Research Article

Organ-oriented phytochemical profiling and radical scavenging activity of *Alcea* spp. (Malvaceae) from Iran

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Abstract
Polyphenol content and antioxidant activity of flowers and herbage of three species of the genus *Alcea* were determined. Flowers showed high total phenolic content and yielded values ranging from 26.83 (*Alcea aucheri* var. *lobata*) to 82.59 (*A. aucheri* var. *aucheri*) mg GAE/g DW. Total anthocyanin content was highest (934.98 mg/ml) in the flowers of *A. auchrei* var. *aucheri* (A12), whereas the content was minimal (1.34 mg/ml) in the herbage of *Alcea koelzii* (A2). The highest total flavonoid content was recorded in *A. koelzii* (16.15 mg Q/g DW), while the lowest value was achieved in *A. aucheri* var. *lobata* (8.06 mg Q/g DW). The highest concentration of mucilage was recorded in the flowers from *Alcea arbelensis* (32%), whereas those of *A. aucheri* var. *lobata* yielded the lowest value (1.05%). Principal component analysis scatter plot, derived from all data for flowers in 13 *Alcea* specimens revealed a different quantitative phytochemical profile. The strongest radical scavenging activity, measured by DPPH method, was recorded in *A. aucheri* var. *aucheri* with an IC_{50} of 34.06 µg/ml.

Keywords *Alcea* · Antioxidant activity · Anthocyanin · Iran · Mucilage · Polyphenol

Abbreviations

F	Flower
H	Herbage
TPC	Total phenolic content
TFC	Total flavonoid content
TAC	Total anthocyanin content
TMC	Total mucilage content
PCA	Principal component analysis

1 Introduction

Since time immemorial, Iran has been reputed as the treasure-house of precious medicinal plants of the world on account of the vast diversity in climatic condition. Up to 8200 plant species are recognized throughout the country, of which 1900 are endemic [1]. The genus *Alcea*, widely scattered throughout different areas of the country, is a main component of this botanical richness. *Alcea* is a large genus recognized in the family Malvaceae, including about 70 species, mostly perennial, sometimes annual and biennial ones, indigenous to Asia and Europe [2]. The genus has undergone marked species radiation in Iran including 34 endemic species, and numerous taxa are naturally scattered across west and southwest Iran [3].

Given its striking appearance and geometry, *Alcea* could be easily distinguished from other genera; the flowers are either solitary or arranged in racemes or fascicles on an unbranched upright stem rising to over 2 m at a fast rate. This genus is also characterized by having long notched petals ranging in color from white and yellow to pink and purple. The leaves usually appear on long petioles and are often lobed or toothed, and adorn the whole parts of the herbage. From an industrial and medicinal point of view, *Alcea* is among the most important genera recognized in the family Malvaceae.

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During the past decade, traditional systems of medicine have become a topic of global importance. In many developing countries, a large proportion of the population relies heavily on traditional systems of medicine to meet primary health care needs for historical and cultural reasons. The genus has long been traditionally used around the world as e.g. an antitussive, sedative, antibacterial and anti-inflammatory agent for thousands of years. Besides being well appreciated as ornamentals, members of the genus have also found a wide range of applications as either functional foods or nutraceuticals. One of the main claims of the food, cosmetic and dying industries nowadays is for natural colorants to replace synthetic dyes, and anthocyanins are the principal candidates [4]; with high levels of anthocyanins, purple-colored flowers of the genus *Alcea* have been utilized as a healthy food commodity and source of natural food colorants in Asia.

Today we clearly know that the biosynthesis of secondary metabolites in medicinally active plants is tightly regulated and is not by no means a random process. The expression of the genes involved in the biosynthesis of secondary metabolites shows tissue- and organ-specific distribution patterns, and hence the capacity for biosynthesis and accumulation can vary greatly within different tissues or organs of a plant [5, 6]. *Alcea* is a botanical source for various pharmaceutically active components. Even though different parts of the plant including flowers and herbage contain a variety of active substances e.g. starch, mucilage, pectin, sucrose, phenolics etc. [7], little is known about the distribution of these phytochemicals in different plant organs. This paper presents data on the qualitative and quantitative distribution of phenolic and mucilaginous compounds in the flowers and herbage of *Alcea* plants.

Moreover, few ethnobotanically important taxa have been scientifically evaluated for their possible medical application. Safety and efficacy data are available for even fewer plants, active ingredients and their extracts, and the preparations containing them. Furthermore, in most countries the herbal medicines market is poorly regulated, and herbal products are often neither registered nor controlled. Despite the potential importance of the *Alcea*-derived chemicals in several industries, little attention has been devoted to exploring this genus worldwide. Likewise, the industrial importance of the genus has always been regretfully neglected in Iran, and curative application of this national wealth has only been considered from the ethnobotanical point of view among indigenous people of the country. Assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries. Therefore, modern assessments of the genus in Iran e.g. characterization of its active substances and biological activities, is still missing. To fulfill this information gap, here we reported the phytochemical properties and antioxidant activities of three species of the genus *Alcea* growing in Iran aimed at taking the first steps towards introducing the genus for further future investigations.

2 Materials and methods

2.1 Sample collection

The distribution range of three species of the genus i.e. *Alcea koelzii* Riedl, *Alcea arbelensis* Boiss. and Hausskn. and *Alcea aucheri* (Boiss.) Alef. (var. *aucheri* and var. *lobata*) in Iran was determined according to Flora Iranica and other reliable sources. The taxa were collected at the flowering stage from their natural habitats in the west and southwest Iran over the growing season in spring and summer 2016 (Table 1). Herbarium specimens (*Alcea koelzii*, 2420; *A.*

Table 1 Geographical distribution of three *Alcea* populations throughout southwest Iran

Species	Identifier	Locality	Latitude	Longitude	Altitude (m)	Flower color
A. koelzii	A1	Cheshme Rostam	32° 50′	50° 32′	1854	White
	A2	Naghan	31° 55′	50° 43′	1995	Violet
	A3	Alikooh	32° 70′	50° 30′	1896	White
	A4	Karimabad	32° 50′	50° 33′	1876	White
A. arbelensis	A5	Kharaji	32° 50′	50° 49′	2014	White
	A6	Hosainabad	32° 60′	50° 31′	1864	White
A. aucheri var. *lobata*	A7	Farokhshahr	32° 16′	50° 58′	2112	Pink
	A8	Faradonbeh	32° 00′	51° 12′	2174	Pink
	A9	Kaaj	32° 30′	50° 34′	1693	Violet
	A10	Teshniz	32° 40′	50° 47′	1998	White
	A11	Broujen	31° 58′	51° 17′	2241	Violet
A. aucheri var. *aucheri*	A12	Shiasi	31° 68′	50° 61′	2017	Violet
	A13	Ardal	31° 59′	50° 38′	1804	Violet

arbelensis, 1833; *A. aucheri* var. *aucheri*, 3513; *A. aucheri* var. *lobata*, 1949) are kept at Agricultural and Natural Research Center of Chaharmahal and Bakhtiari (C and B) province. Taxonomical identification was made by an expert botanist (Asghar Shahrokhi, Education Organization, C and B Province, Shahrekord, Iran); samples were verified based on some material available at Agricultural and Natural Research Center of C and B province.

2.2 Preparation of the ethanolic extracts

Samples were shade-dried at room temperature, and different plant organs, including petals and herbage (leaves and stems), were powdered to homogenous particle size. Plant materials were macerated in 70% ethanol at room temperature and were regularly shaken for 12 h. After filtration, the crude extract was evaporated under vacuum to dryness and kept for 24 h at 4 °C until assayed.

2.3 Phytochemical characterization

Total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), total mucilage content (TMC) were measured. The TPC of extracts were determined according to the method published by Liang et al. [8] and results were expressed as mg gallic acid equivalents per g dry weight of the sample (mg/g GAE). TFC, expressed in mg quercetin per g dry weight of plant material (mg Q/g DW) was determined according to Nazif [9]. The methods referenced by Lee et al. [10] were used to measure the TMC and TAC, respectively.

2.4 Free radical scavenging activity

The antioxidant activity of the ethanol extracts was measured by the DPPH free radical scavenging assay method as previously described [11]. In the DPPH assay, the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to find the antioxidant activity of flower extracts. In a modified assay, 2 ml of 0.1 mM solution of DPPH radical in methanol was mixed with 2 ml of extract. After mixing, it was left for 15 min at room temperature. The DPPH radical inhibition was measured at 517 nm by using a spectrophotometer (Unico 2100). The antioxidant activity was calculated using the following equation:

$$100 - \left[\frac{(A)sample - (A)blank}{(A)control} \times 100\right]$$

where A stands for the absorbance of the color formed in the spectrophotometer cell, using DPPH as control (no extract), and blank consisted of only methanol. The IC_{50} (µg/ml) of each sample (concentration in µg/ml required to inhibit DPPH radical formation by 50%) was calculated. This was obtained by interpolation and using linear regression analysis.

2.5 Statistical analysis

The data were statistically analyzed using nested ANOVA with organ, population and species effects (SPSS 16.0, SPSS Inc., USA) computer software. In order to understand the relationships among different *Alcea* samples and to determine the main constituents influencing the chemical variability, a covariance data matrix composed of 13 samples and 4 variables was prepared and subjected to Principal Component Analysis using Minitab 16.0 (Minitab Inc., USA).

3 Results and discussion

3.1 Phenolic composition

It has been widely proposed to prepare herbal medicines as mixtures (extracts) rather than isolates, as their activity is potentiated when delivered in mixtures [12]. Phenolic compounds are a vital part of the human diet and are of considerable interest due to their biological and physiological activities (e.g. antioxidant, anticarcinogenic, antimutagenic and anti-inflammatory effects) [13]. They can basically be categorized into several classes, of which flavonoids are bioactive substances occurring widely in food and industrial plants. Anthocyanins are among the most prevalent flavonoids and occur in a vast variety of plants [14].

3.1.1 Total phenolic content

TPC of *Alcea* extracts are shown in Tables 2, 3 and 4. TPC was quantified within and between species in both flowers and herbage. In general, findings showed that TPC in the flowers was higher than that of the herbage of the taxa examined (Table 2). The only exception was the values measured in A10 from *A. aucheri* var. *aucheri*, in which a reverse pattern was observed (Table 4). The highest TPC in flowers was found in A. *aucheri* var. *aucheri*, followed by *A. koelzii*, *A. arbelensis* and *A. aucheri* var. *lobata* (Table 3).

Regarding TPC in the floral organs, with the exception of the specimen A7 from A. *aucheri* var. *lobata* (27.26 mg GAE/g DW), in which TPC was markedly lower than other specimens, no significant difference was observed within the taxa in each species. However, this parameter varied significantly in the herbage of the taxa within each species. TPC in A1 (53.51) and A2 (52.69) from *A. koelzii*, A5 (53.69) from *A. arbelensis* and A10 (58.15) from *A. aucheri*

Table 2 Phytochemical components and antioxidant activity according to organ

Organ	Antioxidant activity (µg/ml)	TPC (mg GAE/g DW)	TFC (mg Q/g DW)	TAC (mg/ml)	TMC (%)
Flower	109.99[b]	55.97[a]	12.05[a]	168.49[a]	0.353[a]
Herbage	168.58[a]	35.44[b]	9.134[b]	27.98[b]	0.121[b]

TPC total phenolic content, *TFC* total flavonoids content, *TAC* total anthocyanins content, *TMC* total mucilage content

Means with similar letters are not significantly different at Duncan's grouping at ($P < 0.05$)

Table 3 Phytochemical components and antioxidant activity of flower and herbage according to species

Taxa	AA (µg/ml)	TPC (mg GAE/g DW)	TFC (mg Q/g DW)	TAC (mg/ml)	Mucilage (%)
Flower					
A. koelzii	157.29[a]	56.62[b]	14.10[b]	11.64[c]	17.20[b]
A. arbelensis	147.44[b]	54.55[c]	12.05[c]	18.32[c]	24.30[a]
A. aucheri var. *lobata*	85.78[c]	50.32[d]	9.66[d]	211.17[b]	15.90[c]
A. aucheri var. *aucheri*	46.49[d]	69.73[a]	15.92[a]	598.20[a]	13.43[d]
Herbage					
A. koelzii	113.50[c]	40.55[b]	8.03[b]	20.80[b]	7.70[a]
A. arbelensis	86.37[d]	41.04[a]	15.85[a]	29.96[a]	5.85[b]
A. aucheri var. *lobata*	263.47[a]	33.73[c]	7.97[c]	29.57[a]	4.82[c]
A. aucheri var. *aucheri*	188.73[b]	28.12[d]	8.03[b]	30.70[a]	3.62[d]

AA antioxidant activity, *TPC* total phenolic content, *TFC* total flavonoids content, *TAC* total anthocyanins content, *TMC* total mucilage content

Means with similar letters are not significantly different at Duncan's grouping at ($P < 0.05$)

var. *lobata*was significantly higher, respectively, than that of other taxa within the species.

Moreover, the variation in TPC between four *Alcea* taxa was quantitatively significant. Generally, flowers showed high TPC, and yielded values ranging from 27.26 (*A. aucheri* var. *lobata*) to 82.59 (*A. aucheri* var. *aucheri*) mg GAE/g, showing a difference in TPC up to three-fold. The highest TPC levels were measured in A13 (*A. aucheri* var. *aucheri*) (82.59 mg GAE/g DW), and the lowest in A7 (*A. aucheri* var. *lobata*) (27.26 mg GAE/g DW). The highest (58.15 mg GAE/g DW) and lowest (26.83 mg GAE/g DW) values of TPC were recorded in the herbage of A10 and A7 samples, both belonging to *A. aucheri* var. *lobata* (Table 4).

3.1.2 Total flavonoid content

TFC for *Alcea* extracts is shown in Tables 3 and 4. TFC was mostly higher in the crude extracts obtained from the flowers (Table 2), ranging from 8.06 to 16.15 mg Q/g DW. TFC was higher in A1, A2, A3 (16.15, 16.12, 16.06 mg Q/g DW, respectively) all of which belonging to *A. koelzii*, while the lower values were achieved in A9 from *A. aucheri* var. *lobata* (8.06 mg Q/g DW), A6 from *A. arbelensis* (8.07 mg Q/g DW) and A4 from *A. koelzii* (8.08 mg Q/g DW). The highest and the lowest TFC in the herbage was detected in A5 from *A. arbelensis* (15.90 mg Q/g DW), and A4 from *A. koelzii* and A11 *A. aucheri* var. *lobata* (7.89 mg Q/g DW), respectively (Tables 3, 4).

The flavonoids are probably the most important single group of phenolic compounds in plants, comprising a group of thousands of aromatic compounds, including anthocyanins, flavonols, flavones, flavanones, flavan-3-ols, and isoflavones [12]. Epidemiological data associate some important biological properties e.g. antioxidant, anti-inflammatory, and antimicrobial activities, as well as cardioprotective and anticancer effects, to flavonoids. They are sensitive to degradation because of their hydroxyl and ketone groups and unsaturated double bonds [15].

3.1.3 Total anthocyanin content

Results showed that the content of anthocyanins markedly varied, as influenced by genetic structure of the examined taxa, environmental condition and the organ of choice [16–19]. As shown in Table 2, the flowers contained markedly higher TAC in comparison to the herbage of the examined taxa. The variation of total anthocyanin content (TAC) observed in the crude extracts of four *Alcea* species are summarized in Tables 3 and 4. TAC was highest (934.97, 871.83 and 261.42 mg/ml) in the flowers of A12

Table 4 Phytochemical components and antioxidant activity according to ecotype and organ for each species

Taxa	Population	Organ	AA (µg/ml)	TPC (mg GAE/g DW)	TFC (mg Q/g DW)	TAC (mg/ml)	TMC (%)
A. koelzii	A1	F	137.58^{g}	57.79^{b}	16.15^{a}	6.67^{jk}	14.60^{f}
		H	84.45^{j}	53.51^{g}	8.14^{g}	44.34^{efg}	6.30^{ijk}
	A2	F	236.09^{d}	56.43^{cd}	16.12^{a}	21.89^{ghijk}	15.60^{ef}
		H	151.58^{fg}	52.69^{h}	8.06^{i}	1.34^{k}	6.80^{hij}
	A3	F	143.40^{fg}	55.52^{ef}	16.06^{b}	8.53^{ijk}	18.20^{d}
		H	112.89^{h}	27.57^{jkl}	8.05^{i}	13.69^{ijk}	5.80^{jk}
	A4	F	112.10^{h}	56.78^{c}	8.08^{hi}	9.46^{ijk}	20.40^{c}
		H	105.12^{hi}	28.45^{i}	7.89^{l}	23.85^{ghijk}	11.90^{g}
A. arbelensis	A5	F	137.07^{g}	55.79^{de}	16.03^{bc}	8.07^{ijk}	16.60^{ed}
		H	109.79^{h}	53.69^{g}	15.90^{e}	11.50^{ijk}	6.40^{ijk}
	A6	F	157.81^{ef}	53.33^{gh}	8.07^{i}	28.57^{fghij}	32.00^{a}
		H	62.96^{k}	28.40^{i}	15.80^{f}	48.43^{ef}	5.30^{jkl}
A. aucheri var. *lobata*	A7	F	61.96^{k}	27.26^{l}	8.08^{hi}	89.80^{d}	11.60^{g}
		H	358.39^{a}	26.83^{l}	7.95^{k}	31.70^{fghi}	5.50^{jkl}
	A8	F	41.88^{lm}	56.82^{c}	8.11^{gh}	22.93^{ghijk}	11.80^{g}
		H	227.91^{d}	28.20^{ij}	7.92^{l}	40.79^{efgh}	3.60^{lm}
	A9	F	51.19^{kl}	56.97^{c}	8.06^{i}	871.84^{b}	27.30^{b}
		H	90.4^{1ij}	28.12^{ijk}	8.08^{hi}	38.22^{fgh}	6.05^{ijk}
	A10	F	168.04^{e}	54.88^{f}	8.13^{g}	10.44^{ijk}	12.80^{g}
		H	307.97^{c}	58.15^{b}	8.06^{i}	18.23^{hijk}	7.90^{hi}
	A11	F	105.86^{hi}	55.70^{de}	15.96^{d}	60.85^{e}	16.00^{ef}
		H	332.72^{b}	27.39^{kl}	7.88^{l}	18.92^{hijk}	1.05^{n}
A. aucheri var. *aucheri*	A12	F	34.06^{m}	56.88^{c}	16.02^{c}	934.98^{a}	8.60^{h}
		H	240.98^{d}	27.49^{jkl}	7.99^{j}	30.24f^{ghi}j	2.65^{mn}
	A13	F	58.94^{k}	82.59^{a}	15.83^{f}	261.43^{c}	18.26^{d}
		H	136.49^{g}	28.76^{i}	8.08^{hi}	31.17^{fghi}	4.60^{kl}

F flower, *H* herbage, *AA* antioxidant activity, *TPC* total phenolic content, *TFC* total flavonoid content, *TAC* total anthocyanin content, *TMC* total mucilage content

In each column, means with similar letters are not significantly different at Duncan's grouping at ($P < 0.05$)

(*A. auchrei* var. *aucheri*), A9 (*A. auchrei* var. *lobata*) and A13 (*A. auchrei* var. *aucheri*), respectively. The minimum levels were found in A1 (6.67 mg/ml), followed by A5 (8.07 mg/ml) and A3 (8.53 mg/ml).

TAC in the flowers of A12 was over 30-fold higher that of the herbage. TAC in the herbage of the taxa yielded values between 1.34 mg/ml in A2 (*A. koelzii*) and 48.43 mg/ml in A6 (*A. arbelensis*). Thus, flowers contained a higher level of anthocyanin than that of the herbage of the same sample. Exceptions include the white-colored flowers taxa A1, A3 (*A. koelzii*) and A5 (*A. arbelensis*).

Anthocyanins, an important class of flavonoids, are water-soluble vacuolar pigments, plentiful in plant foods, many fruits, and vegetables. Anthocyanin-rich plants have long been used in folk medicine throughout the world for the treatment of vision disorders, microbial infections, diarrhea, and many others. It is only in recent years, however, that epidemiological data verified some of the specific, measurable pharmacological properties of isolated anthocyanins.

3.2 Total mucilage content

Total mucilage content (TMC) was measured both in the flowers and herbage of the examined taxa. Results showed that this parameter was under the influence of the genetic structure [20], geographical condition [21] as well as the organ of choice [20]. TMC was predominantly higher in the flowers as compared to the herbage of the taxa (Table 2). TMC in the flowers ranged between 8.6 and 32%. The highest TMC in the flowers was recorded in A6 from *A. arbelensis* (32%) followed by A9 from *A.* var. *lobata* (27.3%), whereas the lowest values were obtained in A12 (8.6%) (*A. aucheri* var. *aucheri*), followed by A7 (11.6%) and A8 (11.8%) from *A. aucheri* var. *aucheri*, respectively. The maximum (11.9%) TMC in the herbage of the taxa was

found in A4 (*A. koelzii*), while A11 from *A. aucheri* var. *lobata* contained minimal content (Tables 3, 4).

Mucilages are large, highly branched polymeric structures built from many different kinds of sugar and uronic acid units [22] that contribute to several important ecological adaptations, particularly tolerance to water stress. Therapeutically, mucilage can reduce bowel irritation, toxin absorption, cough, bronchial and urinary spasm, and it is useful to increase expectoration.

3.3 Principal component analysis (PCA)

Principal component analysis (PCA) scatter plot, derived from TPC, TFC, TAC and TMC of flowers in 13 *Alcea* specimens was designed (Fig. 1). PCA gives a fairly well-resolved picture of the distribution pattern of the specimens in terms of the phytochemical properties. Two factors were extracted with PCA, accounting for 72.76% of the total variance. Results indicate that *Alcea* specimens are distributed across the plot in four main groups (A, B, C, D) based on their phytochemical properties. Three specimens A4 (*A. koelzii*), A6 (*A. arbelensis*) and A9 (*A. aucheri* var. *lobata*) grouped together based on TMC and formed group A. A13 (*A. aucheri* var. *aucheri*) was distinct in terms of TPC and alone took place within group B, while group C was formed by a set of 6 specimens (A1, A2, A3, A5, A11, A12), the representatives of all the taxa examined. As it can be seen in Fig. 1, the specimens representing *A. aucheri* var. *lobata*, (A7, A8, A10) were separated from other taxa by their lower TPC, TFC, TAC and TMC, and formed group D.

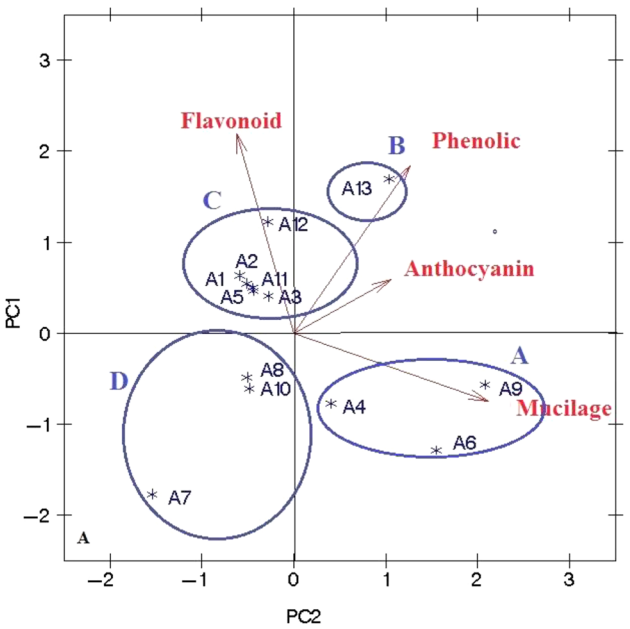

Fig. 1 PCA performed on TPC, TFC, TAC and TMC in the flowers of 13 *Alcea* specimens

They revealed a different quantitative phytochemical profile supporting their chemical distinctiveness from other taxa.

In a general view, specimens placed in four groups with no evident relationships to genotype and bioclimatic zone.

3.4 Free radical scavenging activity

Free radical scavenging activity (DPPH assay) was used to determine the antioxidant capacity of the extracts obtained from both flowers and herbage of the *Alcea* taxa and results were reported as IC_{50} values. DPPH radical is a stable organic free radical with an absorption band at 517 nm [23]. The addition of *Alcea* extracts to the DPPH solution induced a rapid decrease in the optical density at 517 nm. Results of DPPH radical scavenging activity of the examined taxa are summarized in Tables 3 and 4. The samples obtained from flowers gave stronger radical scavenging capacity than those obtained from herbage (Table 2). The extracts obtained from the flowers yielded DPPH radical scavenging activity with IC_{50} values ranging from 34.06 to 236.09 µg/ml. The strongest antioxidant activity of the flowers belonged to A12 from *A. aucheri* var. *aucheri* (34.06 µg/ml), followed by A8 (41.88 µg/ml) and A9 (51.19 µg/ml) from *A. aucheri* var. *lobata*. A2 from *A. koelzii* (236.09 µg/ml), A10 from *A. aucheri* var. *lobata* (168.04 µg/ml) and A6 from *A. arbelensis* (157.81 µg/ml) showed weaker activities. Scavenging activity of the herbage yielded values between 62.96 µg/ml (A6 from *A. arbelensis*) and 358.39 µg/ml (A7 from *A. aucheri* var. *lobata*).

Except for the ecotypes within *A. aucheri* var. *lobata* (A7–A11) and *A. aucheri* var. *aucheri* (A12 and A13), the extracts obtained from the flowers showed more or less a weaker scavenging activity compared to the herbage of the same plants. This comes to mean that anthocyanins play an important role. The correlation matrix (Table 5) revealed a positive correlation between the antioxidant activity of the species studied with TAC ($r = 0.387$, $\alpha = \leq 0.01$), TMC ($r = 0.321$, $\alpha = \leq 0.01$) and TPC ($r = 0.295$, $\alpha = \leq 0.01$). It may be concluded that anthocyanins are likely to play an especially more important role in the overall scavenging activity of the genus *Alcea*. It should be noted that antioxidant activity of the tested ecotypes also correlates strongly ($r = 0.418$, $\alpha = \leq 0.01$) with the attitude of the collection site.

Overall, our findings indicated that the extracts from several specimens of *Alcea* could vary in quantity and quality according to genotype, climate, and the plant part used [24]. It is widely accepted that the production of secondary metabolites in medicinal plants is a complex interplay of intrinsic and extrinsic (e.g. environmental factors) elements. This is particularly modulated at the DNA level,

Table 5 Correlation analysis between altitude and phytochemical properties of the genus *Alcea*

	TFC	TPC	Antioxidant	TAC	TMC	Altitude
TFC	1					
TPC	0.477**	1				
Antioxidant	0.260*	0.295**	1			
TAC	0.147 ns	0.265*	0.387**	1		
TMC	0.185 ns	0.592**	0.321**	0.261*	1	
Altitude	0.005 ns	0.152 ns	0.418**	0.225*	0.336**	1

ns insignificant

*Significant at level 5%; **significant at 1% level

where specific enzymes in the biosynthetic pathway of desired products are encoded [25]. Therefore, the genetic structure can create substantial variability in the distribution and composition of active substances in plants, which is to a large extent genotype-specific [26, 27]. For example, idioblastic mucilage cells typically occur within members of Malvaceae, Liliaceae, Cactaceae, and Orchidaceae [28]. Members of the genus *Alcea* are among those plants known to contain far greater concentrations of mucilage than is typically found in most plants. The accumulation of the mucilage in the genus *Alcea* constitutes an excellent strategy of the plants to adapt to harsh environmental conditions, particularly heat, drought and cold stresses [29–31].

On the other hand, metabolism of SMs is under the influence of extrinsic elements like environmental factors as well. Influenced by environmental elements, each class of secondary metabolites functions as a chemical interface between the environment and the plant. These phytochemicals exert their biological roles, as plastic adaptive drivers in response to the surrounding environment. Such chemical interactions often include variations in the production of plant metabolites, which could possibly be one of the main explanations behind plants metabolic diversity [32]. Therefore, the study of these variations is very useful in the chemical characterization of plants of the same species which are collected from different geographical areas, especially when they have genetic homogeneity [33]. In relation to our study, synthesis of phenolic derivatives (e.g. flavonoids)is reported to be upregulated when plants are overexposed to light [26]. It has also been reported that the color and stability of anthocyanin pigments are influenced by pH [27], light and temperature [34]; they may appear red, purple, or blue depending on the pH of their environment [14].

Moreover, today we clearly know that biosynthesis of secondary metabolites is not a random process, but rather is restricted to specific plant tissues or developmental stages [30]. Anthocyanins are found predominantly in flowers and fruits, but also in leaves, stems, and roots [31]. These are primarily recognized by their strong red to blue coloring [14]. Thus, it is not surprising that we recorded the maximum contents of anthocyanins in the flowers, particularly in those specimens with purple- and pink-colored petals. Interestingly, in the white-flowered taxa, the content of anthocyanins was higher in the herbage rather than floral organs (Tables 1, 4). Moreover, TPC, TFC, and TMC in all of the examined taxa were also abundant in the flowers compared with the herbage (Tables 2, 4).

A large number of medicinal plants contain chemicals with antioxidant activity, among which phenolics and their derivatives are receiving particular attention [29]. Flavonoids possess strong antioxidant activity which may be responsible for some vital biological activities e.g. age-related neurodegenerative disorders [12], prevention or lowering the risk of cancer, diabetes, arthritis and cardiovascular diseases [35]. The phenolic moiety in flavonoids can accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components [33].

Today we know that additive and synergistic effects of phytochemicals in plants are responsible for their potent antioxidant activity and that the benefit of a diet rich in fruit and vegetables is attributed to the complex mixture of phytochemicals present in the body of the plants, not a single compound [36]. It has been shown that the antioxidant capacity of the plant extracts largely depends on the composition of the extracts and conditions of the test system. Therefore, all the aforementioned factors (e.g. genotype, growth factors, laboratory conditions etc.) influencing the recovery of plant extracts would accordingly affect their antioxidant capacity [33, 37, 38]. Our findings suggest that antioxidant activity has a relatively strong correlation with the intensity of the plant color. A12, A8, A9 that showed the strongest activity also owned purple-, pink- and purple-colored flowers, respectively, while in the other taxa having white-colored flowers the antioxidant activity of the herbage was stronger than that of the flowers. Likewise, Sadighara et al. (2012) examined the antioxidant activity of the taxa belonging to *Althaea officinalis* L. and reported that the plants with more pigmentation have more biological effects in association with antioxidant

potentials [39]. However, the stronger free radical scavenging activity of the vegetative organs in white-colored flowers may indicate that other compounds different from phenolics may contribute to the antioxidant activity observed. Accordingly, the strong antioxidant activity of *A. officinalis* to a large extent (approximately 70%) is attributed to the reference compound α-tocopherol [40].

4 Conclusion

In conclusion, flowers and herbage of several taxa belonging to the genus *Alcea* were evaluated for their total phenolics, flavonoids, anthocyanins, mucilage content as well as antioxidant activity, and results showed that the content and biological activity of such phytochemicals were markedly varied, as influenced by genetic structure, environmental condition and the organ of choice. Our findings indicated that in the majority of the taxa the content of the phytochemicals in the flowers was to some extent higher than that of the herbage. We also reasoned that there is a significant relationship between the flower color and its anthocyanin content. Except for the taxa within *A. aucheri* var. *aucheri* and *A. aucheri* var. *lobata*, extracts obtained from flowers showed, more or less, weaker antioxidant activity compared with the herbage of the same plants. Our findings suggest that the antioxidant activity has a relatively strong correlation with the intensity of the plant color, as the strongest activity was observed in the purple- and pink-colored flowers, while in the white-colored taxa, the antioxidant activity of the herbage was stronger than that of the flowers.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kiani M, Mohammadi S, Babaei A, Sefidkon F, Naghavi MR, Ranjbar M, Razavi SA, Saeidi K, Jafari H, Asgari D (2017) Iran supports a great share of biodiversity and floristic endemism for *Fritillaria* spp. (Liliaceae): a review. Plant Divers 39:245–462. https://doi.org/10.1016/j.pld.2017.09.002
2. Uzunhisarcikli ME, Vural M (2012) The taxonomic revision of Alcea and Althaea (Malvaceae) in Turkey. Turk J Bot 36:603–636. https://doi.org/10.3906/bot-1108-11
3. Pakravan M (2008) A new species and a new combination in Iranian Alcea (Malvaceae). Ann Bot Fenn BioOne 45:133–137. https://doi.org/10.5735/085.045.0207
4. Rymbai H, Sharma R, Srivastav M (2011) Bio-colorants and its implications in health and food industry: a review. Int J PharmTech Res 3:2228–2244
5. Shaghaghi A, Alirezalu A, Nazarianpour E, Sonboli A, Nejad-Ebrahimi S (2019) Opioid alkaloids profiling and antioxidant capacity of Papaver species from Iran. Ind Crops Prod 142:111870. https://doi.org/10.1016/j.indcrop.2019.111870
6. Shameh S, Alirezalu A, Hosseini B, Maleki R (2019) Fruit phytochemical composition and color parameters of 21 accessions of five Rosa species grown in North West Iran. Agric Food Sci J 99:5740–5751. https://doi.org/10.1002/jsfa.9842
7. Shah SA, Akhtar N, Akram M, Shah PA, Saeed T, Ahmed K, Asif H (2011) Pharmacological activity of *Althaea officinalis* L. J Med Plants 5:5662–5666
8. Liang T, Yue W, Li Q (2010) Comparison of the phenolic content and antioxidant activities of *Apocynum venetum* L. (Luo-Bu-Ma) and two of its alternative species. Int J Mol Sci 11:4452–4464. https://doi.org/10.3390/ijms11114452
9. Nazif NM (2002) Phytoconstituents of *Zizyphus spina-christi* L. fruits and their antimicrobial activity. Food Chem 76:77–81. https://doi.org/10.1016/S0308-8146(01)00243-6
10. Lee J, Durst RW, Wrolstad RE (2005) Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J AOAC Int 88:1269–1278
11. Moein S, Farzami B, Khaghani S, Moein MR, Larijani BA (2007) Antioxidant properties and protective effect on cell cytotoxicity of *Salvia mirzayani*. Pharm Biol 45:458–463. https://doi.org/10.1080/13880200701389144
12. Lila MA (2004) Anthocyanins and human health: an in vitro investigative approach. Biomed Res Int 2004:306–313. https://doi.org/10.1155/S111072430440401X
13. Balasundram N, Sundram K, Samman S (2006) Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem 99:191–203. https://doi.org/10.1016/j.foodchem.2005.07.042
14. Holton TA, Cornish EC (1995) Genetics and biochemistry of anthocyanin biosynthesis. Plant Cell 7:1071–1083. https://doi.org/10.1105/tpc.7.7.1071
15. Qiao L, Sun Y, Chen R, Fu Y, Zhang W, Li X, Chen J, Shen Y, Ye X (2014) Sonochemical effects on 14 flavonoids common in citrus: relation to stability. PLoS ONE 9:e87766. https://doi.org/10.1371/journal.pone.0087766
16. Azevedo J, Fernandes I, Faria A, Oliveira J, Fernandes A, Freitas V, Mateus N (2010) Antioxidant properties of anthocyanidins, anthocyanidin-3-glucosides and respective portisins. Food Chem 119:518–523. https://doi.org/10.1016/j.foodchem.2009.06.050
17. Bystrická J, Vollmannová A, Margitanová E (2010) Dynamics of polyphenolics formation in different plant parts and different growth phases of selected buckwheat cultivars. Acta Agric Slov 95:225. https://doi.org/10.2478/v10014-010-0014-0
18. Laleh GH, Frydoonfar H, Heidary R et al (2006) The effect of light, temperature, pH and species on stability of anthocyanin pigments in four Berberis species. Pak J Nutr 5:90–92. https://doi.org/10.3923/pjn.2006.90.92
19. Vial P, Crisosto C, Crisosto G (2005) Early harvest delays berry skin browning of 'Princess' table grapes. Calif Agric 59:103–108. https://doi.org/10.3733/ca.v059n02p103
20. Pakravan M, Abedinzadeh H, Safeepur J (2007) Comparative studies of mucilage cells in different organs in some species of Malva, Althaea and Alcea. Pak J Biol Sci 10:2603–2605
21. Hohtola A (2007) Northern plant as a source of bioactive products. In: Taulavuori E, Tauravuori K (eds) Physiology of northern plants under changing environment. Research Signpost India, pp 291–307
22. Bone K, Simon Mills M, FNIMH M (2012) Principles and practice of phytotherapy: modern herbal medicine. Elsevier Health Sciences

23. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH (2014) Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food drug Anal 22:296–302. https://doi.org/10.1016/j.jfda.2013.11.001
24. Angioni A, Barra A, Coroneo V, Dessi S, Cabras P (2006) Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. stoechas essential oils from stem/leaves and flowers. J Agric Food Chem 54:4364–4370. https://doi.org/10.1021/jf0603329
25. Kurosaki F (2012) Induction and activation of plant secondary metabolism by external stimuli. In: Drug discovery research in pharmacognosy. IntechOpen
26. Pavarini DP, Pavarini SP, Niehues M, Lopes NP (2012) Exogenous influences on plant secondary metabolite levels. Anim Feed Sci Technol 176:5–16. https://doi.org/10.1016/j.anifeedsci.2012.07.002
27. Wrolstad RE, Durst RW, Lee J (2005) Tracking color and pigment changes in anthocyanin products. Trends Food Sci Technol 16:423–428. https://doi.org/10.1016/j.tifs.2005.03.019
28. Bosabalidis AM (2014) Idioblastic mucilage cells in *Teucrium polium* leaf. Anatomy and histochemistry. Mod Phytomorphol 5:49–52. https://doi.org/10.5281/zenodo.161003
29. Dorman HD, Koşar M, Kahlos K, Holm Y, Hiltunen R (2003) Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties, and cultivars. J Agric Food Chem 51:4563–4569. https://doi.org/10.1021/jf034108k
30. Ncube B, Finnie J, Van Staden J (2012) Quality from the field: the impact of environmental factors as quality determinants in medicinal plants. S Afr J Bot 82:11–20. https://doi.org/10.1016/j.sajb.2012.05.009
31. Tanaka Y, Sasaki N, Ohmiya A (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J 54:733–749. https://doi.org/10.1111/j.1365-313X.2008.03447.x
32. Kiani M, Sefidkon F, Babaei A, Naghavi MR (2015) Phytochemical profiling of medicinal isosteroidal alkaloids of Iranian Fritillaria spp. (Liliaceae). Ind Crops Prod 70:451–458. https://doi.org/10.1016/j.indcrop.2015.03.064
33. Shamloo M, Babawale EA, Furtado A, Henry RJ, Eck PK, Jones PJ (2017) Effects of genotype and temperature on accumulation of plant secondary metabolites in Canadian and Australian wheat grown under controlled environments. Sci Rep 7:9133. https://doi.org/10.1038/s41598-017-09681-5
34. Khoo HE, Azlan A, Tang ST, Lim SM (2017) Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res 61:1361779. https://doi.org/10.1080/16546628.2017.1361779
35. Einbond LS, Reynertson KA, Luo XD, Basile MJ, Kennelly EJ (2004) Anthocyanin antioxidants from edible fruits. Food Chem 84:23–28. https://doi.org/10.1016/S0308-8146(03)00162-6
36. Liu RH (2003) Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 78:517S–520S. https://doi.org/10.1093/ajcn/78.3.517S
37. Raut JS, Karuppayil SM (2014) A status review on the medicinal properties of essential oils. Ind Crops Prod 62:250–264. https://doi.org/10.1016/j.indcrop.2014.05.055
38. Saeidi K, Moosavi M, Lorigooini Z, Maggi F (2018) Chemical characterization of the essential oil compositions and antioxidant activity from Iranian populations of *Achillea wilhelmsii* K. Koch. Ind Crops Prod 112:274–280. https://doi.org/10.1016/j.indcrop.2017.12.007
39. Sadighara P, Gharibi S, Jafari AM, Khaniki GJ, Salari S (2012) The antioxidant and Flavonoids contents of *Althaea officinalis* L. flowers based on their color. Avicenna J Phytomed 2:113–117
40. Kardošová A, Machová E (2006) Antioxidant activity of medicinal plant polysaccharides. Fitoterapia 77:367–373. https://doi.org/10.1016/j.fitote.2006.05.001