



## RESEARCH ARTICLE

# Taxonomic study of some species of the subfamily Dipsacoideae Eaton (Caprifoliaceae) by phenolic acid profiles

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## ABSTRACT

Dipsacoideae has always been problematic for taxonomic delimitation of the taxa because of their morphological similarities and diversity amongst the taxa. Phenolic compounds are found in various organs of plants and are important in terms of chemotaxonomy and pharmacognosy. In this study, the phenolic acid compounds of 12 species of Dipsacoideae were analyzed using high-performance liquid chromatography photodiode array detection (HPLC-PDA) and also evaluated their significances as chemotaxonomic markers. The main phenolic acids were found to be caffeic acid, p-coumaric acid, ferulic acid and salicylic acid. The principal components analysis (PCA) bi-plot indicated that ferulic acid, caffeic acid, cinnamic acid, p-coumaric acid and rosmarinic acid were principal components in the studied species dispersion. The species were separated from each other in a principal coordinate analysis (PCoA) plot in terms of their phenolic acid profile. Regarding the results, the high amount of caffeic acid and cinnamic acid could be considered a chemotaxonomic marker for genus *Pterocephalus* Vaill. and *Cephalaria* Schrad. respectively. The results indicated that *Scabiosa koelzii* Rech. and *S. amoena* Jacq. were placed as a distinct group regarding their phenolic acid profile and established the opinion supported by Greuter and Raus. Consequently, phenolic contents could be applied as a significant marker in the chemotaxonomy of Dipsacoideae. Considering it, we suggest the study of interaction among ecological and genetically factors as well as the studied chemical compounds.

## Introduction

Dipsacoideae Eaton (APG III; Caprifoliaceae, Dipsacales) contains ca. 150 species of perennial or biennial herbs and shrubs classified in 14 genera distributed in Europe, Asia and Africa (1). Some species are ornamental, noxious weeds and identified as herbal medicine sources (2, 3). Dipsacoideae has always been discussed for delimitation of the taxa, concerning morphological similarities and high diversity amongst the taxa, especially calyx and fruit characters in genera *Scabiosa* L. and *Pterocephalus* Vaill. (4). Therefore, the taxonomy and phylogenetic of taxa within the Dipsacoideae subfamily have been significantly changed over time.

First, Linnaeus distinguished three genera that include *Scabiosa* L., *Dipsacus* L. and *Knautia* L. The genera *Pterocephalus* Vaill., *Succisa* Haller, *Cephalaria* Schrad. ex Roem & Schult. were later segregated from *Scabiosa* L. (5).

Dipsacoideae was divided into two tribes by De Candolle, viz. Morineae (including genus *Morina* L.) and Scabioseae (including *Dipsacus* L., *Cephalaria* Schrad., *Pterocephalus* Vaill., *Knautia* L. and *Scabiosa* L.) (6). In addition, Verlaque divided this sub-family into three tribes with nine genera (4). Major revisions to Scabioseae divided *Scabiosa* sensu lato into 6 genera: *Lomelosia* Raf., *Scabiosa* sensu stricto, *Pseudoscabiosa* Dev., *Pterocephalidium* (Lag.) G. Lopez, *Pycnocomon* Hoffmanns. and Link and *Sixalix* Raf. (7, 8).

Phylogeny has widespread advantages in interdisciplinary studies including taxonomy, evolutionary biology, biogeography, ecology, conservation and even medicine (9, 10). Relatively little phylogenetic work has focused on Dipsacoideae. Caputo and Cozzolino (11) used morphology traits for the first phylogenetic study of Dipsacoideae, widely supported the traditional definitions described above. On the contrary, molecular phylogenetic results were

in contrast to the previous morphological works and supported the revisions to tribes and genera (12). In this way, two major clades in Dipsacoideae were identified: Scabioseae sensu stricto and a clade containing Dipsaceae, Knautieae and the groups excluded from Scabioseae sensu lato [*Pseudoscabiosa* Dev., *Pterocephalidium* (Lag.) G. Lopez, *Succisa* Haller, *Succisella* Beck] (11). As a result, taxonomic evidence of this sub-family can be very effective in classifying the status rank of taxa within different genera and species.

Most of the recent chemotaxonomic knowledge was applied in order to study the phylogenetic relationships of plants (13). Secondary metabolites, in particular phenolic compounds, are extensively used in chemotaxonomy investigations in terms of their widespread dissemination in vascular plants, structural variation and chemical stability (14-19). The phenolic compounds value has been proven to be the appropriate taxonomic marker in different taxonomic ranks (13). In Dipsacoideae, the fatty acid and sterol composition of eight taxa belonging to three genera *Scabiosa*, *Cephalaria* and *Pterocephalus* were investigated (20). Moreover, reports are on studied chemotaxonomic status and fatty acid compositions of eleven species of *Scabiosa* (21).

This study aimed to investigate the phenolic acid compounds diversity in the aerial parts of 12 species of Dipsacoideae from Iran, using HPLC method and their significance evaluation as chemical markers for taxonomic purposes.

## Materials and Methods

### Plant material

In this study, 12 species (one accession per species) belonged to 4 Dipsacoideae genera were collected from seven provinces of Iran during spring and summer in 2015-2017. The species identification was performed using literature (22, 23). The list of voucher specimens and localities were presented in Table 1.

### Extraction of free phenolic acids

Free phenolic acids were extracted using the method described (24). In this method, 5 ml of 80% ethanol were added to 0.5 g of dried powdered sample (aerial parts of plant) and they were mixed at 3000 rpm on a vortex mixer for 5 min. The mixture was centrifuged (Rotina 420R Hettich, German) at a speed of 6000 × g

for 5 min. The supernatant was decanted, evaporated and was stored in an amber glass vial (-20 °C) for additional analysis.

### Analysis of phenolic acids

The phenolic compounds analysis was accomplished using an HPLC (Waters 2695, USA) system equipped with a diode-array detector, a 20 µl loop and a C18 analytical column (250 × 0.46 mm, 5 µm). Separation was performed by the use of a gradient programme run at room temperature, which consist two solvents: solvent A (0.1% TFA in methanol) and solvent B (0.1% TFA in water, v/v) as following: 20% A, at 0 min; 30% A, (from 0 to 10 min); 60% A, (from 10 to 30 min); 80% A, (30 to 40 min); 100% A, (40 to 45 min); 20% A, (from 45 to 52 min); isocratic, 6 min. The flow rate of the mobile phase was maintained at 1 ml/min, and the wavelength was adjusted at 254, 275 and 320 nm. The identification of studied phenolic acids was evaluated by retention times and the analysis of spiked crude extract with standards solution. For the quantitative analysis of phenolic acid compounds, calibration curves were obtained via the injection of different concentrations of standard compounds (5, 20, 40, 60, 80 and 100 ppm). The results were expressed as micrograms per g of dry weight extract. Experiments were performed in triplicate repeats.

### Statistical analysis

The most variable phenolic acids were identified by Principal Components Analysis (PCA biplot) in order to avoid from the misclassification of the taxa. Box plots of significance phenolic acids were applied to display the means and variable characters range amongst different taxa. Principal Coordinate Analysis (PCoA) was performed for the variable phenolic acid content of investigated taxa with Euclidean as similarity index. Statistical analysis was accomplished using the PAST ver. 2.17c programme.

## Results

This study was the first study that quantifies phenolic acid compounds of 12 species of Dipsacoideae in Iran. In general, 11 phenolic acid compounds were evaluated in methanol extracts of studied species using the HPLC-PDA (Fig. 1). Totally, 10, 9, 8, 7, 9, 8, 8, 9, 9, 9, 8 and 8 phenolic acid compounds were identified in *D. pilosus*, *C. procera*, *P. plumosus*, *C.*

**Table 1.** Name, place of collection and vouchers of studied taxa of Dipsacoideae sub-family.

Genus	Species	Locality	Voucher No.
<i>Cephalaria</i> Schrad.	<i>C. kotschy</i> Boiss.	Mazndaran, Chalus	ASMUH95001
	<i>C. procera</i> Fisch.	Ardebil, Khalkhal	ASMUH95002
	<i>C. syriaca</i> (L.) Schrad.	Tehran, Darakeh	ASMUH95010
<i>Dipsacus</i> L.	<i>D. pilosus</i> L.	Tehran, Darakeh	ASMUH95011
	<i>D. strigosus</i> Willd.	Mazndaran, Chalus	ASMUH95005
<i>Pterocephalus</i> Vaill.	<i>P. canus</i> Coul.	Tehran, Ab-ali	ASMUH95004
	<i>P. plumosus</i> (L.) Coult.	Mazndaran, Chalus	ASMUH95003
<i>Scabiosa</i> L.	<i>S. caucasica</i> M. B.	Ardebil	ASMUH95006
	<i>S. amoena</i> Jacq.	Masuleh	ASMUH95007
	<i>S. koelzii</i> Rech. f.	Bojnord	ASMUH95008
	<i>S. rotata</i> Bieb.	Mashhad	ASMUH95009
	<i>S. persica</i> Boiss.	Piranshahr	HSBU4000

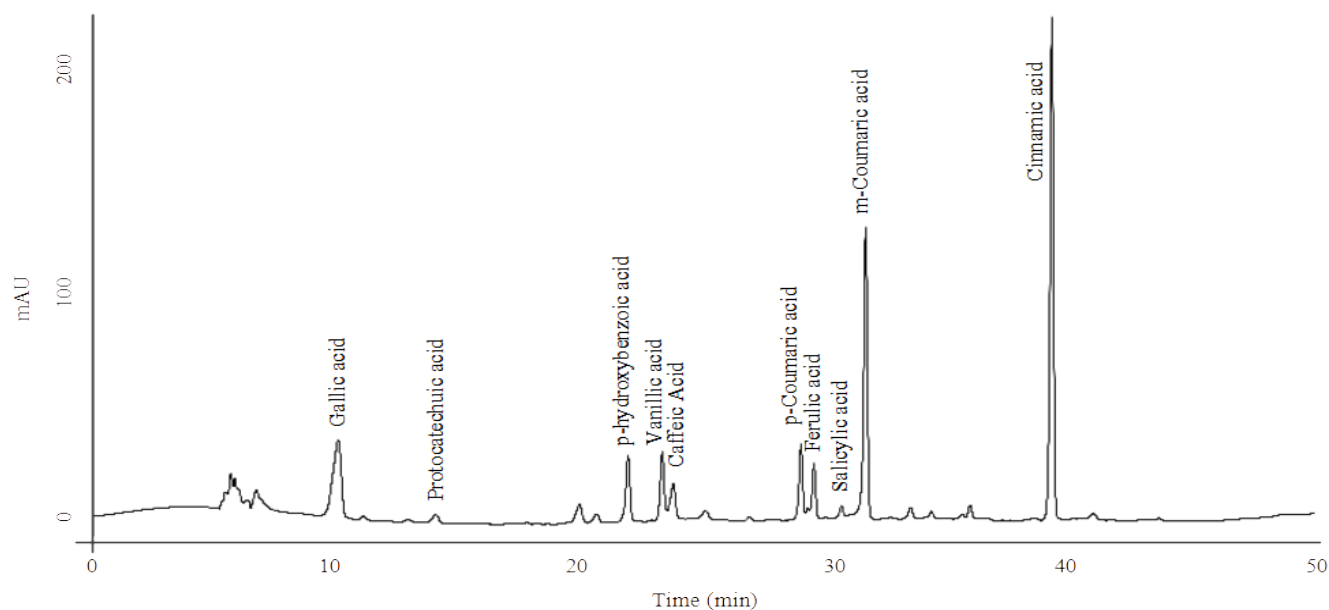


Fig. 1. HPLC chromatogram of phenolic acid extract from *Dipsacus pilosus* L.

*syriaca*, *S. persica*, *D. strigosus*, *C. kotschyi*, *S. rotata*, *S. amoena*, *S. koelzii*, *S. caucasica* and *P. canus* with the total amount of 5.752, 10.207, 11.383, 11.317, 6.719, 5.702, 12.388, 8.274, 6.077, 6.087, 6.597 and 6.853 mg/g dried weight respectively (Table 2). The dominant components included caffeic acid, p-coumaric acid, ferulic acid and salicylic acid (Table 3). Gallic acid, caffeic acid, p-coumaric acid, m-coumaric acid and salicylic acid were present in all taxa, and their

3.145±0.100 mg/g dried weight respectively. Ferulic acid was not observed in *P. canus*, whereas the highest amounts were associated with *C. kotschyi*, *S. amoena* and *S. koelzii* with values of 1.452±0.100, 1.259±0.010 and 1.087±0.069 mg/g dried weight respectively. p-Hydroxybenzoic acid was another phenolic acid compound in all the investigated species, except *P. plumosus* and *P. canus*.

Table 2. Amount of phenolic acid compounds (mg/g dried weight; mean ± S.E.) in some species of Dipsacoideae sub-family.

	GA	PCA	PHBA	VA	CaA	PCoA	FA	MCA	CiA	RA	SA
<i>D. pilosus</i>	0.020±0.002	0.010±0.001	0.020±0.002	0.453±0.032	3.278±0.124	0.458±0.024	0.409±0.034	0.139±0.017	0.067±0.009	0.000	0.898±0.014
<i>C. procera</i>	0.019±0.001	0.000	0.011±0.001	0.040±0.004	8.048±0.251	0.888±0.029	0.193±0.014	0.177±0.015	0.079±0.003	0.000	0.752±0.016
<i>P. plumosus</i>	0.021±0.002	0.000	0.000±0.000	0.000	7.823±0.207	0.317±0.031	0.176±0.009	0.162±0.011	1.996±0.098	0.051±0.001	0.837±0.031
<i>C. syriaca</i>	0.019±0.001	0.000	0.010±0.000	0.000	9.586±0.211	0.637±0.011	0.220±0.011	0.161±0.015	0.000	0.000	0.685±0.019
<i>S. persica</i>	0.024±0.002	0.010±0.000	0.016±0.001	0.124±0.017	5.038±0.30	1.019±0.084	0.120±0.009	0.164±0.014	0.000	0.000	0.566±0.012
<i>D. strigosus</i>	0.019±0.001	0.000	0.012±0.001	0.255±0.021	3.595±0.079	0.456±0.012	0.289±0.010	0.143±0.009	0.000	0.000	0.932±0.040
<i>C. kotschyi</i>	0.019±0.002	0.000	0.013±0.001	0.000	8.021±0.239	0.981±0.017	1.452±0.100	0.176±0.009	0.000	0.919±0.029	0.808±0.019
<i>S. rotata</i>	0.023±0.001	0.009±0.000	0.013±0.001	0.178±0.042	6.468±0.270	0.866±0.011	0.081±0.007	0.136±0.011	0.000	0.000	0.499±0.017
<i>S. amoena</i>	0.025±0.002	0.013±0.001	0.017±0.001	0.077±0.005	3.145±0.100	0.803±0.014	1.259±0.010	0.173±0.010	0.000	0.000	0.565±0.010
<i>S. koelzii</i>	0.030±0.002	0.010±0.001	0.019±0.002	0.090±0.004	3.312±0.081	0.627±0.012	1.087±0.069	0.175±0.006	0.000	0.000	0.738±0.014
<i>S. caucasica</i>	0.023±0.001	0.000	0.015±0.001	0.371±0.021	4.470±0.102	0.921±0.051	0.137±0.015	0.144±0.009	0.000	0.000	0.516±0.015
<i>P. canus</i>	0.020±0.001	0.000	0.000	0.288±0.019	3.536±0.187	0.577±0.041	0.000	0.151±0.011	1.256±0.112	0.085±0.005	0.940±0.026

GA: Gallic acid; PCA: Protocatechuic acid; PHBA: p-Hydroxybenzoic acid; VA: Vanillic acid; CaA: Caffeic acid; PCoA: p-Coumaric acid; FA: Ferulic acid; MCA: m-Coumaric acid; CiA: Cinnamic acid; RA: Rosmaric acid; SA: Salicylic acid

highest amount was related to *S. koelzii*, *C. syriaca*, *S. persica*, *C. procera* and *P. canus* with values of 0.030±0.002, 9.586±0.211, 1.019±0.084, 0.177±0.015 and 0.940±0.026 mg/g dried weight respectively. The highest amounts of protocatechuic acid, cinnamic acid and rosmarinic acid were related to *S. amoena*, *P. plumosus* and *C. kotschyi* with values of 0.013±0.001, 1.996±0.098 and 0.919±0.029 mg/g dried weight respectively. Moreover, the highest and lowest caffeic acid amounts were associated with *C. syriaca* and *S. amoena* with the following values 9.586±0.211 and

The PCA bi-plot of the 11 distinct phenolic acids demonstrated that the ferulic acid, caffeic acid, rosmarinic acid, cinnamic acid and p-coumaric acid were principal components in the studied taxa dispersion (Fig. 2). For example, the highest and lowest amount of ferulic acid was in *C. kotschyi* (1.452±0.100 mg/g dried weight) and *S. rotata* (0.081±0.007 mg/g dried weight) respectively. The caffeic acid amount varied between 3.145±0.100 mg/g dried plant in *S. amoena* to 9.586±0.211 mg/g dried weight in *C. syriaca*.

**Table 3.** Eigen-values, estimated and cumulative variance for seven factors obtained from principal components.

Compounds	Principal components						
	1	2	3	4	5	6	7
Caffeic acid	18.02	-0.06	-0.07	-0.12	-0.02	-0.02	0.01
p-Coumaric acid	-0.31	0.62	-0.29	0.45	0.51	0.09	-0.04
Ferulic acid	-1.27	1.18	1.09	-0.06	0.01	-0.15	0.04
Cinnamic acid	-1.65	-1.93	0.59	0.03	0.17	-0.01	0.02
Rosmaric acid	-2.26	0.24	0.08	-0.62	-0.03	0.44	-0.01
Gallic acid	-2.57	-0.02	-0.31	-0.21	-0.04	-0.14	-0.03
p-Hydroxybenzoic acid	-2.61	-0.01	-0.32	-0.22	-0.05	-0.14	-0.03
Protocatechuic acid	-2.63	-0.02	-0.31	-0.23	-0.05	-0.14	-0.03
m-Coumaric acid	-2.13	0.03	-0.23	-0.07	-0.02	-0.13	-0.04
Vanillic acid	-2.27	0.01	-0.40	0.32	-0.12	0.06	0.21
Salicylic acid	-0.33	-0.03	0.17	0.73	-0.34	0.14	-0.10
Eigen-value	36.44	0.56	0.22	0.14	0.04	0.03	0.01
Variance (%)	97.35	1.49	0.58	0.37	0.12	0.08	0.01
Cumulative variance	97.35	98.84	99.42	99.79	99.91	99.99	100

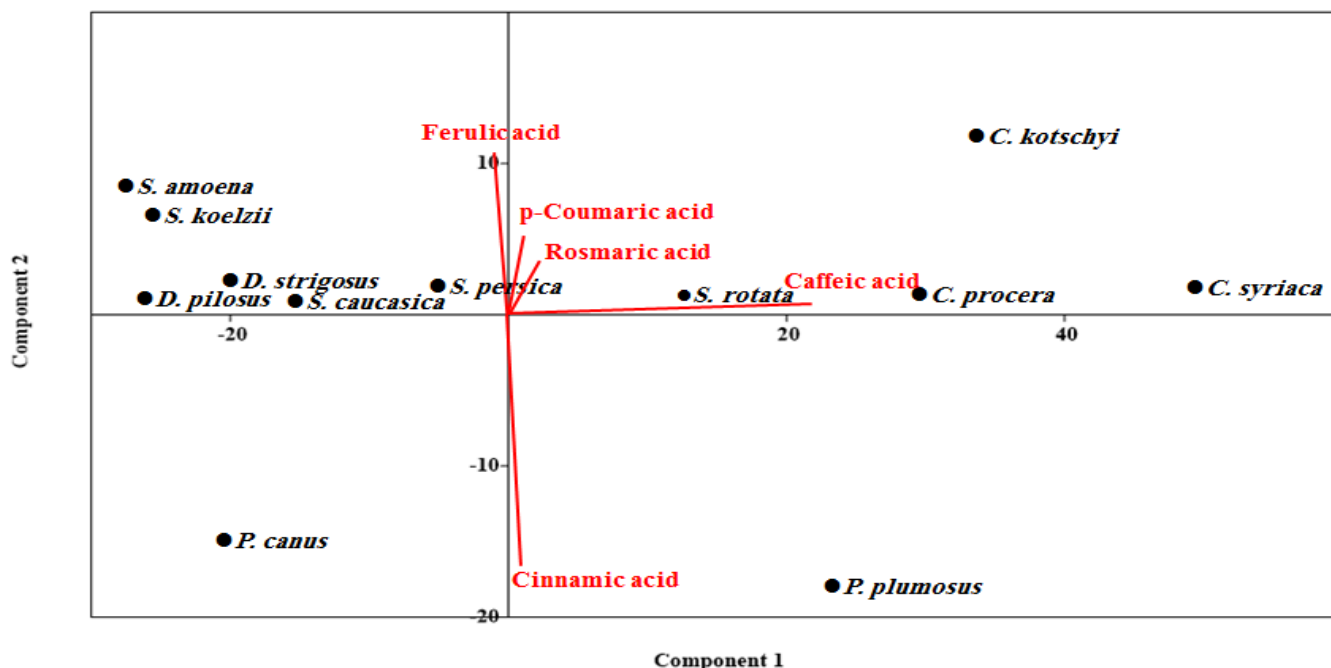
The main PC (component 1) explained 97.40% of the variation and had a positive correlation with caffeic acid. Also, PC2 (component 2) had a positive correlation with ferulic acid, p- coumaric acid and rosmaric acid.

Box-Plot graphs were applied for highlighting the phenolic acid compounds distributions amongst those investigated taxa. Box plots indicated that some quantitative phenolic acid compounds were more

in addition, *D. pilosus* and *D. strigosus* were placed close to each other and the species of *Scabiosa* were placed in another group.

### Discussion

Phenolic acid compounds are considered an important class of secondary metabolites in Dipsacoideae. As phenolic compounds have high



**Fig. 2.** Principal Component Analysis (PCA) among studied species based on phenolic acid compounds.

valuable in the taxa identification (Fig. 3). For example, cinnamic acid quantity was a useful character in *P. canus* and *P. plumosus* identification from other taxa. A high amount of rosmaric acid can be used for recognizing *C. kotschyi*.

PCoA analysis revealed each studied species distribution due to phenolic acid contents (Fig. 4). Due to high amount of caffeic acid (>8 mg/g dried weight), *Cephalaria* were categorized into separate groups. In

evolutionary rates compared to molecular markers like DNA sequences, they are important in phylogenetic studies and have been used at lower taxonomic ranks (25). The evolution within the Dipsacoideae followed complex paths and several genera were polyphyletic (25, 26). This sub-family has always been subjected to the argument for the taxa delimitation. de Castro and Caputo indicated that there is a high degree of homoplasy at the generic level in Dipsacoideae (27). This research indicated the

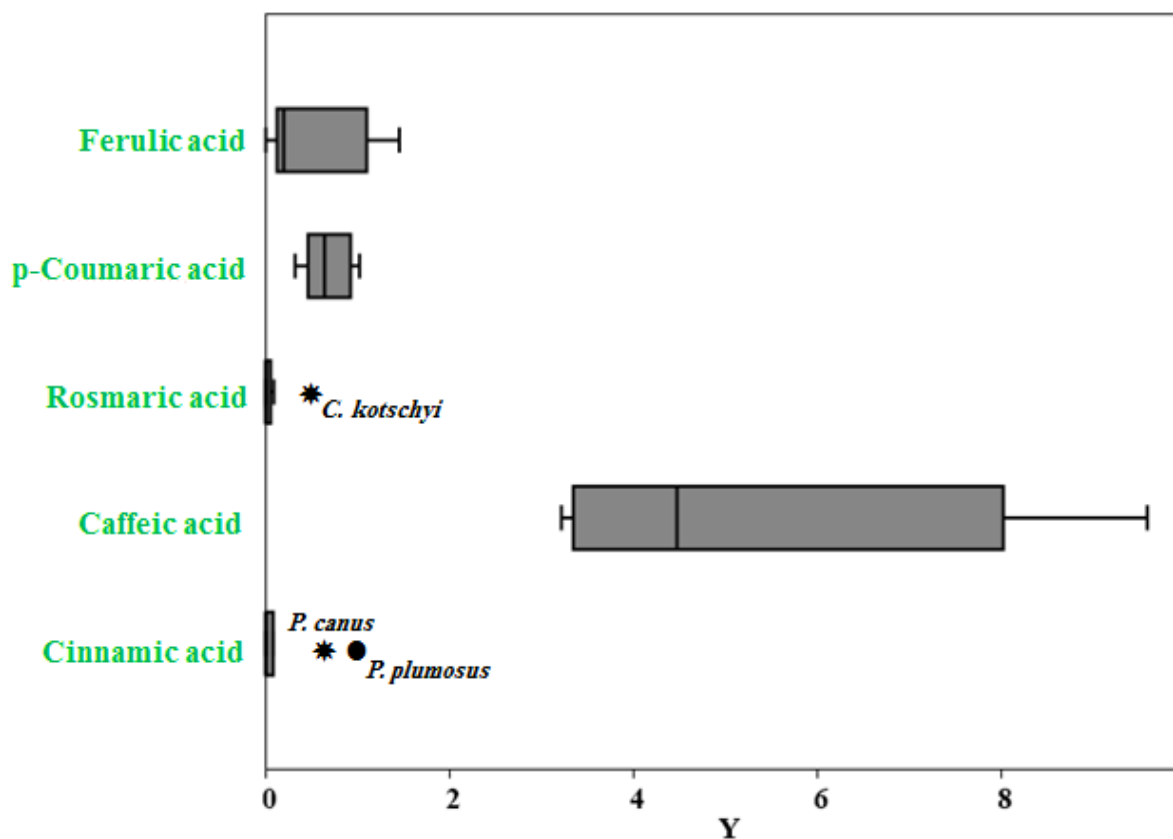


Fig. 3. Box plot of the most variable phenolic acid content among studied species of Dipsacoideae sub-family.

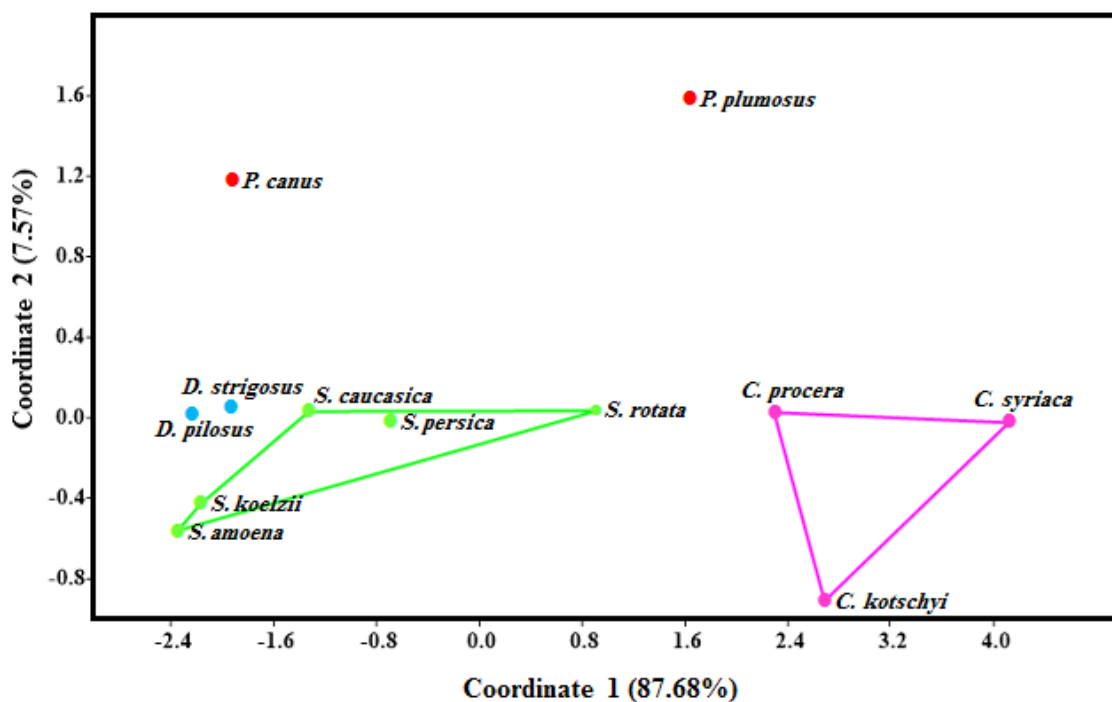


Fig. 4. Two-dimensional plot of Principal Coordinate Analysis (PCoA) of studied species based on phenolic acid compounds.

high variation of phenolic acid content amongst the Dipsacoideae. In this study, the PCoA plot showed that there are significant differences amongst the various species and genera, with respect to phenolic acid compounds.

Given to the taxonomical aspect, the *Scabiosa* is the most important genus of the Dipsacoideae due to ambiguous in the number of reported species (28, 29). It was reported that there are 22 species of *Scabiosa* L. in the Flora Iranica, which belonged to three sections:



*Scabiosa*, *Astrocephalus* and *Olivierianae* (23). Greuter and Raus categorized Iranian species of *Scabiosa* into two genera as *Lomelosia* Raf. (= *Scabiosa* sect. *Astrocephalus* Coult. and sect. *Olivierinae* Coult.) and *Scabiosa* s. str. (= *Scabiosa* sect. *scaboisa* L.) (30). On the basis of the plant list, situation of *S. koelzii* and *S. amoena* is unresolved. However, based on one study, involucre tubes of *Lomelosia* had eight apical pits, whereas *Scabiosa* included eight longitudinal grooves (30). Therefore, among the studied species, *S. koelzii* and *S. amoena* could be remaining as species of the genus *Scabiosa* and three other species could be transferred to *Lomelosia*. Given to character of pitted epicalyx, the relocation of four species from *Scabiosa* to *Lomelosia* was reported (31).

According to our earlier reports, *S. koelzii* and *S. amoena* were placed as a distinct group from other *Scabiosa* species due to their fatty acid profile and palynological characters and confirmed the opinion supported (21, 32). The taxonomic differentiation between two sections (*Scabiosa* and *Astrocephalus*) was followed by variation in their phenolic acid compounds. In conclusion, in this study, the phenolic acid results established the opinion supported by a previous study, that *Scabiosa* sect. *Astrocephalus* was well transferred taxonomically to *Lomelosia* (30).

In recent studies, phytochemical studies of *Cephalaria* species indicated salicylic acid, p-coumaric acid, vanillic acid, gallic acid and caffeic acid presence (33, 34), consistent with this study results. In the PCoA plot, species of *Cephalaria* were located in a group because their phenolic acid compositions were similar. This means that these compounds, especially the high content of caffeic acid, can be considered useful to recognize the *Cephalaria* spp. It was reported that caffeic acid was the main phenolic acid in n-butanol extract of *C. davisiana* (34). In addition, *C. kotschyi* was separated from *C. syriaca* and *C. procera* based on the presence of ferulic acid and rosmarinic acid.

The high amount of cinnamic acid was distinguished at *P. plumosus* and *P. canus*. This compound has been identified as an interesting compound with anti-inflammatory, antioxidant and cytotoxic properties. Although *P. plumosus* and *P. canus* were placed apart from each other with respect to the PCoA plot, the cinnamic acid content of these two taxa was high and could be considered as a biomarker for this genus.

The extract of *D. asperoides* has been reported to have various phenolic acids compounds such as p-coumaric acid, o-coumaric acid, ferulic acid, caffeic acid and vanillic acid, which is in agreement with our results (35). Moreover, the caffeic acid, cinnamic acid derivative and vanillic acid were identified in *D. asper* and *D. fullonum* (36, 37). In addition, salicylic acid for the first time was identified at *D. pilosus* and *D. strigosus* in our study.

## Conclusion

The investigated taxa of Dipsacoideae were recognized using the phenolic acid profile and these compounds could be applied in the Dipsacoideae chemotaxonomy as a significance marker. Regarding future

phylogenetic studies in this subfamily, it is highly recommended to investigate the interaction of molecular marker and chemotaxonomy, as well as environmental factors.

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## Authors' contributions

All authors contributed equally.

## Compliance with ethical standards

**Conflict of interests:** The authors declare that there exists no conflict of interest.

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