Chemical Profiling and Discrimination of Medicinal Himalayan *Iris* Species Using Direct Analysis in Real Time Mass Spectrometry Combined with Principal Component Analysis

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Graphical Abstract

Metabolic profiling of *I. crocea*, *I. ensata*, *I. germanica*, *I. hookeriana*, *I. kashmiriana* and *I. spuria*, was carried out using direct analysis in real-time mass spectrometry (DART-MS) to generate the chemical fingerprints for the differentiation. Phytochemical analysis showed presence of twenty-two flavonoids and isoflavonoids in the intact leaf and root of these species. The DART-MS data have been subjected to principal component analysis (PCA) which showed clear differentiation among the species and plant parts. It clearly indicated that the DART-MS technique followed by PCA is a quick and reliable method for the direct profiling and discrimination of *Iris* species and their plant parts.

Keywords: DART-MS, flavonoids, isoflavonoids, *Iris* species, Principal Component Analysis (PCA).

INTRODUCTION

Traditional health care system involved the medicinal use of plants throughout the world. In India the practice of ethno medicine by rural and tribal communities evolved through the system of Ayurveda, Siddha and Unani [1]. The Indian Himalayan region is a biodiversity hotspot hosting a remarkably rich variety of medicinal plants [2]. The genus *Iris* belongs to the family Iridaceae which comprises over 300 species, of which twelve are reported in India, mostly in the Kashmir, Himalaya from the valley to high alpine [3,4]. The most common *Iris* species found in India include *I. crocea*, *I. ensata*, *I. germanica*, *I. hookeriana*, *I. kashmiriana* and *I. spuria*.

Iris plants are widely used in traditional medicine to treat liver bacterial, dysfunction, inflammation, and viral infections [5,6]. They are also used as antispasmodic, emetic, haemostasis and laxative agents [6]. The rhizomes of *I. germanica* and *I. spuria* have been used as aperient, blood purifier, diuretic, and stimulant to treat gall bladder, venereal diseases and cancer [7]. Because of the violet-like scent of their flowers *Iris* species also find use in the perfume and cosmetic industries [8]. They are considered rich sources of secondary metabolites. The phytochemicals isolated from *Iris* species possess antibacterial [9], anticancer, anticholinesterase, antihelmintic, antiinflammatory, antimicrobial, antioxidant, antiparasitic, antituberculosis, antiulcer, cytotoxic, free radical scavenging [10,11], hepatoprotective, hypolipidemic, immunomodulatory, molluscicidal and pesticidal activities [5,6,9,12,13].

A variety of secondary metabolites including flavonoids, isoflavonoids, iridal type triterpenoids, irones, phenolics, quinines, stilbenes glycosides and xanthenes have been isolated from *Iris* plants [9,13-15]. The *Iris* rhizomes showed characteristic isoflavonoids and iridals (mono and bicyclic triterpenoids), whereas the leaves contained C-glycosylflavones, flavonoid aglycones, isoflavones, phenolics and xanthone glycosides [16]. About 50 different isoflavonoids in the form of diglucosides, triglucosides or aglycones are reported from the *Iris* plants [17]. The preventive role of isoflavones is well-known in diseases like cancer, cardiovascular, osteoporosis, and menopausal symptoms [18,19]. Since the genus *Iris* is rich in bioactive flavonoids, isoflavonoids, phenolics and several *Iris* species having different contents of these components are used in traditional medicine, their metabolite analysis is essential for the quality control of herbal drugs [20-22]. Earlier reports showed the metabolite profiling of *Iris* species using gas chromatography-mass spectrometry (GC-MS) [23] for volatile components, high-performance liquid chromatography (HPLC) [24,25] and high-performance thin layer chromatography (HPTLC) [25]. Electrospray ionization mass spectrometry (ESI-MS) combined with high performance liquid chromatography and diode array detector (HPLC-DAD) was used to identify the flavonoids and other constituents in the rhizomes of three *Iris* species namely *I. crocea*, *I. germanica* and *I. spuria* [6,13,25]. The flavonoids and isoflavonoids of *Iris* species were also identified by ¹H-NMR [5,15,26], ¹³C-NMR [27], IR [28], UV [16,29]. But all these techniques require elaborate sample preparation and are time and labor consuming. Direct analysis in real-time mass spectrometry (DART-MS)-based metabolic profiling is quicker profiling strategy as there is no need of sample preparation [30-34]. It is therefore an appropriate technique for the metabolic profiling of plant species [35,36]. Besides DART, there are others ambient ionization technique like Desorption electrospray ionization (DESI), Desorption atmospheric pressure chemical ionization (DAPCI), Atmospheric solids analysis probe (ASP) employed to access metabolic profiles of plants [37]. Metabolic profiling followed by statistical analysis is a preferred method for the differentiation of plants [37-40]. Hence, it was decided to profile the chemical constituents in the roots and leaves of six *Iris* species namely *I. crocea*, *I. ensata*, *I. germanica*, *I. hookeriana*, *I. kashmiriana* and *I. spuria* collected from Kashmir Himalaya using DART-MS and utilize the chemical fingerprint data to run principal component analysis (PCA) for discrimination.

MATERIALS AND METHODS

Plant Materials

The leaf and root of *I. crocea*, *I. ensata*, *I. germanica*, *I. hookeriana*, *I. kashmiriana* and *I. spuria* were collected in June 2015 from Kashmir Himalaya. Voucher specimens of all collected *Iris* species were deposited in Plant Herbarium of CSIR-IIIM, Jammu India. Details are available in supplementary Table SI.

Samples for DART MS analysis

Intact plant parts (leaf and root) were thoroughly washed with tap water followed by distilled water in order to remove foreign particles from the surface and dried at room temperature.

DART MS Analysis

The mass spectrometer used was a JMS-T100LC, Accu Tof atmospheric pressure ionization time-of-flight mass spectrometer (Jeol, Tokyo, Japan) fitted with a DART ion source. The mass spectrometer was operated in positive-ion mode with a resolving power of 6000 (full-width at half-maxima). The orifice 1 potential was set to 28 V, resulting in minimal fragmentation. The ring lens and orifice 2 potentials were set to 13 and 5 V, respectively. Orifice 1 was set at 100 °C and RF ion guide potential at 300 V. The DART ion source was operated with helium gas flowing at approximately 4.0 L min⁻¹ and gas heater was set at 300 °C. The potential on the discharge needle electrode of the DART source was set to 3000 V, electrode 1 at 100 V and the grid at 250 V. Data acquisition was from *m/z* 10 to 1050. All the leaf and root samples were analyzed in 15 repeats to check the reproducibility of spectra. Mass calibration was accomplished by including a mass spectrum of neat polyethylene glycol (PEG) (1:1 mixture PEG 200 and PEG 600) in the data file. The mass calibration was accurate to within ±0.002 u. Using the Mass Centre software, the elemental composition was determined on selected peaks.

Statistical analysis

Principal component analyses (PCAs) was performed with the STATISTICA software, Windows version 7.0 (Stat Soft, Inc., USA). Data for PCA analysis was extracted from DART-MS spectra of fifteen repeats of each sample. All ions having ≥5% peak intensity were selected for principal component analysis.

RESULTS AND DISCUSSION

Comparison of DART-MS fingerprints of *Iris* species

Comparative DART-MS fingerprints of the leaf and root of *I. crocea*, *I. ensata*, *I. germanica*, *I. hookeriana*, *I. kashmiriana* and *I. spuria* are shown in Figures 1 and 2 respectively. The structures of detected compounds are given in Figure 3.

Figure 1. Comparative DART-MS fingerprint spectra of the leaf of six *Iris* species.

Figure 2. Comparative DART-MS fingerprint spectra of the root of six *Iris* species.

Figure 3. Structures of identified compounds in *Iris* species.

Table I. Exact mass data for the identified constituents and their distribution in the roots and leaves of six *Iris* species^a

S. N°	Class of Compounds	Compounds	Cal. mass [M+H] ⁺	Meas. mass [M+H] ⁺	M. formula	Error (ppm)	Root						Leaf						Biological activity	Ref.
							lc	le	lg	lh	lk	ls	lc	le	lg	lh	lk	ls		
1	Alkyl-phenylketones	Acetovanillone	167.0708	167.0701	C ₉ H ₁₀ O ₃	-4.2	-	-	+	-	-	-	-	+	+	+	+	-	Free radical scavenging activity	[14]
2	Iron oxide	β-Irone	207.1991	207.1960	C ₁₄ H ₂₂ O	-15.0	-	-	-	-	-	-	-	+	+	-	+	+		[10]
3	Isoflavonoids	Alpinone	287.2724	287.2739	C ₁₆ H ₁₄ O ₅	5.2	-	+	+	-	-	-	-	+	-	-	-	-		[14]
4	Isoflavonoids	Irilone	299.0602	299.0556	C ₁₆ H ₁₀ O ₆	-15.4	-	+	+	+	+	+	+	+	-	+	+	-	Cancer chemo preventive potential, free radical scavenging activity	[6]
5	Isoflavonoids	Tectorigenin	301.9810	301.9773	C ₁₆ H ₁₂ O ₆	-12.3	+	+	+	+	+	-	-	+	-	-	-	-	Antibacterial, antiinflammatory, antioxidative and antitumor	[6]
6	Isoflavonoids	5,2',3'-Trihydroxy-7-methoxyflavone	303.1251	303.1233	C ₁₆ H ₁₄ O ₆	-5.9	-	-	-	-	-	-	-	+	-	-	+	+		[20]
7	Isoflavonoids	5-Methoxy-4'-hydroxy-6,7-methylenedioxyisoflavone	313.0579	313.0581	C ₁₇ H ₁₂ O ₆	0.6	+	+	+	-	+	+	+	+	-	-	-	+	Free radical scavenging activity	[6]
8	Isoflavonoids	5,7-Dihydroxy-4'6-dimethoxyisoflavone	315.0732	315.0716	C ₁₇ H ₁₄ O ₆	-5.1	+	+	+	+	+	+	-	+	-	-	-	-	Cancer chemo preventive potential	[14]
9	Isoflavonoids	Irisoid A	329.2042	329.2038	C ₁₇ H ₁₂ O ₇	-1.2	+	+	+	+	+	-	-	-	-	-	-	-	Free radical scavenging activity	[14]
10	Isoflavonoids	Iristectorigenin A	331.0793	331.0818	C ₁₇ H ₁₄ O ₇	7.6	+	+	+	+	-	-	+	+	-	+	+	-		[14]

Table I. Exact mass data for the identified constituents and their distribution in the roots and leaves of six *Iris* species (Cont.)^a

S. N°	Class of Compounds	Compounds	Cal. mass [M+H] ⁺	Meas. mass [M+H] ⁺	M. formula	Error (ppm)	Root						Leaf						Biological activity	Ref.
							lc	le	lg	lh	lk	ls	lc	le	lg	lh	lk	ls		
11	Isoflavonoids	Iriskashmirianin	343.2637	343.2683	C ₁₈ H ₁₄ O ₇	13.4	+	+	+	+	+	+	+	+	-	+	+	-	Cancer chemo preventive potential	[10]
12	Isoflavonoids	Irisflavone C	361.0923	361.0917	C ₁₈ H ₁₆ O ₈	-1.7	-	-	+	-	+	+	-	+	-	-	-	-		[28], [43]
13	Xanthonoid	Mangiferin	423.3591	423.3627	C ₁₉ H ₁₈ O ₁₁	8.5	-	-	-	-	-	-	-	-	-	+	+	-		[43]
14	Xanthonoid	7-O-methylmangiferin	437.3632	437.3631	C ₂₀ H ₂₀ O ₁₁	-0.2	-	-	-	-	+	-	-	+	-	-	-	-		[14]
15	Flavonoids	Swertisin	447.3287	447.3267	C ₂₂ H ₂₂ O ₁₀	-4.5	-	-	-	-	-	-	-	+	+	+	-	-		[8] [14]
16	Isoflavonoid o-glycosides	Irilone 4'-O-glucoside	461.1401	461.1448	C ₂₂ H ₂₀ O ₁₁	10.2	+	+	+	+	+	+	+	-	-	-	+	+		[6]
17	Flavonoids	Tectoridin	463.3205	463.3212	C ₂₂ H ₂₂ O ₁₁	1.5	+	+	+	-	+	+	+	+	+	+	+	+	Antibacterial, antiinflammatory, antioxidative and antitumor	[6] [14]
18	Flavonoids	Germanaism B	475.1392	475.1380	C ₂₃ H ₂₂ O ₁₁	-2.5	-	+	+	+	-	+	+	+	-	-	-	-		[14]
19	Flavonoids	Iriflogenin 4'-O-glucoside	491.4783	491.4828	C ₂₃ H ₂₂ O ₁₂	9.2	+	+	-	-	-	-	+	-	-	-	-	-	Cancer chemo preventive potential	[6]
20	Flavonoids	Iristectorigenin B 7-O-glucoside	493.4984	493.4972	C ₂₃ H ₂₄ O ₁₂	-2.4	-	+	+	-	+	+	+	+	-	-	-	+		[6]
21	Flavonoids	Iridin	523.4028	523.4018	C ₂₄ H ₂₆ O ₁₃	-1.9	-	-	-	-	-	-	-	-	+	-	-	-		[6]
22	Flavonoids	Germanaism D	611.3387	611.3373	C ₂₇ H ₃₀ O ₁₆	-2.3	-	-	-	-	-	-	+	+	-	-	-	-		[11]

^a(+): detected, (-) not detected, lc: *Iris crocea*, le: *Iris ensata*, lg: *Iris germanica*, lh: *Iris hookeriana*, lk: *Iris kashmiriana* and ls: *Iris spuria*.

The variation and distribution of bioactive compounds in the leaves and roots of six *Iris* species could be observed from their fingerprints. Twenty-two constituents were tentatively identified based on their exact mass and molecular formula (Table I). These constituents were directly ionized from the leaves and roots during analysis and appeared as protonated molecular ions $[M+H]^+$ in the resulting spectra. Phytochemical analysis of *Iris* species showed mainly the presence of flavonoids and isoflavonoids.

The major isoflavonoids obtained in the DART-MS of *Iris* species at m/z 299.0556 ($C_{16}H_{10}O_6$), 301.9773 ($C_{16}H_{12}O_6$), 313.0581 ($C_{17}H_{12}O_6$), 315.0716 ($C_{17}H_{14}O_6$), 329.2038 ($C_{17}H_{12}O_7$) and 343.2683 ($C_{18}H_{14}O_7$), could be due to irilone (4) [6], tectorigenin (5) [6], 5-methoxy-4'-hydroxy-6,7-methylenedioxyisoflavone (7) [6], 5,7-dihydroxy-4'6-dimethoxyisoflavone (8) [14], irisoid A (9) [14] and iriskashmirianin (11) [10] respectively. Compounds irilone (4), tectorigenin (5), 5-methoxy-4'-hydroxy-6,7-methylenedioxyisoflavone (7) and irisoid A (9) are reported for their anticancer activity were detected in the roots of all the species except (4) in *I. crocea*, (5) and (9) in *I. spuria*, and (7) in *I. hookeriana*. Compound irilone 4'-O-glucoside (16) [6] was detected in the roots of all the species. While compound tectoridin (17) [6,14] m/z 463.3212 ($C_{22}H_{22}O_{11}$) was present in all the roots and leaves except *I. hookeriana*. Compound iriflogenin 4'-O-glucoside (19) [6] m/z 491.4828 ($C_{23}H_{22}O_{12}$) was detected only in the roots and leaves of *I. crocea* and *I. ensata*. Compound mangiferin (13) [43] m/z 423.3627 ($C_{19}H_{18}O_{11}$) was found only in the leaf of *I. hookeriana* and *I. kashmiriana*, while swertisin (15) [8,14] m/z 447.3267 ($C_{22}H_{22}O_{10}$) was detected in the leaves of *I. ensata*, *I. germanica* and *I. hookeriana*. The compounds iridin (21) [6] m/z 523.4018 ($C_{24}H_{26}O_{13}$) and germanaism D (22) [11] m/z 611.3373 ($C_{27}H_{30}O_{16}$) were detected in the leaves of *I. germanica*, *I. crocea* and *I. ensata* as shown in Table I.

The relative content of nine bioactive compounds was tentatively converted in terms of percent ionization, which was obtained as the ratio of the expression of the peak to the sum of all the expressions within the spectra ranging from m/z 100–800. All the ions with the relative intensity above 5% were taken and compared based on the percent ionization. Fifteen repeats of each sample were carried out and the averaged value was utilized for the comparison as shown in Figure 4 and in supplementary Figure S1. The relative content is given in Table II.

Figure 4. Comparison of nine bioactive compounds in roots of six *Iris* species.

Table II. Relative percent ionization of nine bioactive compounds in *Iris* species

Peaks	<i>I. crocea</i>		<i>I. ensata</i>		<i>I. germanica</i>		<i>I. hookeriana</i>		<i>I. kashmiriana</i>		<i>I. spuria</i>	
	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
287 (3)	nd	nd	43.7	nd	24.7	nd	nd	nd	nd	nd	nd	nd
299 (4)	nd	29.4	63.1	39.6	17.1	nd	16.3	43.1	23.7	51.4	23.3	nd
301 (5)	79.9	nd	42	27.9	nd	nd	28.1	nd	20.2	nd	nd	nd
313 (7)	98.6	54.7	36	40	96.1	nd	89.4	nd	78.9	nd	44.6	76
315 (8)	68.5	nd	31.5	25.7	28.1	nd	24.7	nd	39	nd	95.2	nd
329 (9)	27.5	nd	25	nd	22.5	nd	29.8	nd	29.2	nd	nd	nd
343 (11)	32.7	30.6	30.6	14	32.3	nd	42.6	51.1	24.2	23.3	39.4	nd
463 (17)	31.1	25.7	95	23.6	39.5	43.5	nd	53.3	32.3	42.1	49.3	58.7
491 (19)	26.5	21.8	27.5	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd: not detected

Highest abundance of cancer chemo preventive compound, irilone (4) [41] was detected in the root of *I. ensata* (63.1%) followed by leaf of *I. kashmiriana* (51.4%), *I. hookeriana* (43.1%) and *I. ensata* (39.6%). Similarly, 5,7-dihydroxy-4'-6-dimethoxyisoflavone (8) was relatively high in the roots of *I. spuria* (90%) and *I. crocea* (68.5%). Compound iriskashmirianin (11) was detected relatively more in the leaf of *I. hookeriana* (50.1%) and roots of *I. hookeriana* (42.6%) and *I. spuria* (39.4%) whereas iriflogenin 4'-O-glucoside (19) was found high in the roots of *I. ensata* (27.5%) and *I. crocea* (26.5%). High content of irisoid A (9) was found in the roots of *I. hookeriana* (29.8%), *I. kashmiriana* (29.2%) and *I. crocea* (27.5%).

The compounds alpinone (3) and 5-methoxy-4'-hydroxy-6,7-methylenedioxyisoflavone (7) were detected high in the roots of *I. crocea* (98.6%), *I. germanica* (96.1%), *I. hookeriana* (89.4%), *I. kashmiriana* (78.9%) and *I. ensata* (43.7%) respectively. Similarly, tectorigenin (5) and tectoridin (17) were found high in the roots of *I. ensata* (95%) and *I. crocea* (79.9%) respectively. This observation could be helpful in selecting the most suitable plant/parts of *Iris* species for their medicinal purposes and quality control.

Discrimination of root and leaf of six *Iris* species using principal component analysis

The chemical fingerprint data when combine with principal component analysis serves as an effective means for identifying the natural components as markers which can be used to discriminate among the species [38-40,42]. PCA is an unsupervised procedure that determines the directions of the largest variations in the data set and the data are generally presented as a two-dimensional plot (score plot) where the coordinate axis represents the directions of two largest variations [38-40,42]. The DART MS data obtained from root and leaf of *I. crocea*, *I. ensata*, *I. germanica*, *I. hookeriana*, *I. kashmiriana* and *I. spuria* was subjected to PCA. The scores and loading plots (PC1 vs. PC2) are given in Figures 5 and 6. The data for PCA analysis was taken from the *m/z* 100 to 800 Da. All the *m/z* considered for PCA analysis were pseudo molecular $[M+H]^+$ ions with defined isotopic peak patterns. Fifty-three peaks were identified from fifteen repeats of each sample of the six *Iris* species. Averages of 15 repeats of all the samples were used for PCA analysis. In the case of leaf samples of six *Iris* species, 71 peaks were taken for analysis. These extracted 53 and 71 peaks of roots and leaves respectively were used as variables for PCA analysis using correlation matrix of data. The PCA scores plots of the roots of *I. crocea*, *I. ensata*, *I. germanica*, *I. hookeriana*, *I. kashmiriana* and *I. spuria* (Figure 5a) showed clustering of the data according to the plant species.

Figure 5a. PCA Score plot discriminating the root of six *Iris* species.

Figure 5b. PCA loading plot of root of six *Iris* species.

The PCA extracted 18 marker peaks out of 53 peaks at m/z 133, 163, 237, 261, 271, 297, 299, 377, 387, 393, 427, 439, 441, 443, 475, 491, 499 and 507 which were contributing for discrimination of roots. These 18 marker peaks clearly separated roots of all the plants, which accounted for total 78.86% variance as shown in Figure 5a and 5b.

Similar, clustering and differentiation was clearly observed among the leaves of *I. crocea*, *I. ensata*, *I. germanica*, *I. hookeriana*, *I. kashmiriana* and *I. spuria*. The principal component analysis was initially done for 71 variables (m/z) detected in the fingerprints of leaves. The PC1 vs PC2 plot showed that the first two principal components were able to explain highest variance of 73.71% information contained in the 16 marker peaks at m/z 123, 153, 167, 183, 237, 277, 313, 327, 331, 343, 397, 415, 441, 493, 557 and 638 which discriminated among the leaves Figure 6a and 6b. By using the PCA as a chemometric tool, the number of PCs were identified which were able to differentiate among the leaves and roots of six *Iris* species. It is, therefore, clear that DART MS followed by PCA is an appropriate method for the clear identification of different *Iris* species.

Figure 6a. PCA Score plot discriminating the leaf of six *Iris* species.

Figure 6b. PCA loading plot of leaf of six *Iris* species.

CONCLUSION

The DART-MS method has been developed and applied successfully for the first time for the chemical profiling of flavonoids and isoflavonoids in the roots and leaves of *I. crocea*, *I. ensata*, *I. germanica*, *I. hookeriana*, *I. kashmiriana* and *I. spuria*. Significant difference in the mass spectra was observed. A total of twenty-two phyto-constituents were tentatively identified and their variations were studied. Compounds acetovanillone (1), irilone (4), tectorigenin (5), 5-methoxy-4'-hydroxy-6,7-methylenedioxyisoflavone (7), 5,7-dihydroxy-4'-dimethoxyisoflavone (8), irisoid A (9), iriskashmirianin (11), tectoridin (17), and iriflogenin-4'-O-glucoside (19) reported to possess several biological activities are among the main identified bioactive compounds. PCA analysis was able to classify and identify marker peaks for discrimination among roots and leaves of *Iris* species. This analysis underscores the importance of DART-MS method for high throughput analysis and identification of bioactive compounds for selection of the best plant/part according to need, authentication and quality control of these *Iris* species.

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Supplementary Material

Table SI. The sample code, voucher specimen number and collection location of *Iris* species from J&K, India

S. N°	Voucher Specimen N°	Iris species	Sample Code	Place of collection
1	RRLH 8643	<i>Iris crocea</i> Jacq. ex R.C. Foster	lc	Srinagar
2	RRLH 52992	<i>Iris ensata</i> Thunb.	le	Bandipora
3	RRLH 52990	<i>Iris x germanica</i> L.	lg	Pahalgam
4	RRLH 53176	<i>Iris hookeriana</i> Foster	lh	Razdhan Pass
5	RRLH 52991	<i>Iris kashmiriana</i> Baker	lk	Srinagar
6	RRLH 8642	<i>Iris spuria</i> L.	ls	Srinagar

Table SII. Variance contributions of each principal component in the root of six *Iris* species

	Variable contributions based on correlations				
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
133	0.092791	0.004990	0.017997	0.050937	0.033285
163	0.036368	0.044221	0.113450	0.135111	0.091540
237	0.042414	0.099133	0.008150	0.074854	0.037519
261	0.092791	0.004990	0.017997	0.050937	0.033285
271	0.013311	0.071328	0.210276	0.039550	0.041972
297	0.092791	0.004990	0.017997	0.050937	0.033285
299	0.092791	0.004990	0.017997	0.050937	0.033285
377	0.051031	0.082844	0.034164	0.035257	0.010497
387	0.051031	0.082844	0.034164	0.035257	0.010497
393	0.051031	0.082844	0.034164	0.035257	0.010497
427	0.023319	0.068255	0.088416	0.151777	0.173980
439	0.059184	0.045572	0.063246	0.006869	0.164783
441	0.051031	0.082844	0.034164	0.035257	0.010497
443	0.013311	0.071328	0.210276	0.039550	0.041972
475	0.042414	0.099133	0.008150	0.074854	0.037519
491	0.059184	0.045572	0.063246	0.006869	0.164783
499	0.042414	0.099133	0.008150	0.074854	0.037519
507	0.092791	0.004990	0.017997	0.050937	0.033285

Table SIII. Variance contributions of each principal component in the leaf of six *Iris* species

	Variable contributions based on correlations				
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
123	0.071466	0.057625	0.086823	0.013097	0.041480
153	0.120635	0.001805	0.009805	0.079409	0.004345
167	0.052878	0.089544	0.054694	0.076012	0.000327
183	0.021815	0.158362	0.024677	0.005847	0.054842
237	0.103264	0.020605	0.001516	0.109099	0.016853
277	0.031894	0.002110	0.166062	0.305526	0.038281
313	0.048839	0.101673	0.038750	0.072000	0.008419
327	0.018637	0.101194	0.042743	0.021079	0.295006
331	0.001706	0.175003	0.044722	0.005172	0.085057
343	0.002482	0.160381	0.000066	0.041501	0.185119
397	0.120450	0.011834	0.005839	0.052407	0.002905
415	0.000269	0.057402	0.253889	0.179570	0.011065
441	0.101471	0.004826	0.130796	0.000204	0.006385
493	0.106080	0.038860	0.004805	0.038459	0.000597
557	0.096643	0.013952	0.004016	0.000413	0.242934
638	0.101471	0.004826	0.130796	0.000204	0.006385

Figure S1. Comparison of nine bioactive compounds in leaves of six *Iris* species.

Figure S2. PCA Score plot (PC1 x PC3) of root of six *Iris* species

Figure S3. PCA Score plot (PC1 x PC4) of root of six *Iris* species

Figure S4. PCA Score plot (PC1 x PC3) of leaf of six *Iris* species

Figure S5. PCA Score plot (PC1 x PC4) of leaf of six *Iris* species