidiosa infection by HPLC-UV and MALDI imaging mass spectrometry. Phytochemistry. 115. 161-170.

Syafni, N., Putra, D.P., Arbain, D. 2012. 34-Dihydroxybenzoic acid and 34-dihydroxy benzaldehyde from the fern Trichomanes chinense; isolation antimicrobial and antioxidant properties. Indonesian Journal of Chemistry. 12. 273–278.

Tarnawski, M., Depta, K., Grejciun, D., Szelepin, B. 2006. HPLC determination of phenolic acids and antioxidant activity in concentrated peat extract–a natural immunomodulator. Journal of Pharmaceutical and Biomedical Analysis. 41. 182–188.

Vang, O. 1994. Anticarcinogenic substances in cruciferous vegetables - mechanisms and models. In: Deutsche Gesellschaft für Qualitätsforschung (Pflanzliche Nahrungsmittel) e.V.: Neue spekte der gesundheitlichen Wirkung Pflanzlicher Nahrungsmittel (pp 74-85). Quedlinburg: DGQ, 1995 (DGQ-Veröffentlichungen, Bd. 29).

Vauzour, D., Corona, G., Spencer, J.P.E. 2010. Caffeic acid, tyrosol and p-coumaric acid are potent inhibitors of 5-S-cysteinyl-dopamine induced neurotoxicity. Archives of Biochemistry and Biophysics. 501. 106-111.

Williams, K.M., Martin, W.E., Smith, J., Williams, B.S. 2012. Production of protocatechuic acid in Bacillus thuringiensis ATCC33679. International Journal of Molecular Science.13. 3765–3772.

Ziakova , A., Brands teterova, E. 2003. Validation of HPLC determination of phenolic acids present in some Lamiaceae family plants. Journal of Liquid Chromatography & Related Technologies. 26. 443–453.

(Received for publication 21 October 2016; The date of publication 15 April 2017)