

Research paper

**Interspecific morphological and phytochemical variations in the willow herb (*Epilobium* spp.) medicinal plant**

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**Abstract**

Willow herb (*Epilobium* spp.) is a medicinal plant that is used for various purposes in the traditional medicine and food industries. In this research, an attempt was made to assess morphological and phytochemical variations in eight *Epilobium* species (*E. hirsutum*, *E. Parviflorum*, *E. roseum*, *E. algidum*, *E. anatolicum*, *E. confusum*, *E. frigidum*, and *E. lanceolatum*). The species were collected from natural habitats during the flowering period. Analysis of variance showed a significant variation among the studied species for measured morphological and phytochemical traits. Total phenolic content varied from  $194.64 \pm 1.17$  to  $309.10 \pm 5.59$  mg g<sup>-1</sup> DW, total flavonoids ranged from  $39.03 \pm 0.21$  to  $56.14 \pm 0.67$  mg g<sup>-1</sup> DW, and antioxidant activity differed from  $43.03 \pm 2.20$  to  $101.36 \pm 3.68$  mg g<sup>-1</sup> DW. The results showed that *E. frigidum* is the richest source of natural antioxidants among the studied species, and also has the highest seed and leaf lengths. Antioxidant activity was significantly correlated with bioclimatic variables. However, the dendrogram based on morphological traits was relatively different from that based on all phytochemical variables. In conclusion, the remarkable variation among species for the morphological and phytochemical traits may be exploited in the breeding programs of the genus *Epilobium*.

**Keywords:** antioxidant activity, *Epilobium*, morphological traits, total phenols, total flavonoids

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**Introduction**

The *Epilobium* genus which consists of approximately 200 species, is an extensive medicinal herb that has various applications in the food, pharmaceutical, and cosmetic industries (Vitalone *et al.* 2001). Some *Epilobium* extracts have valuable pharmacological properties and biological activities, including antioxidant, anti-

microbial, anti-inflammatory, antinociceptive, and anti-exudative substances (Kiss *et al.* 2011; Granica *et al.* 2014; Dreger *et al.* 2016). In folk medicine, the extracts have been suggested for the treatment of skin diseases and inflammation, such as eczema, acne, burns, and ulcers, and also as a poultice for the treatment of mouth wounds (Sõukand *et al.* 2020). Furthermore, the unique chemical

composition of *Epilobium* is used as a traditional remedy for the treatment of prostate diseases and is also successfully applied for male health maintenance, hormonal imbalances, and urinary system health (Constantin and Coste 2013). *Epilobium* plants are rich sources of secondary metabolites, especially polyphenols, such as flavonoids, phenolic acids, and tannins (Oenothin B and Oenothin A) which are the main and more important constituents of their polar extracts and accumulated in leaves, roots, and flower parts (Vital *et al.* 2006; Granica *et al.* 2014)

Nowadays, the demand for this herb for the pharmaceutical industry and medicinal usage has risen. In this regard, breeding programs are needed to select suitable genotypes and hybridization would be important and is a common feature in the *Epilobium* genus due to cross-pollination which has had important effects on the diversity of morphological and phytochemical characteristics in this genus (Lewis and Moore 1962). However, variation in *Epilobium* has been attributed to both environmental and genetic factors (Hay and Waterman 1993).

Evaluation of the morphological and phytochemical characteristics provides useful information about the species relationships and promotes further insight for plant breeders (Khadivi-Khub *et al.* 2014) which

can be used in breeding programs to introduce new cultivars with desirable traits. Eighteen species of *Epilobium* have been reported in the flora of Iran, occurring in various geographical areas (Akbari and Azizian 2006). Due to the lack of information about the morphological and phytochemical traits in the willow herb, the present investigation aimed to examine the morpho-phytochemical variation of eight species of this genus.

## Materials and Methods

### *Plant materials*

Aerial parts of eight species of Willow herb (*Epilobium* spp., Onagraceae), comprising *E. hirsutum*, *E. parviflorum*, *E. roseum*, *E. algidum*, *E. anatolicum*, *E. confusum*, *E. frigidum*, and *E. lanceolatum* were collected from natural habitats during the flowering period. The identification, geographic coordinates, and climate data of plant species are shown in Table 1. The plants are deposited in the herbarium and were used for both morphological and phytochemical measurements. For each species, samples were prepared from four randomly selected individual plants, and then plant samples were dried at room temperature. Finally, the plant samples of each species were pooled together and used for phytochemical analysis.

### *Morphological characterization*

To determine the morphological variability

Table 1. Geographical information, bioclimatic data, collection sites, and accession number of endemic *Epilobium* spp. of Iran.

Species name	Locality	Longitude (E) Latitude (N)	Altitude (m)	Bio1	Bio2	Bio3	Bio4	Bio5	Bio6	Bio7	Bio8
<i>E. hirsutum</i>	Chalus	36°38' 51°24'	80	6.19	24.87	34.00	631.00	18.13	-5.00	24.00	125
<i>E. parviflorum</i>	Urmia	37°10' 45°7'	1719	6.11	24.48	33.00	610.00	18.27	-5.50	24.30	122
<i>E. roseum</i>	Liqvan	37°49' 46°23'	2516	10.37	28.80	-5.90	20.33	22.38	-1.65	19.42	146
<i>E. algidum</i>	Hovir	35°41' 52°24'	2221	5.54	28.19	35.00	644.00	17.42	-5.53	23.20	123
<i>E. anaticum</i>	Sorkheh Dizaj	36°35' 48°51'	1978	7.68	19.18	32.00	538.00	19.57	1.50	26.20	136
<i>E. confusum</i>	Shahrud	36°32' 54°49'	2154	6.97	22.37	33.00	603.00	19.35	0.33	25.50	130
<i>E. frigidum</i>	Tange Vashi	35°54' 52°43'	2591	5.53	28.04	35.00	634.00	17.38	-5.45	23.40	122
<i>E. lanceolatum</i>	Evan lake	36°29' 50°27'	1857	4.50	31.16	32.00	634.00	16.07	-1.45	22.20	122

Bio1=Annual mean temperature, Bio2=Max temperature of warmest month, Bio3=Min temperature of coldest month, Bio4 = Mean temperature of wettest quarter, Bio5 = Mean temperature of driest quarter, Bio6 = Mean temperature of warmest quarter, Bio7 = Mean temperature of coldest quarter, Bio8 = Precipitation seasonality.

among *Epilobium* species, seven morphological traits including plant height (from base to top, mm), capsule length (mm), inflorescence length (mm), leaf length (mm), leaf width (mm), seed length (mm), and seed width (mm) were measured. To measure seed length and width, seeds were first photographed, using an Olympus 8ZX12 Stereo microscope (Olympus Optical Co., Tokyo, Japan) equipped with an Olympus DP12 digital camera (Olympus Optical Co., Tokyo, Japan). Then, the width and length were measured, using MicroMeasure 3.3 software.

### Phytochemical analysis

**Chemicals and reagents:** Folin-Ciocalteu phenol reagent, 2, 2-Diphenyl-picrylhydrazyl

(DPPH), Gallic acid (GA), quercetin, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sodium carbonate, sodium acetate, and aluminum chloride from Methanol Dae-Jung (South Korea).

**Plant extraction:** Dried aerial parts of the leaves were ground to a fine powder with a porcelain crucible, and 2 g of tissue was then mixed with 4 ml of distilled water and 16 ml methanol (80% v/v). Then, the samples were incubated in the shaker for 24 h at room temperature. The content of each sample was then filtered through a filter paper and immediately centrifuged at 3100 rpm for 4 min and the supernatant was transferred into 2

ml test tubes and stored at  $-20\text{ }^{\circ}\text{C}$  before the phytochemical assays (Rohloff *et al.* 2015).

***Determination of total phenolic content:***

Total phenolic content (TPC) was quantified using colorimetric Folin-Ciocalteu's reagent as described by Barreca *et al.* (2015) with some modifications. At first, 20  $\mu\text{l}$  of the extract was added to 1.6 ml of distilled water and mixed with 100  $\mu\text{l}$  of Folin-Ciocalteu reagent. Then, after 8 min, 300  $\mu\text{l}$  of sodium carbonate solution (20% w/v) was added and incubated in a water bath ( $60\text{ }^{\circ}\text{C}$ ) for 30 min in the dark, and absorbance was determined at 760 nm by using a spectrophotometer (Agilent Technologies Cary 60 UV-Vis, Santa Clara, California, USA). The total phenolic content was calculated concerning the standard curve of gallic acid and the results were expressed as mg gallic acid equivalent per g of dry weight (mg GAE  $\text{g}^{-1}$  DW). The concentration range of the standard was from 1 to 50  $\mu\text{g}\times\text{ml}^{-1}$ . The determinations were performed in triplicate ( $n = 3$ ).

***Determination of total flavonoid content:***

Total flavonoid content was determined using the aluminum chloride colorimetric assay (Barreca *et al.* 2015). Briefly, an aliquot (200  $\mu\text{l}$ ) of extracts was mixed with 200  $\mu\text{l}$  of 10% aluminum chloride and 2 ml of sodium

acetate ( $25\text{ mg ml}^{-1}$ ). Following 2.5 h incubation, absorbance was read at 440 nm, using a spectrophotometer (Agilent Technologies Cary 60 UV-Vis, Santa Clara, California, USA). The total flavonoid content was estimated by using the standard curve of quercetin and the results were expressed as mg quercetin equivalent per g dry weight (mg QE  $\text{g}^{-1}$  DW). The concentration range of the standard was from 1 to 50  $\mu\text{g}\times\text{ml}^{-1}$ . All determinations were carried out in triplicate ( $n = 3$ ).

***Antioxidant activity (DPPH assay):***

Antioxidant activity was determined based on DPPH radical scavenging activity following the modified method of Lalegani *et al.* (2018). The primary extract was diluted in 80% (v/v) methanol, and then, 100  $\mu\text{l}$  of the extract was added to 900  $\mu\text{l}$  of the 0.1 mM DPPH solution in methanol. After, the reaction mixture was incubated in the dark for half an hour at room temperature. Following the incubation, the absorbance was read at 516 nm by using a spectrophotometer (Agilent Technologies Cary 60 UV-Vis, Santa Clara, California, USA). The inhibition of DPPH scavenging activity was calculated using the standard Trolox curve equation. All assays were performed in triplicates ( $n = 3$ ).

### **Statistical analysis**

The statistical analyses of all data, including the analysis of variance and the normality tests (Shapiro-Wilk) were done via SPSS 22 (SPSS Inc, Chicago, IL, USA). The data were analyzed based on the completely randomized design (CRD) and the least significant differences (LSD) test was used for the mean comparisons among the species. Bivariate correlation coefficients between phytochemical characteristics and climate data were calculated by SPSS (Ver. 22) software. The morphological and phytochemical traits of samples were used to group the different *Epilobium* species by Ward's method of hierarchical clustering with the Euclidean distance as the measure of dissimilarity, using Minitab software (Ver. 17).

## **Results**

### **Morphological traits**

The results showed significant differences ( $p \leq 0.01$ ) among the studied morphological traits of *Epilobium* species. The mean comparisons of those are presented in Table 2. The longest (71.33 mm) and the shortest (52.33) capsules were recorded for *E. confusum*, and *E. hirsutum* species, respectively. The inflorescence length varied from 30 mm (*E. anatolicum*) to 72 mm (*E. algidum*). The longest leaf was recorded for *E. hirsutum* (77.33 mm) and the widest leaf

for *E. frigidum* (22.33 mm). The lowest values of those traits were identified in *E. lanceolatum* (26.66 mm) and *E. confusum* (8.33 mm), respectively.

Hierarchical cluster analysis based on morphological traits resulted in three groups (Figure 1). The first group comprised four species (*E. hirsutum*, *E. parviflorum*, *E. roseum*, and *E. frigidum*). The species of this group had higher plant height, seed length and width, capsule length, and leaf length. *E. algidum* was separately located in a second cluster group. The third group was formed by three species (*E. anatolicum*, *E. confusum*, and *E. lanceolatum*) represented by lower values in the majority of morphological traits. Morphological traits showed no significant correlations with either phytochemical characteristics or climatic variables.

### **Phytochemical analysis**

Analysis of variance indicated significant differences ( $p \leq 0.01$ ) among the *Epilobium* species regarding the three-phytochemical data, supported by the LSD test. Mean values and the ranges of variability are presented in Table 3. Total phenolic content varied from 19.46 mg g<sup>-1</sup> DW (*E. roseum*) to 30.91 mg g<sup>-1</sup> DW (*E. parviflorum*; 1.6-fold increase), antioxidant activity from 43.03 (*E. roseum*) to 101.36 mg g<sup>-1</sup> DW (*E. frigidum*; 2.3-fold increase), and total flavonoid content from 39.03 mg g<sup>-1</sup> DW (*E. algidum*) to 58.76 mg g<sup>-1</sup>

<sup>1</sup> DW (*E. lanceolatum*; 1.5-fold increase). Moreover, cluster analysis (Figure 2) separated the eight *Epilobium* species into three groups, containing cluster 1 (*E. hirsutum*, *E. algidum*, and *E. roseum*), cluster 2 (*E. parviflorum*), and cluster 3 (*E.*

*anatolicum*, *E. confusum*, *E. lanceolatum*, and *E. frigidum*). The first group had high total flavonoid content and antioxidant activity, and the second group had the highest total phenolic content.

Table 2. Means ( $\pm$  SE) of different morphological traits for eight *Epilobium* spp.

Species name	Seed width (mm)	Seed length (mm)	Capsule length (mm)	Inflorescence length (mm)	Leaf width (mm)	Leaf length (mm)	Plant height (mm)
<i>E. hirsutum</i>	0.550 $\pm$ 0.010	1.154 $\pm$ 0.001	71.33 $\pm$ 1.76	36.66 $\pm$ 0.88	20.33 $\pm$ 0.33	77.33 $\pm$ 2.66	607.67 $\pm$ 3.93
<i>E. parviflorum</i>	0.545 $\pm$ 0.010	1.190 $\pm$ 0.010	69.33 $\pm$ 0.67	38.33 $\pm$ 1.67	15.66 $\pm$ 0.88	58.33 $\pm$ 1.67	629.00 $\pm$ 4.58
<i>E. roseum</i>	0.567 $\pm$ 0.010	1.250 $\pm$ 0.018	67.00 $\pm$ 1.53	40.00 $\pm$ 2.89	21.00 $\pm$ 1.00	62.00 $\pm$ 1.53	430.00 $\pm$ 5.77
<i>E. algidum</i>	0.413 $\pm$ 0.010	1.044 $\pm$ 0.011	58.00 $\pm$ 1.00	72.33 $\pm$ 1.44	16.00 $\pm$ 1.00	47.00 $\pm$ 0.57	395.67 $\pm$ 7.88
<i>E. anatolicum</i>	0.462 $\pm$ 0.009	1.103 $\pm$ 0.005	63.33 $\pm$ 1.67	30.00 $\pm$ 2.89	15.33 $\pm$ 0.88	55.00 $\pm$ 2.89	353.33 $\pm$ 8.82
<i>E. confusum</i>	0.521 $\pm$ 0.002	1.310 $\pm$ 0.032	52.33 $\pm$ 1.45	30.67 $\pm$ 1.20	8.33 $\pm$ 0.67	32.33 $\pm$ 1.20	412.30 $\pm$ 14.00
<i>E. frigidum</i>	0.530 $\pm$ 0.009	1.430 $\pm$ 0.011	68.33 $\pm$ 1.76	35.00 $\pm$ 2.88	22.33 $\pm$ 0.67	44.00 $\pm$ 2.08	360.00 $\pm$ 11.50
<i>E. lanceolatum</i>	0.419 $\pm$ 0.020	1.265 $\pm$ 0.006	54.67 $\pm$ 0.88	41.33 $\pm$ 0.88	11.66 $\pm$ 0.33	26.66 $\pm$ 0.88	370.00 $\pm$ 5.77
LSD <sub>1%</sub>	0.06	0.06	5.72	8.37	3.15	7.65	34.92

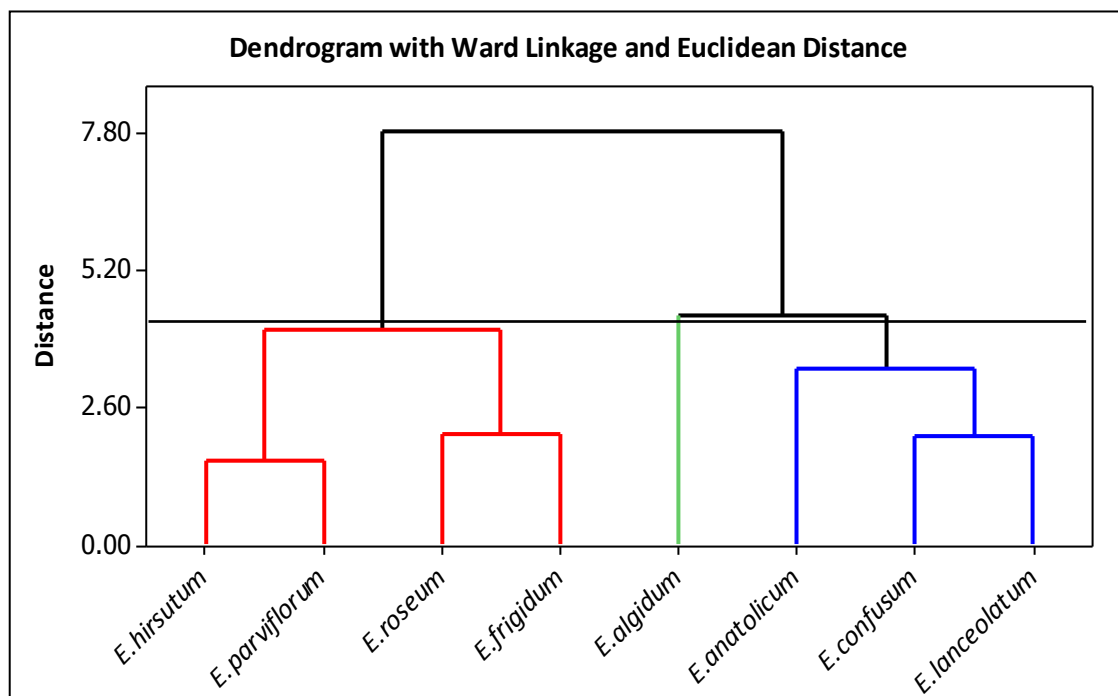
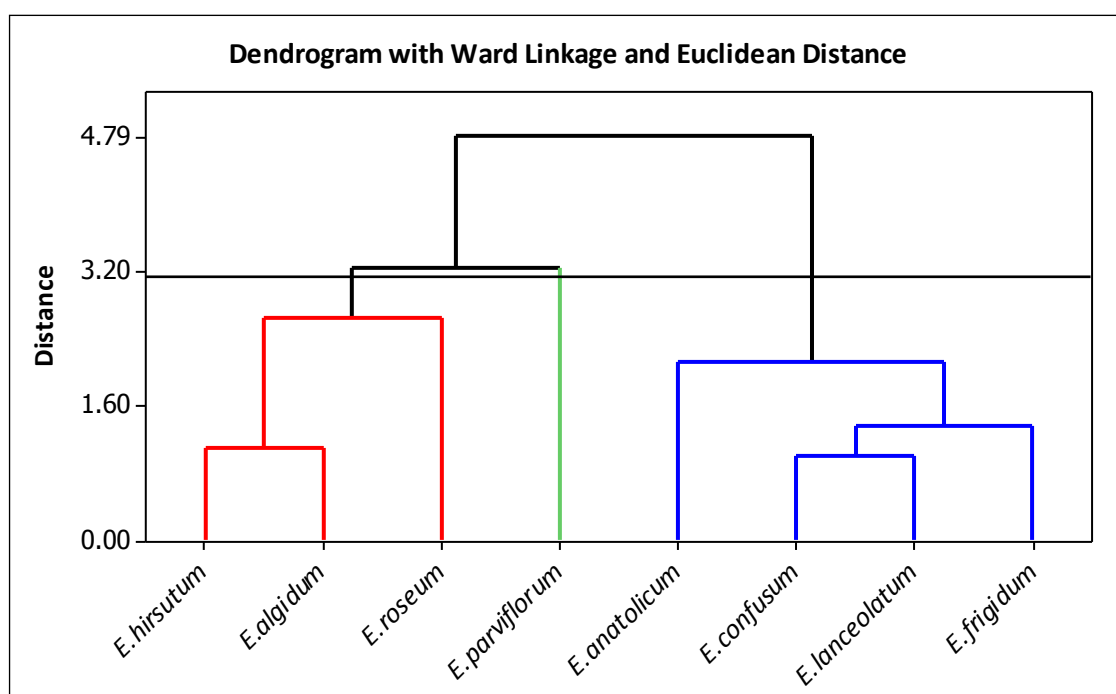


Figure 1. Ward cluster analysis for the studied *Epilobium* species based on seven morphological characteristics.

Table 3. Means ( $\pm$  SE) of total phenolic content (TPC), antioxidant activity (AOX), and total flavonoid content (TFC) of eight *Epilobium* spp.

Species name	TPC (mg g <sup>-1</sup> DW)	AOX (mg g <sup>-1</sup> DW)	TFC (mg g <sup>-1</sup> DW)
<i>E. hirsutum</i>	23.43 $\pm$ 0.90	80.95 $\pm$ 1.90	45.92 $\pm$ 0.62
<i>E. parviflorum</i>	30.91 $\pm$ 0.56	64.46 $\pm$ 0.98	46.47 $\pm$ 0.25
<i>E. roseum</i>	19.46 $\pm$ 0.11	43.03 $\pm$ 2.20	44.59 $\pm$ 1.28
<i>E. algidum</i>	22.42 $\pm$ 0.28	75.02 $\pm$ 1.20	39.03 $\pm$ 0.21
<i>E. anatolicum</i>	21.09 $\pm$ 0.38	72.72 $\pm$ 2.21	54.68 $\pm$ 1.77
<i>E. confusum</i>	26.05 $\pm$ 0.23	78.97 $\pm$ 1.04	56.14 $\pm$ 0.67
<i>E. frigidum</i>	24.39 $\pm$ 0.56	101.36 $\pm$ 3.68	53.09 $\pm$ 0.68
<i>E. lanceolatum</i>	26.86 $\pm$ 0.36	95.09 $\pm$ 3.00	58.76 $\pm$ 1.40
LSD <sub>1%</sub>	5.11	9.15	3.03

DW: Dry weight

Figure 2. Ward cluster analysis for the studied *Epilobium* species based on total phenolic content, total flavonoid content, and antioxidant activity.

All bioclimatic variables were significantly and negatively correlated with antioxidant activity, except for the mean temperature of the coldest quarter, which had a significant positive correlation with the antioxidant activity. The total phenolic

content was only negatively and significantly correlated with the minimum temperature of the coldest month among the bioclimatic variables. Total flavonoid content was not significantly correlated with any of the bioclimatic variables (Table 4).

Table 4. Pearson's correlation coefficient of phytochemical traits with bioclimatic factors of the studied *Epilobium* spp.

Trait	Bio1	Bio2	Bio3	Bio4	Bio5	Bio6	Bio7	Bio8
TFC	-0.22	-0.15	-0.29	-0.28	-0.23	-0.22	0.08	-0.07
AOX	-0.83**	-0.81*	-0.76*	-0.78*	-0.84**	-0.79*	0.72*	-0.74*
TPC	-0.60	-0.54	-0.73*	-0.35	-0.54	-0.67	0.44	-0.69

Bio1 = Annual mean temperature, Bio2 = Max temperature of warmest month, Bio3 = Min temperature of coldest month, Bio4 = Mean temperature of wettest quarter, Bio5 = Mean temperature of driest quarter, Bio6 = Mean temperature of warmest quarter, Bio7 = Mean temperature of coldest quarter, Bio8 = Precipitation seasonality. \*, \*\*Significant at 0.05 and 0.01 probability levels, respectively. TFC: Total flavonoid content, AOX: Antioxidant activity, TPC: Total phenolic content.

## Discussion

Some studies have reported that there is inter-specific hybridization and morphological overlap in the sec. *Epilobium* (Feliner 1994; Walter *et al.* 2007; Rahimi *et al.* 2022). In the current study, eight species of willow herb collected from different natural habitats of Iran were evaluated for morphological characterization and phytochemical variation. Substantial differences were found among the species of *Epilobium* for various morphological traits. Morphological, anatomical, and cytological diversity were reported among different species of *Epilobium* (Sheidai *et al.* 2018; Abbasi Karin *et al.* 2021; Rahimi *et al.* 2022). Among morphological characteristics, seed length, seed width, and capsule size are important features as physical indicators in the classification of flowering plants and their adaptation to different ecological niches (Skvortsov and Rusanovitch 1974; Aydın 2020). Seed morphological traits, including seed length and width, could be important in determining the seed size and shape (Wyllie-Echeverria *et al.* 2003; Diantina *et al.* 2020) which can influence water imbibition, seed

moisture content, and consequently germination rate, seed dormancy, and seed quality (Balkaya and Odabas 2002; Cerdà and García-Fayos 2002; Mandal *et al.* 2010). It was shown that larger seeds of common bean (*Phaseolus vulgaris* L.) had higher germination percentages and greater germination speed as compared to small seeds. Furthermore, plant size is the most important character in medicinal plant breeding programs (Falster and Westoby 2003).

Several phytochemical surveys have been performed on *Epilobium* extracts due to their importance in folk medicine and their rich biologically active compounds (Ducrey *et al.* 1995; Hiermann 1995; Hevesi Tóth *et al.* 2009; Kiss *et al.* 2011; Granica *et al.* 2014; Monschein *et al.* 2015; Mohammadi Bazargani 2019). In the present study, spectrophotometric analysis of total phenolic content and total flavonoid content as well as antioxidant activity (DPPH assay) in *Epilobium* also showed remarkable interspecific variation among the eight species studied.

Evaluation of the antioxidant potential in



several medicinal plants has been carried out by spectrophotometric assays such as ABTS, FRAP, and DPPH (Hevesi Tóth *et al.* 2009; Deng *et al.* 2018). According to previous studies, it was verified that *Epilobium* extracts, possess a high antioxidant capacity and are also good sources of natural antioxidants (Wojdyło *et al.* 2007; Sheikh *et al.* 2017). Hevesi Tóth (2009) observed no significant difference in total flavonoid content among five *Epilobium* species. Hevesi Tóth (2009) reported that among five *Epilobium* species growing in Hungary (*E. parviflorum* Schreb, *E. roseum* Schreb, *E. tetragonum*, *E. montanum*, and *E. angustifolium*), *E. parviflorum* showed the highest level of total phenolic content (TPC; 34.8 mg g<sup>-1</sup> DW) compared to other species, that was agreed with our results for this compound in *E. parviflorum* (309.10 mg g<sup>-1</sup> DW). However, the amount of total phenolic content in the present experiment was nine times higher than that reported by Hevesi Tóth (2009).

Variation in morphological and phytochemical traits of medicinal plant species can be attributed to the effect of various geographical conditions and also, developmental and genetic factors (Blumthaler *et al.* 1997; Heywood 2002; Labra *et al.* 2004). In the current study, the bioclimatic variables such as temperature exhibited a profound effect on the level of

antioxidant activity. Mohammadi Bazargani *et al.* (2021) reported that secondary metabolites of *E. hirsutum* were significantly correlated with altitude and found extensive inter- and intra-specific variability. Several experimental data in the literature support that the total phenolic content contributes to the antioxidant activity of some plants (Gheldof *et al.* 2003; Jaakola *et al.* 2004; Spitaler *et al.* 2006; Sultana *et al.* 2007). The antioxidant activity assessed by DPPH assay did not correlate with the total phenolic content in our study, contradicting that these compounds contribute to antioxidant activity. In the present study, a slight disagreement between dendrograms from the morphological variables and the phytochemical traits could be attributed to the different geographical characteristics of the studied species. Similar results were obtained by Moghaddam and Ghasemi Pirbalouti (2017) who reported a significant difference between morphological traits and phytochemical variables in 20 accessions of *Cuminum cyminum* L. The current study intended to provide suitable information for breeders to develop superior genotypes with desired traits to be used in future breeding programs.

## Conclusion

This study provided new information regarding the phytochemicals and morphological characteristics of interspecific

variability among *Epilobium* species of Iran. The species were found to be significantly diverse phenotypically and phytochemically in terms of plant height, length and width of fully grown leaves, length and width of seeds, capsule length, inflorescence length, total phenolic content, total flavonoid content, and antioxidant activity. This diversity can be useful in the breeding programs of the *Epilobium* genus. Also, the negative correlation of antioxidant activity with the majority of the studied climatic factors may help breeders in the selection of suitable genotypes.

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## Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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## تنوع بین گونه‌ای مورفولوژیکی و فیتوشیمیایی در گیاه دارویی بید علفی (*Epilobium spp.*)

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### چکیده

بید علفی (*Epilobium spp.*) یک گیاه دارویی است که برای اهداف متنوع در طب سنتی و صنایع غذایی استفاده می‌شود. در این تحقیق تلاش شده است که تنوع مورفولوژیکی و فیتوشیمیایی در هشت گونه گیاه اپیلوبیوم بررسی شود. گونه‌های گیاهی از رویشگاه طبیعی در طول دروره گلدهی جمع آوری شدند. تجزیه واریانس تنوع مورفولوژیکی معنی‌داری را میان گونه‌های مورد مطالعه برای صفات مورفولوژیکی و فیتوشیمیایی نشان داد. فنل کل از  $194.64 \pm 1.17$  تا  $309.1 \pm 5.59$   $\text{mg g}^{-1}$  DW، فلاونوئید کل از  $39.03 \pm 0.21$  تا  $56.14 \pm 0.67$   $\text{mg g}^{-1}$  DW و فعالیت آنتی‌اکسیدانی از  $2.20 \pm 43.03$  تا  $101.36 \pm 3.68$   $\text{mg g}^{-1}$  DW نوسان داشت. از یافته‌های ما استنتاج شد که *E. frigidum* دارای غنی‌ترین منبع آنتی‌اکسیدانی در بین گونه‌های مورد مطالعه و همچنین بیشترین طول بذر و طول برگ است. فعالیت آنتی‌اکسیدانی با داده‌های اقلیمی ارتباط معنی‌دار داشت. دندروگرام بر اساس صفات مورفولوژیکی با دندروگرام بر اساس متغیرهای فیتوشیمیایی نسبتاً متفاوت بود به طور کلی، تنوع زیاد بین گونه‌ها برای صفات مورفولوژیکی و فیتوشیمیایی می‌تواند در برنامه‌های اصلاحی جنس *Epilobium* مورد بهره‌برداری قرار گیرد.

**واژه‌های کلیدی:** فعالیت آنتی‌اکسیدانی، اپیلوبیوم، صفات مورفولوژیک، فلاونوئید کل، فنل کل