



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 referred 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 constituents 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 H₂O₂ 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 room temperature and then the samples were pulverized with a laboratory mill and stored at 4°C 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 ^{2c}	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

^bParent ion (m/z): Molecular ions of the standard compounds (mass to charge ratio)

^cR²: coefficient of determination

^dRSD: relative standard deviation

^eLOD/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

^hN.D: not detected.

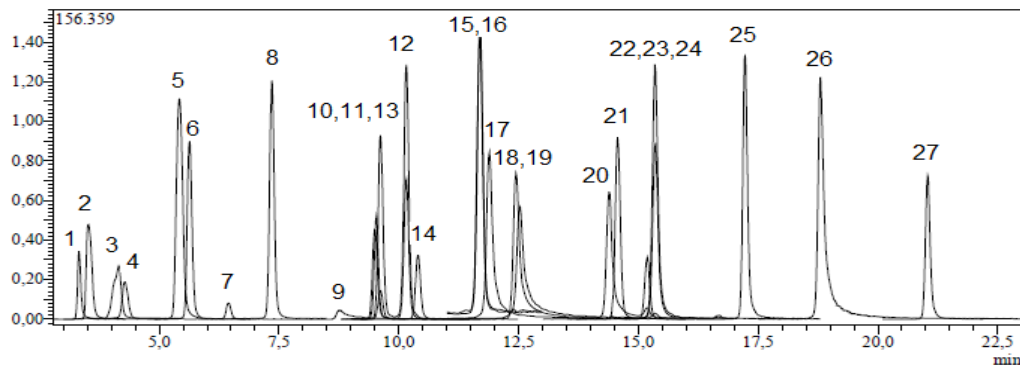


Figure 1. LC-MS/MS chromatograms of 250 ppb standard mix. quinic acid: 1, malic acid: 2, tr-aconitic acid:3, gallic acid:4, chlorogenic acid:5, protocatechuic acid:6, tannic acid:7, tr-caffeic acid:8, vanillin:9, *p*-coumaric acid:10, rosmarinic acid:11, rutin:12, hesperidin:13, hyperoside:14, 4-OH benzoic 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.

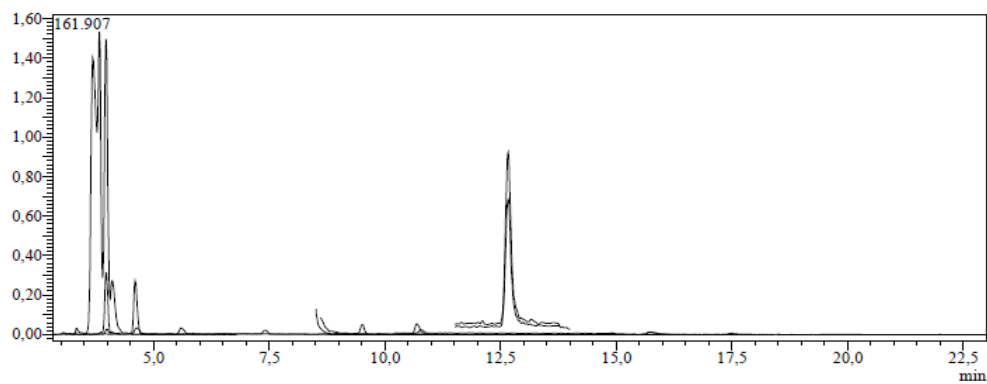


Figure 2. LC-MS/MS chromatogram of leaf in vegetative season.

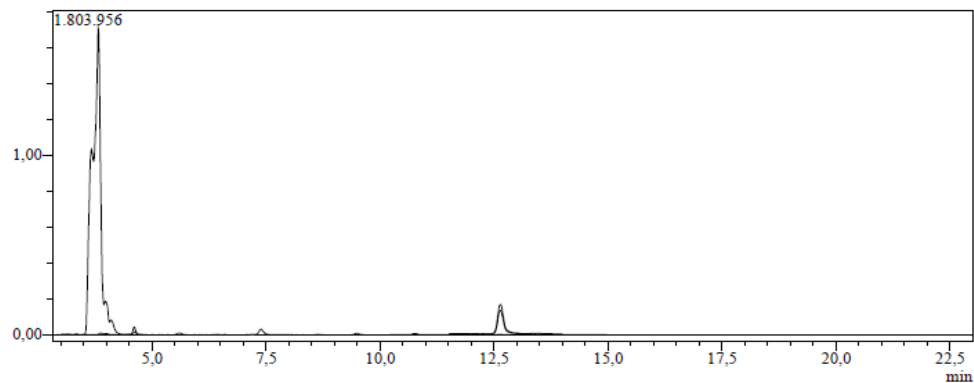


Figure 3. LC-MS/MS chromatogram of root in vegetative season

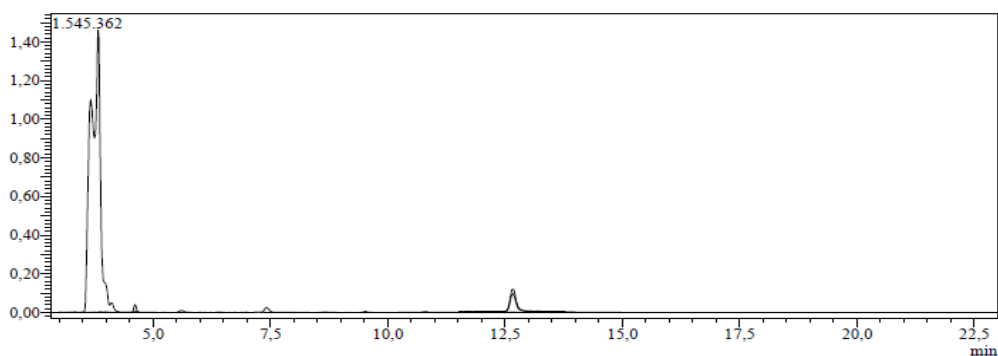


Figure 4. LC-MS/MS chromatogram of root in full flowering season

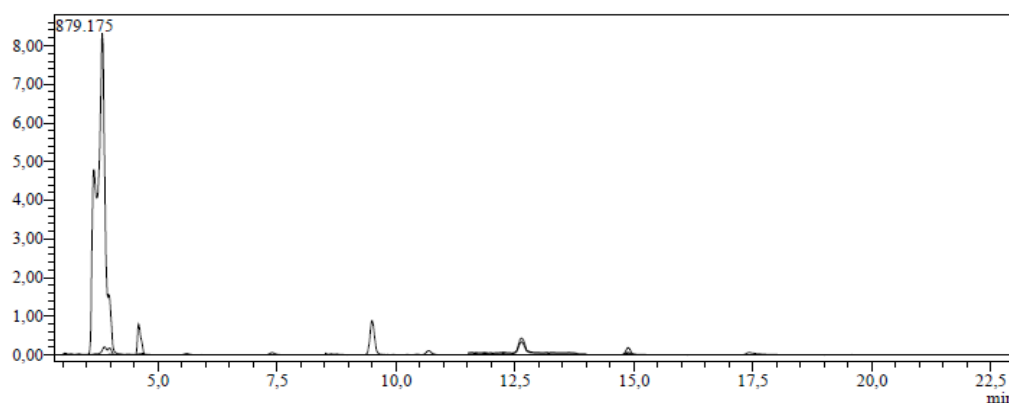


Figure 5. LC-MS/MS chromatogram of flower in full flowering season

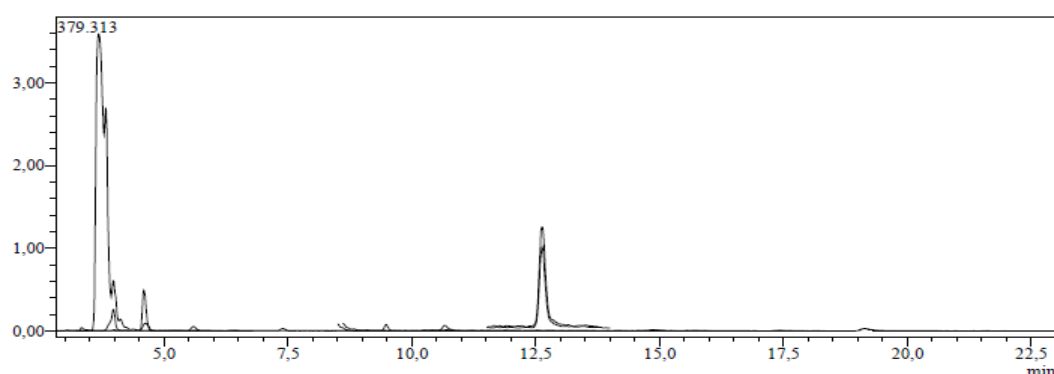


Figure 6. LC-MS/MS chromatogram of leaf in full flowering season

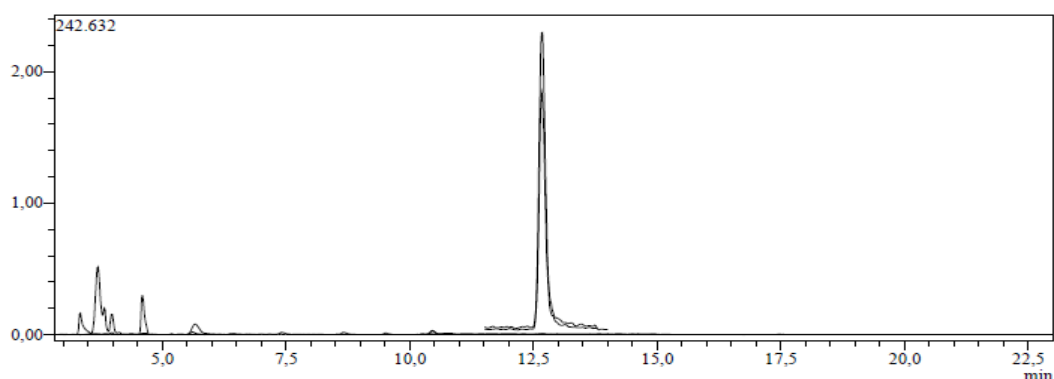


Figure 7. LC-MS/MS chromatogram of fruit

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 $\mu\text{g g}^{-1}$), tr-Aconitic acid (105.2 -298.62 $\mu\text{g g}^{-1}$), salicylic acid (35.94-216.27 $\mu\text{g g}^{-1}$), p-coumaric acid (19.13-1457.45 $\mu\text{g g}^{-1}$) and 4-OH Benzoic acid (27.99-176.57 $\mu\text{g g}^{-1}$) and lower amounts of gallic acid (0.58-2.05 $\mu\text{g g}^{-1}$) (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 $\mu\text{g g}^{-1}$ 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 $\mu\text{g g}^{-1}$ 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 $\mu\text{g g}^{-1}$ 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 $\mu\text{g g}^{-1}$ and flowering leaf 1087.19 $\mu\text{g g}^{-1}$) were greater amounts from root extracts (vegetative root 550.74 $\mu\text{g g}^{-1}$ and flowering root 221.18 $\mu\text{g g}^{-1}$). Similarly, 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) although the distribution of this compound is rare (Nierhaus and Kinzel, 1971). The highest amount of *trans*-aconitic acid was obtained from flower stage with 298.62 $\mu\text{g g}^{-1}$ (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 $\mu\text{g g}^{-1}$), protocatechuic acid (59.4 - 66.91 $\mu\text{g g}^{-1}$), tannic acid (40.98- 25.92 $\mu\text{g g}^{-1}$) and tr-caffeic acid (34.09- 29.41 $\mu\text{g g}^{-1}$) 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 $\mu\text{g g}^{-1}$), naringenin (22.96 $\mu\text{g g}^{-1}$), rhamnetin (72.74 $\mu\text{g g}^{-1}$), and hyperoside (49.19 $\mu\text{g g}^{-1}$) and non phenolics tr-aconitic acid (298.62 $\mu\text{g g}^{-1}$), and p-coumaric acid (1457.45 $\mu\text{g g}^{-1}$). 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 $\mu\text{g g}^{-1}$ 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 $\mu\text{g g}^{-1}$), hesperetin (0-0.21 $\mu\text{g g}^{-1}$), luteolin (0-0.42 $\mu\text{g g}^{-1}$) and apigenin (0-0.35 $\mu\text{g g}^{-1}$) were the most abundant flavonoids in the extracts of *I. demiriziana* (Table 2).

According to our result, the highest amounts of hesperidin (27.61 $\mu\text{g g}^{-1}$), chlorogenic acid (110.4 $\mu\text{g g}^{-1}$), rutin (29.67 $\mu\text{g g}^{-1}$), 4-OH benzoic acid (176.57 $\mu\text{g g}^{-1}$) and salicylic acid (216.27 $\mu\text{g g}^{-1}$) 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 $\mu\text{g g}^{-1}$ 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.

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