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Research paper

Evaluation of morphological traits and chemical compounds of chicory (*Cichorium intybus*) populations in central Iran (Markazi province)

Golpar Rahmati and Mansour Ghorbanpour*

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Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, Arak, Iran
*Corresponding author; Email: m-ghorbanpour@araku.ac.ir

Abstract
Chicory has a wide range of uses in the pharmaceutical and food industries owing to its valuable bioactive compounds such as phenolic acids, terpenoids, vitamins, and inulin. The presence and content of these compounds are influenced by genetic and environmental factors, as well as the processing method. The present research aimed to evaluate the morphological and biochemical characteristics of wild chicory populations from the Markazi province, Iran. The essential oil was extracted through the distillation technique using Clevenger-type apparatus. GC and GC/MS were used to identify the compounds of the essential oil, and a spectrophotometer was used to determine the amount of inulin. The results showed high and significant diversity between populations for the measured traits. The maximum and minimum variations were observed for the plant height and branch weight, respectively. Significant correlations were observed between different traits. The yield and composition of essential oil in the studied populations showed considerable variation. The main components of the essential oil were chamazulene, 1,8-cineole, carvacrol, cuminic aldehyde, thymol, cinnamic aldehyde, camphor, borneol, linalool, and carvone. Cluster analysis separated the populations into two different groups based on morphological traits and chemical components of essential oils. In conclusion, the existence of considerable diversity in morphological traits and chemical compounds among the chicory populations can be useful in the breeding programs of this plant.

Keywords: chemical compounds; chicory; essential oil; genetic variation

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Introduction

Chicory (*Cichorium intybus* L.) is one of the most important medicinal plants of Asteraceae family (Khan *et al*. 2020). This species is widely distributed in different regions of Iran (Mozaffarian 2012). The chemical composition of chicory includes polyphenols, alkaloids, proteins, polyacetylene, lipids, anthocyanins, tannins, and types of terpenoids, especially sesquiterpene lactones, and the plant is a rich source of minerals such as potassium, calcium, and phosphorus (Nwafor *et al*. 2017; Zlatic and Stankovic 2017; Emam *et al*. 2019; Choudhary *et al*. 2021; Janda *et al*. 2021). Furthermore, it has a high potential for use in the food industry owing to the presence of useful carbohydrates such as starch, cellulose, hemicellulose, pectin, and inulin in the roots

(Puhlmann and de Vos 2020; Perovic *et al*. 2021). The compounds of the chicory have a wide range of biological and pharmacological effects, including antioxidant, anti-inflammatory, antibacterial, anti-diabetic, anti-malarial, anti-parasitic, anti-allergic, and pain-relieving effects, and are used in the treatment of cardiac-vascular, gastric ulcer, and liver diseases, as well as digestive disorders. They also have a high potential in healing wounds and inhibiting cancer cells (Zlatic and Stankovic 2017; Pena-Espinoza *et al*. 2018; Emam *et al*. 2019; Khan *et al*. 2020; Choudhary *et al*. 2021; Nasimi *et al*. 2021;). Due to the presence of these valuable compounds, the wide range of uses, and the economic importance of chicory, breeding and industrial and targeted production of this medicinal plant has received great attention in many parts of the world (Bais and Ravishankar 2001; Barcaccia *et al*. 2016).

The plants' diversity is associated with many ecological processes such as the stability of communities, fertility, evolution, transformation, ecological nest architecture, and competition. Plants show variation in their morphological and physiological traits in different ecological conditions (Bartels and Chen 2013). Genetic diversity is an important factor for the adaptation and survival of populations, especially wild types, in different environments, which is resulted from the interaction of genetic structure and environmental conditions (Baricevic *et al*. 2015).

Given the importance of sustainable exploitation and protection of medicinal species, it is necessary to domesticate and introduce them into production systems (Wang *et al*. 2020). Diversity is the basis of selection, and the evaluation of genetic diversity is the first and, of course, the most important and costly step towards the domestication of a particular species. Wild species of medicinal plants are important resources of valuable medicinal metabolites and contain genes associated with resistance against pests, diseases, and adverse environmental conditions (Neel and Ellstrand 2003).

A variety of marker systems such as morphological and biochemical characteristics have been used to study the genetic diversity of medicinal plants (Cirak *et al*. 2007). Evaluating the morphological characteristics is the first step for describing and classifying a plant collection. Using these indicators, descriptors can be developed for any species for description and identification or definition of standards (Julsing *et al*. 2006). Morphological indicators often correspond to qualitative traits that are scored visually. These markers are found in natural populations or are created as a result of mutation experiments. Furthermore, due to the advantages such as having a wide range of genes controlling phenotypic traits, low cost, simple

measurement, visibility, and permanent presence in different stages of ontogeny, they are widely used in diversity and breeding studies and are an integral part of breeding programs (Chesnokov *et al*. 2020).

In addition to morphological characteristics, examining chemical compounds allows the possibility of identification of new types of medicinal compounds (Carlen 2016). Plants produce secondary metabolites to adapt to different ecological conditions. Therefore, the populations of medicinal species grown in different ecological conditions show different quantities and quality of effective substances, and this diversity leads to differences in the scope of their medicinal and biological activities (Heywood 2002; Razavi and Enferadi 2022).

Given the importance and widespread use of chicory, the present research was carried out to investigate the habitat potential and diversity among chicory populations using different morphological and chemical characteristics in the Markazi province of Iran

Materials and Methods

Collection and morphological evaluations

In the current research, after studying sources related to flora, receiving local information, matching herbarium samples, and existing reports on identifying distribution areas and habitats, nine habitats from Markazi province of Iran, including Zalian pass, Gheinar village (Shazand county), Toreh, Astaneh, Darband (Shazand-Astaneh connecting road), Golpaygan, FarajAbad village, Varcheh, and Heshmatieh (Khomein county) were selected for the study (Table 1).

Ten samples were collected in the second half of September during full bloom. After recording and transferring the samples, they were dried in the shade and away from direct light. Finally, morphological characteristics including qualitative traits (flower color, stem color, canopy density, and root type) and quantitative traits (plant height, number of branches, number of flowers, main stem weight, lateral branch weight, root weight, and plant weight) were measured. A digital caliper and a precise laboratory scale were used for the quantitative evaluation, and a coding method was used for the evaluation of the qualitative traits (Table 2).

Biochemical evaluation

***Extraction and identification of inulin*:** To extract inulin, roots were completely cleaned and freed from the soil particles after harvesting. To remove the contamination, the roots were washed with cold water and, after being dried, were kept at 4 °C until the extraction. To extract inulin, the roots were placed in 96% ethanol solution at 40 °C for 12 h. After this period, inulin was precipitated in alcohol in the form of spherical or star-shaped

crystals. The UV-VIS spectrophotometry method was used to determine the amount of inulin in this solution. The absorbance of standard inulin solutions was first measured in the visible wavelength, then the absorbance of the samples was measured according to the corresponding standard curve, and finally, the percentage of inulin in the sample was calculated (Steegmans *et al*. 2004; Roberfroid 2005; Saengkanuk *et al*. 2011).

***Extraction and identification of the essential oil compounds*:** To determine the amount of essential oil and its compounds, the samples were dried in the shade at ambient temperature. Then, the essential oil of the samples was isolated by distillation method using Clevenger-type apparatus for 3 h. After separating from water and dehumidifying with dry sodium sulfate, the essential oil was weighed and, after calculating the content of the essential oil in weight/weight (v/w), it was stored in the dark glass containers in the refrigerator for further analyses (Rustaiyan *et al*. 2011b; Haghi *et al*. 2012).

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) were used to identify the compounds of the essential oil. The GC/MS analysis was carried out on an Agilent 3400 apparatus gas chromatograph fitted with an Agilent mass selective detector in the EI mode at the 70 eV electron energy. The column was a semi-polar DB-5 (30 m × 0.25 mm i.d.; 0.25 μm film thicknesses). The helium was the carrier gas at a flow rate of 0.7 mL/min. The temperature range was programmed at a rate of 4 °C/min to a final temperature of 250 °C. The temperatures of the injector and the

Table 1. Geographical characteristics of the studied sites.

NO.	Location	Code	Altitude	Latitude	Longitude
1	Zalian	S_1	2262	33˙ 58' 18.145"	49˙ 4' 58.759"
2	Gheinar	S_2	1890	33˙ 56' 3.671"	49˙ 28' 33.159"
3	Toreh	S_3	1870	34˙ 2' 46.683"	49˙ 17' 46.926"
4	Astaneh	S_4	1999	33˙ 53' 18.756"	49˙ 21' 13.448"
5	Darband	S_5	1973	33˙ 54' 3.929"	49˙ 22' 43.633"
6	Golpayegan	S_6	2068	33˙ 34' 56.914"	50˙ 14' 28.233"
7	Faraj Abad	S_7	2209	33˙ 27' 44.580"	49˙ 52' 34.604'
8	Varcheh	S_8	2025	33˙ 46' 0.123"	49˙ 56' 49.868"
9	Heshmatieh	S_9	1858	33˙ 35' 57.261"	50˙ 1' 17.807"

Table 2. Codes of the qualitative traits in the studied chicory populations.

Qualitative traits	Code					
	1	3	5	7	9	11
Flower color (FCo)	Purple	Purple willing to violet	Light violet	Violet	Bluish purple	Dark violet
Stem color (SCo)	Whitish green	Yellowish green	Pale green	Green	Cedar green	Dark green
Canopy density (CanD)	Low	Medium	Relatively high	High	Very high	-
Root type (RtTy)	Woody	Thick and woody	-	-	-	-

temperatures of the injector and the GC/MS interface were held at 260 °C and 270 °C, respectively. The compounds were identified using the retention indices and recorded mass spectra (McLafferty and Stauffer 1998; Adams 2007).

Data analysis

The data were analyzed based on the one-way analysis of variance. Means were compared using the LSD test. The Pearson correlation coefficients among the quantitative traits were also determined. Cluster analysis was performed by the UPGMA method using the square of the Euclidian distance. To analyze the data, the SPSS statistical software was used.

Results and Discussion

Diversity in the studied populations The results of the analyses of variance for quantitative morphological traits showed that there were significant differences among the populations for all traits (Tables 3 and 4), indicating different responses to the ecological conditions due to genetic diversity. The presence of large variations among the populations allows the selection of proper populations for the desired traits. Phenotypic differences are the result of genotypic and environmental effects, and their interaction. If the phenotypic observations are made based on a sufficient sample size, they can be an indicator of genetic diversity (Panguluri and Kumar 2016; Carpentier *et al*. 2019). In this context, part of the diversity in the morphological traits measured in this study can be due to the differences in climatic and habitat conditions such as average rainfall and humidity, annual temperature, length of the dry season, and soil fertility, and another part is the result of genetic diversity among populations (Araus and Cairns 2014). Although morphological differences are not only related to genetic differences, they depend largely on the genetic content and even the ploidy level of different populations (Fasoula *et al*. 2020).

The characteristics of the studied traits are presented in Table 5. As the results showed, the branch weight, flower number, plant weight, and root weight displayed high coefficients of variation. Considering that the branch weight and root weight are important traits affecting the magnitude of metabolites in this medicinal plant, these indicators can be effectively used for breeding purposes. Traits that have the least diversity compared to other traits are less affected by the environment (Bernardo 2002).

Regarding the content and yield of the essential oil, populations of Astaneh and Gheinar showed the lowest and highest values, respectively. Maximum and minimum amounts of inulin were obtained from the

Table 3. Analysis of variance of morphological and biochemical traits in the studied chicory populations.

SV	df	Plant height	No. of branches	No. of flowers	Stem weight	Branches' weight
Treatments	8	13549.2**	877.022*	1685.68**	253.00**	6722.00**
Error	81	32241.3	4113.70	6076.10	829.10	16204.02
CV (%)		22.37491	59.66338	85.00	57.47	135.34

*, **: Significant at p≤ 0.05 and p≤ 0.01, respectively.

Table 4. Comparison of chicory populations for morphological and biochemical traits.

Locations	Plant height	No. of branches	No. of flowers	Stem weight	Branches' weight	Root weight	Total plant weight
Faraj Abad	105.7 a	11.9abc	8.2bcd	6.9a	12.3bc	5.9a	21.5bc
Heshmatieh	82.5bc	12.8ab	15.9ab	5.2ab	6.5c	6.5a	18.3c
Darband	79.4bc	10.6bc	5.8cd	3.2bc	4.5c	4.1abc	9c
Varcheh	67.2c	11.8abc	5.9cd	2.2c	4.1c	1.35d	8.55c
Gheinar	80.8bc	17.9a	13.1abc	6.4a	23ab	3.35bcd	35.85b
Toreh	91.3ab	13.9ab	10.3abcd	6.4a	4.3c	2.6cd	11.85c
Astaneh	107.5a	9.1bc	11.8abc	5.5ab	8.7c	3.05cd	17.85c
Golpayegan	94.7ab	13.5ab	16.8a	7.3a	28.2a	5.6ab	56.3a
Zalian	93.4ab	6.0c	3.9d	7a	2.45c	1.95cd	10.1c

In each column, means followed by the same letters are not significantly different (p≤ 0.05).

Table 4 continued

Location	Flower color	Stem color	Canopy density	Root type	Inulin content	Essential oil content	Essential oil yield
Faraj Abad	11	5	5	3	3.16i	0.14e	0.0308b
Heshmatieh	5	3	3	1	57.05a	0.06g	0.0119b
Darband	7	5	3	1	38.26g	0.216c	0.0191b
Varcheh	5	11	3	1	39.08f	0.26b	0.0218b
Gheinar	3	9	9	1	46.60d	0.281a	0.101a
Toreh	7	1	3	1	35.27h	0.076f	0.009b
Astaneh	7	7	3	1	48.43b	0.0415i	0.007b
Golpayegan	9	5	7	1	44.81e	0.0522h	0.0297b
Zalian	1	3	1	1	48.03c	0.148d	0.0148b

In each column, means followed by the same letters are not significantly different (p≤ 0.05).

populations of Heshmatieh (57.05 mg/g DW) and Faraj Abad (3.16 mg/g DW), respectively (Table 4). The amount and type of effective substances in medicinal plants, in addition to the nature and potential of the species or variety, depend on the ecological conditions such as climatic, topographical, and edaphic conditions. Climatic factors can affect plant genotypes to some extent, and thus result in morphological changes and changes in effective substances (Formisano *et al*. 2011). Different studies also show the fact that habitat conditions affect the quantity and quality of secondary compounds (Ghani *et al.* 2022; Saeidi *et al.* 2018; Rahimmalek *et al.* 2017; Figueiredo *et al*. 2008).

The results also demonstrated that there were differences among the investigated populations regarding qualitative traits of stem color, leaf color, and flower color (Table 6). The highest frequency was observed in the chicory with purple flower color, pale green stem, medium canopy density, and woody roots (Table 6). In contrast to qualitative traits such as the color and shape of organs that are less affected by the environment and are thus

Table 5. Descriptive statistics of the quantitative traits in studied chicory populations.

No.	Trait	Abbreviation	Unit	Min	Max	Mean	SD	CV
1	Plant height	PH	cm	52	150	92.16	24.39	26.47
2	Number of branches	NoBr	-	2	47	1.06	8.21	68.05
3	Number of flowers	NoF	-	0	49	10.2	10.90	106.91
4	Stem weight	SW	g	1	17	5.26	3.38	64.35
5	Weight of branches	BrW	g	1	100	11.32	18.72	165.94
6	Root weight	RW	g	0.5	19	3.46	3.34	96.58
7	Plant weight	TpW	g	2.5	113	20.05	21.89	109.14
8	Inulin content	IC	mg/g DW	3.33	57.61	39.96	15.26	38.18
9	Essential oil content	EoC	%V/W	0.04	0.29	0.14	0.09	63.87
10	Essential oil yield	EoY	ml/plant	0.01	0.24	0.03	0.32	126.38

SD: Standard deviation; CV: coefficient of variation

Table 6. Frequency of qualitative traits in the studied chicory populations.

Qualitative traits		Code					
		1	3	5	7	9	11
Flower color	Absolute	7	7	13	21	5	7
	Relative	11.66	11.66	21.66	35	8.33	11.66
Stem color	Absolute	7	13	19	7	7	7
	Relative	11.66	21.66	31.66	11.66	11.66	11.66
Canopy density	Absolute	7	34	7	5	7	
	Relative	11.66	56.66	11.66	8.33	11.66	
Root type	Absolute	53	7				
	Relative	88.33	11.66				

suitable criteria for evaluating diversity (Causse *et al*. 2007), quantitative traits are more important for domestication and breeding.

Variation of the essential oil compounds

According to the results of GC/MS analysis, 28 compounds were identified in the essential oil of the examined populations (Table 7). This number was higher than the number reported in other studies. The number of identified compounds in the chicory essential oil in other studies has been reported to be 14 (Rustaiyan *et al*. 2011a) and 20 (Haghi *et al*. 2012). Moreover, the results showed a large diversity among the studied populations in terms of the magnitude of chemical compounds in the essential oil. The main compounds of the essential oil in the studied populations were chamazulene (28.8%), 1,8-cineole (22.7%), carvacrol (18.6%), cuminic aldehyde (18.6%), thymol (18.4%), cinnamic aldehyde (18.3%), camphor (15.6%), borneol (12.3%), linalool (12.1%), and carvone (11.7%). The lowest amounts were observed for α-pinene and sesqui-lavendolol (0.1%). The most important compounds of chicory essential oil in the previous studies have been reported to be carvacrol, thymol, cinnamic aldehyde, camphor, carvone, linalool, and α-terpineol in Janda *et al*. (2021), carvacrol, thymol, cinnamic aldehyde, camphor, carvone,

Table 7. Chemical compounds of the essential oil in the studied chicory populations.

No.	Compound (%)	RI	Faraj Abad	Heshmatieh	Darband	Varcheh	Gheinar	Toreh	Astaneh	Golpayegan	Zalian
1	α-pinene	936	0.2	0.4	0.8	7.3	0.5	3.1	0.1	1.3	0.7
2	Sabinene	976	1.4	0.6	1.1	5.6	2.2	1.6	2.6	0.4	0.3
3	Myrcene	992	0.6	1.2	0.4	0.2	0.7	1.5	0.6	1.2	0.8
4	Limonene	1032	1.8	2.7	0.6	0.4	1.5	0.8	3.5	2.3	0.7
5	1,8-cineole	1034	22.7	13.4	2.3	2.1	19.6	7.6	1.4	16.3	12.5
6	*Trans*-linalool oxide	1074	0.9	0.3	3.4	1.4	3.1	2.1	0.8	0.3	0.4
7	Linalool	1107	7.1	12.1	0.8	0.3	2.8	0.4	4.5	1.6	2.1
8	6-Methyl-3,5-heptadiene-2-one	1116	0.6	0.4	1.5	0.5	1.4	3.1	0.3	1.2	0.6
9	Camphor	1155	5.2	1.6	1.7	0.6	0.3	15.6	12.4	8.6	1.4
10	Borneol	1168	3.1	0.4	0.6	0.2	0.5	12.3	7.2	0.8	0.5
11	Terpinen-4-ol	1177	1.1	2.8	3.4	0.7	1.3	1.4	3.1	0.3	1.4
12	1-methyl adamantane	1179	0.8	0.3	1.2	0.4	2.1	0.8	0.4	1.1	0.5
13	Naphthalene	1182	0.4	1.7	3.1	1.4	0.3	3.1	1.1	2.4	2.1
14	α-terpineol	1195	3.7	0.6	6.8	0.5	0.9	2.5	5.5	3.6	2.4
15	Cuminic aldehyde	1246	0.4	0.2	2.5	3.4	18.6	0.7	0.5	0.3	0.4
16	Carvone	1248	7.3	3.1	2.7	0.8	11.7	0.3	0.3	0.2	0.6
17	Cinnamic aldehyde	1267	6.2	5.2	1.4	0.3	0.2	1.5	18.3	11.6	14.2
18	Thymol	1300	8.4	2.3	14.6	18.4	0.6	4.7	7.9	14.3	7.8
19	Carvacrol	1309	4.1	15.6	3.7	3.2	4.1	0.3	3.1	5.5	18.6
20	Eugenol	1367	0.3	0.2	1.1	0.8	0.3	2.4	0.2	0.3	0.6
21	Neryl acetate	1374	0.9	0.4	0.2	0.5	0.3	1.3	0.2	1.1	2.1
22	(E)-caryophyllene	1420	1.3	0.3	0.5	0.3	0.7	0.9	0.2	0.4	0.7
23	β-Bisabolene	1516	0.5	6.4	0.3	1.6	1.5	3.6	0.2	1.6	0.4
24	Spathulenol	1583	0.9	0.5	1.4	1.8	2.7	0.5	1.3	1.5	1.1
25	(E)-sesquilavandulol	1594	0.2	0.3	0.6	0.4	0.1	1.4	0.7	0.3	0.3
26	α-bisabolol	1665	1.4	4.3	.03	2.1	0.5	0.2	0.5	0.4	0.6
27	Chamazulene	1707	0.5	0.6	24.9	28.8	8.3	3.4	1.3	2.4	8.2
28	(E)-hexyl cinnamaldehyde	1728	0.6	0.2	3.6	2.3	0.4	2.3	0.4	0.3	1.4

RI: The Kovats retention indices relative to C8-C20 n-alkanes were determined on the DB-5 ms capillary column.

linalool, and α-terpineol in Haghi *et al*. (2012), and hexadecanoic acid, nonadecane, and trans-α-bergamotene in Rustaiyan *et al*. (2011a), which are relatively consistent with the results of the present study.

Studies have shown that the maximum amounts of essential oil in medicinal species are obtained at the full bloom stage (Goudarzi *et al*. 2013), therefore, this stage was considered as an indicator of the harvest phase for the extraction and evaluation of essential oil compounds in the present study.

Synthesis and accumulation of secondary metabolites in medicinal plants are influenced by the interaction of genes and environmental conditions. Essential oils are influenced by these factors in terms of the amount and type of constituent compounds (Rustaiyan *et al*. 2011b). Indeed, any changes in the path of biosynthesis, including reduction to affect the sequential conversion, number of the genes involved in the path, are under environmental conditions and the induction of environmental stresses, which ultimately lead to differences

in the genetic constitution (Figueiredo *et al*. 2008).

The dominant compounds in the studied populations were as follows: 1,8-cineole in Faraj Abad; carvacrol, 1,8-cineole, and linalool in Heshmatieh; chamazulene and thymol in Darband and Varcheh; 1,8-cineole, cuminic aldehyde, and carvone in Gheinar; camphor and borneol in Toreh; cinnamic aldehyde and camphor in Astaneh; 1,8-cineole, thymol, and cinnamic aldehyde in Golpayegan; carvacrol, cinnamic aldehyde, and 1,8-cineole in Zalian. In general, the three compounds of 1,8-cineole, thymol, and cinnamic aldehyde had dominant distribution among the populations.

In the Faraj Abad population, there was a greater tendency toward the production of a high amount of 1,8-cineole compound, while in Gheinar, Golpayegan, and Heshmatieh populations, besides 1,8-cineole, the tendency was toward the production of other major compounds such as thymol, carvacrol, linalool, and cinnamic aldehyde. The tendency to produce a specific chemical compound was more pronounced in the Faraj Abad population. Since the effects of biologically active substances are the result of their compounds (Polatoglu *et al*. 2012), this population is more distinctive than other populations and therefore, can be used to achieve the breeding goals. The superior genotypes in terms of compounds such as thymol and carvacrol can be used in the pharmaceutical industry as an antibacterial, for the treatment of bronchitis and the production of anti-flatulent, antifungal, and oral drugs, and as a natural preservative for food products in the food industry (Costa *et al*. 2018).

Toreh population was also distinguished by the predominance of camphor and borneol monoterpenes. Camphor is a bicyclic oxygenated monoterpene ketone that is pharmacologically stimulant. It has strong biological effects and is used as an analeptic (revival and invigorating), respiratory stimulant, and local pain reliever in the treatment of muscle and joint pains such as fibrositis and neuralgia. Also, it has strong antiseptic properties and is considered a strong anti-pathogen in the treatment of fungal and bacterial infections (Zuccarini 2009). Borneol is also a valuable compound with antimicrobial, anti-inflammatory, and antifungal biological effects and is widely used in the treatment of lung disorders, digestive pains, and cleaning products (Li *et al*. 2021).

Cluster analysis

Cluster analysis categorized the populations based on the similarity of their traits (Figure 1). This analysis classified chicory populations into two main groups, the first group included two separate subgroups. In the first group, subgroup 1 included the populations of Faraj Abad, Astaneh, Heshmatieh, Toreh, Darband,

and Zalian, and subgroup 2 included Varcheh. The second group encompassed the populations of Ghainar and Golpayegan.

One of the methods used in plant breeding is selection along with progeny testing. The success of the selection depends on the diversity occurring by genetic recombination and heterosis. Increasing the genetic distance increases the probability of heterosis in crossing programs. In crossing between genotypes with greater genetic distance, more heterosis occurs through genetic recombination. Indeed, when several traits are studied simultaneously, grouping genotypes based on genetic distance is effective in the breeding programs (Panguluri and Kumar 2015; Darkwa *et al*. 2020; Fasoula *et al*. 2020). If groups with the maximum distance (for example, Faraj Abad and Astaneh) have superior traits, they can be crossed in hybridization programs.

Based on the magnitude of chemical compounds of the essential oil, cluster analysis classified the populations into two separate groups; the first group included the populations of Gheinar, Toreh, Astaneh, Golpayegan, Faraj Abad, Zalian, and Heshmatieh, and the second group included Darband and Varcheh populations. It should be noted that the first group included two main subgroups, where the Gheinar population was included in one subgroup and the rest were placed in the other subgroup (Figure 2).

The presence of Darband and Varcheh populations in a separate group is due to the predominance of chamazulene and thymol compounds. In terms of the magtitude of essential oil compounds and especially the main compounds, the maximum distance was observed between Gheinar and Varcheh populations. This advantage can be used in hybridization programs together with paying attention to other yield-related traits. The results of the biochemical analyzes of the present study are consistent with the results of many such studies, showing that sometimes environmental factors, even at the microclimate level, have great effects on the occurrence of genetic differentiation, synthesis of metabolites, and separation of a species. These effects lead to the maximum difference and differentiation not only in the magnitude of metabolites but also in the type of specific compounds (Bergonzi *et al*. 2001; Torras *et al.* 2007; Satyal *et al*. 2016; Karimi *et al*. 2020).

Conclusion

The present study showed considerable diversity in quantitative and qualitative morphological traits and biochemical compounds which can provide a suitable basis for the breeding of this valuable medicinal plant. In addition to identifying the compounds of each population and determining their affinity and divergence, the chemical potential of the studied germplasm can be monitored

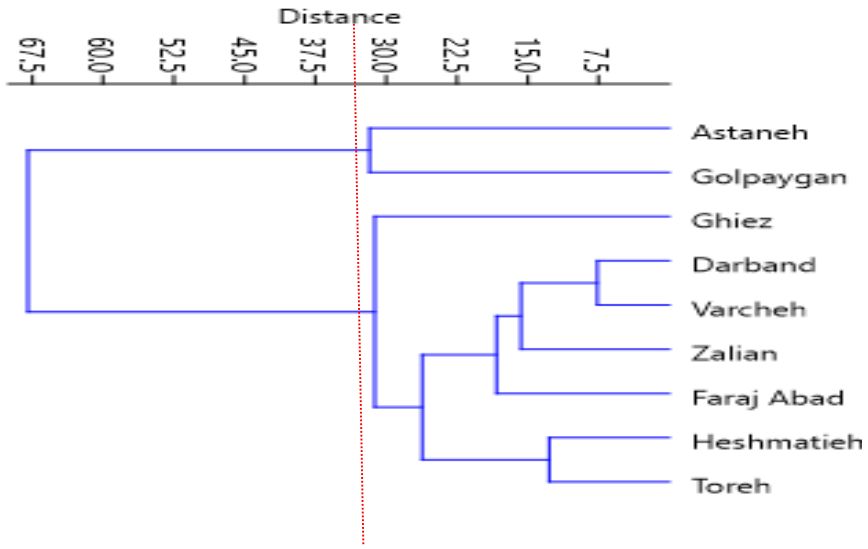

Figure 1. Cluster analysis of the studied chicory populations based on the quantitative morphological traits using the UPGMA method.

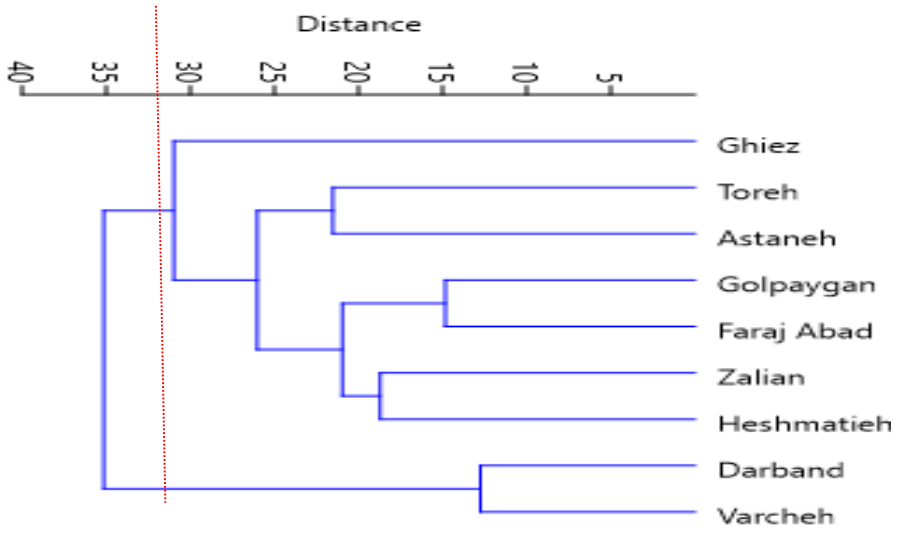

Figure 2. Cluster analysis of the studied chicory populations based on the chemical compositions of the essential oil using the UPGMA method.

and used based on the needs of different industries. The current research was the first study in Iran as the basis of a breeding program to obtain desirable types of chicory by evaluating morphological and biochemical characteristics.

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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.

References

Adams RP, 2007. Identification of essential oil components by gas chromatography/mass spectrometry. 4th Edition. Allured Publ., Carol Stream, IL, USA.

Araus JL and Cairns JE, 2014. Field high-throughput phenotyping: the new crop breeding frontier. Trends in Plant Science 19(1): 52-61.

Bais HP and Ravishankar GA, 2001. *Cichorium intybus* L. cultivation, processing, utility, value addition and biotechnology, with an emphasis on current status and future prospects. Journal of the Science of Food and Agriculture 81(5): 467-484.

Barcaccia G, Ghedina A, and Lucchin M, 2016. Current advances in genomics and breeding of leaf chicory (*Cichorium intybus* L.). Agriculture 6(4):1-24.

Baricevic D, Mathe, A, and Bartol T, 2015. Conservation of wild crafted medicinal and aromatic plants and their habitats. In: Máthé Á (ed.). Medicinal and Aromatic Plants of the World. Springer. Pp. 131-144.

Bartels SF and Chen HY, 2013. Interactions between overstorey and understorey vegetation along an overstorey compositional gradient. Journal of Vegetation Science 24(3): 543-552.

Bergonzi MC, Bilia AR, Gallori S, Guerrini D, and Vincieri FF, 2001. Variability in the content of the constituents of *Hypericum perforatum* L. and some commercial extracts. Drug Development and Industrial Pharmacy 27: 491-497.

Bernardo R, 2002. Breeding for Quantitative Traits in Plants. Stemma press. Woodbury, MN, USA. 369 pp.

Bhandari HR, Bhanu AN, Srivastava K, Singh MN, and Shreya HA, 2017. Assessment of genetic diversity in crop plants-an overview. Advances in Plants and Agriculture Research 7(3): 279-286.

Carlen C, 2016. Breeding and cultivation of medicinal plants. In: Bagetta G, Cosentino M, Corasaniti MT, and Sakurada S (eds). Herbal Medicines: Development and Validation of Plant-derived Medicines for Human Health. Pp. 79-91. CRC Press.

Carpentier S, Costa C, Schurr U, Loreto F, and Menesatti P, 2019. Plant phenotyping research trends, a science mapping approach. Frontiers in Plant Science 9: 1933.

Causse M, Damidaux R, and Rousselle P, 2007. Traditional and enhanced breeding for quality traits in tomato. In: Razdan MK (ed.) Genetic Improvement of Solanaceous Crops: Volume 2 Tomato. Pp. 153-192. Science Publ., NH, USA.

Chesnokov YV, Kosolapov VM, and Savchenko IV, 2020. Morphological genetic markers in plants. Russian Journal of Genetics 56(12): 1406-1415.

Choudhary S, Kaurav H, and Chaudhary G, 2021. Kasani beej (*Cichorium intybus*): Ayurvedic view, folk view, phytochemistry and modern therapeutic uses. International Journal for Research in Applied Sciences and Biotechnology 8(2): 114-125.

Cirak C, Radusiene J, Karabuk B, Janulis V, and Ivanauskas L, 2007. Variation of bioactive compounds in *Hypericum perforatum* growing in Turkey during its phenological cycle. Journal of Integrative Plant Biology 49(5): 615-620.

Costa MF, Durço AO, Rabelo TK, Barreto RS, and Guimaraes AG, 2019. Effects of carvacrol, thymol and essential oils containing such monoterpenes on wound healing: a systematic review. Journal of Pharmacy and Pharmacology 71(2): 141-155.

Darkwa K, Agre P, Olasanmi B, Iseki K, Matsumoto R, Powell A, and Asfaw A, 2020. Comparative assessment of genetic diversity matrices and clustering methods in white Guinea yam (*Dioscorea rotundata*) based on morphological and molecular markers. Scientific Reports 10: 13191.

Fasoula DA, Ioannides, IM, and Omirou, M, 2020. Phenotyping and plant breeding: overcoming the barriers. Frontiers in Plant Science 10: 1713.

Figueiredo AC, Barroso JG, Pedro LG, and Scheffer JJ, 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour and Fragrance Journal 23(4): 213-226.

Formisano C, Rigano D, and Senatore F, 2011. Chemical constituents and biological activities of Nepeta species. Chemistry and Biodiversity 8(10): 1783-1818.

Fu YB, 2015. Understanding crop genetic diversity under modern plant breeding. Theoretical and Applied Genetics 128(11): 2131-2142.

Ghani A, Amini S, Mohtashami S, and Neamati SH, 2022. Physicochemical and biochemical variations of jujube (*Ziziphus jujuba* Mill.) populations: a comparison of Iranian and imported types. Industrial Crops and Products 183: 1-11.

Goudarzi T, Saharkhiz MJ, and Rowshan V, 2015. Ontogenetic variation of essential oil content and constituents in tansy (*Tanacetum vulgare* L.). Journal of Applied Research on Medicinal and Aromatic Plants 2(2): 48-53.

Haghi G, Arshi R, Ghazian F, and Hosseini H, 2012. Chemical composition of essential oil of aerial parts of *Cichorium intybus* L. from Iran. Journal of Essential Oil Bearing Plants 15(2): 213-216.

Heywood VH, 2002. The conservation of genetic and chemical diversity in medicinal and aromatic plants. In: Şener B (ed.) Biodiversity. Springer, Boston, MA, USA, pp. 13-22.

Imam, KMSU, Xie Y, Liu Y, Wang F, and Xin F, 2019. Cytotoxicity of *Cichorium intybus* L. metabolites. Oncology Reports 42(6): 2196-2212.

Janda K, Gutowska I, Geszke-Moritz M, and Jakubczyk K, 2021. The common chicory (*Cichorium intybus* L.) as a source of extracts with health-promoting properties: a review. Molecules 26(6): 1-4.

Karimi A, Krahmer A, Herwig N, Schulz H, Hadian J, and Meiners T, 2020. Variation of secondary metabolite profile of *Zataria multiflora* Boiss. populations linked to geographic, climatic, and edaphic factors. Frontiers in Plant Science 11: 969.

Julsing MK, Quax WJ, and Kayser O. 2006. The Engineering of medicinal plants: Prospects and limitations of medicinal plant biotechnology. In: Kayser *et al.* (eds). Medicinal Plant Biotechnology: From Basic Research to Industrial Applications. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, pp. 1-8.

Khan MF, Nasr FA, Noman OM, Alyhya NA, Ali I, Saoud M, and Hussain H, 2020. Cichorins D–F: three new compounds from *Cichorium intybus* and their biological effects. Molecules 25(18): 1-11.

Li Y, Ren M, Wang J, Ma R, Chen H, Xie Q, Li H, Li J, and Wang J, 2021. Progress in borneol intervention for ischemic stroke: a systematic review. Frontiers in Pharmacology 4(12): 606682.

McLafferty FW, Zhang MY, Stauffer DB, and Loh SY, 1998. Comparison of algorithms and databases for matching unknown mass spectra. In: McLafferty FW and Stauffer DB (eds.). Wiley/NBS Registry of Mass Spectral Data Vol. 1. Wiley, New York, USA, pp. 31-39.

Mozaffarian V, 2012. Identification of medicinal and aromatic plants of Iran. Farhang Moaser Publishers, Tehran. 1444 pp. (In Persian).

Nasimi Doost Azgomi R, Karimi A, Tutunchi H, and Moini Jazani A, 2021. A comprehensive mechanistic and therapeutic insight into the effect of chicory (*Cichorium intybus*)

supplementation in diabetes mellitus: A systematic review of literature. International Journal of Clinical Practice 75(12): 1-14.

Neel MC and Ellstrand NC, 2003. Conservation of genetic diversity in the endangered plant *Eriogonum ovalifolium* var. vineum (Polygonaceae). Conservation Genetics 4(3): 337-352.

Nwafor IC, Shale K, and Achilonu MC, 2017. Chemical composition and nutritive benefits of chicory (*Cichorium intybus*) as an ideal complementary and/or alternative livestock feed supplement. Scientific World Journal 2017: 1-12.

Panguluri SK and Kumar AA, 2016. Phenotyping for Plant Breeding. Springer-Verlag, NY, USA. 222 pp.

Pena-Espinoza M, Valente AH, Thamsborg SM, Simonsen HT, Boas U, Enemark HL, and Williams AR, 2018. Antiparasitic activity of chicory (*Cichorium intybus*) and its natural bioactive compounds in livestock: a review. Parasites and Vectors 11(1): 1-14.

Perovic J, Saponjac VT, Kojic J, Krulj J, Moreno DA, Garcia-Viguera C, and Ilic N, 2021. Chicory (*Cichorium intybus* L.) as a food ingredient–nutritional composition, bioactivity, safety, and health claims: a review. Food chemistry 336: 1-12.

Polatoglu K, Demirci B, Demirci F, Goren N, and Baser KC, 2012. Biological activity and essential oil composition of two new *Tanacetumc hiliophyllum* (Fisch. and Mey.) Schultz Bip. var. Chiliophyllum chemotypes from Turkey. Industrial Crops Production 39: 97-105.

Puhlmann ML and De Vos WM, 2020. Back to the roots: revisiting the use of the fiber-rich *Cichorium intybus* L. taproots. Advances in Nutrition 11(4): 878-889.

Rahimmalek M, Fadaei Heidari E, Ehtemam MH, and Mohammadi, S, 2017. Essential oil variation in Iranian ajowan (*Trachyspermum ammi* (L.) Sprague) populations collected from different geographical regions in relation to climatic factors. Industrial Crops and Products 95: 591-598.

Razavi S and Enferadi M, 2022. Essential oil composition and metabolites changes of *Artemisia melanolepis* Boiss at different phenological stages. Journal of Plant Physiology and Breeding 12(1): 39-49.

Roberfroid MB, 2005. Introducing inulin-type fructans. British Journal of Nutrition 93(1): 13-25.

Rustaiyan A, Masoudi S, Ezatpour L, Danaii E, Taherkhani M, and Aghajani Z, 2011a. Composition of the essential oils of *Anthemis Hyalina* DC., *Achillea Nobilis* L. and *Cichorium intybus* L. three asteraceae herbs growing wild in Iran. Journal of Essential Oil Bearing Plants 14(4): 472-480.

Rustaiyan A, Masoudi S, Eghbali H, and Salehi HR, 2011b. Phenological variation on essential oil composition of *Stachys inflata* Benth. from Iran. Journal of Essential Oil Bearing Plants 14(6): 679-683.

Saeidi K, Moosavi M, Lorigooini Z, and Maggi F, 2018. Chemical characterization of the essential oil compositions and antioxidant activity from Iranian populations of *Achillea wilhelmsii* K. Koch. Industrial Crops and Products 112: 274-280.

Saengkanuk A, Nuchadomrong S, Jogloy S, Patanothai A, and Srijaranai S, 2011. A simplified spectrophotometric method for the determination of inulin in Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. European Food Research and Technology 233(4): 609-616.

Satyal P, Murray BL, McFeeters RL, and Setzer WN, 2016. Essential oil characterization of *Thymus vulgaris* from various geographical locations. Foods 5(4): 1-13.

Steegmans M, Iliaens S, and Hoebregs H, 2004. Enzymatic, spectrophotometric determination of glucose, fructose, sucrose, and inulin/oligofructose in foods. Journal of AOAC International 87(5): 1200-1207.

Swarup S, Cargill EJ, Crosby K, Flagel L, Kniskern J, and Glenn KC, 2021. Genetic diversity is indispensable for plant breeding to improve crops. Crop Science 61(2): 839-852.

Torras J, Grau MD, Lopez JF, and de las Heras FC, 2007. Analysis of essential oils from chemotypes of *Thymus vulgaris* in Catalonia. Journal of the Science of Food and Agriculture, 87(12): 2327-2333.

Wang W, Xu J, Fang H, Li Z, and Li M, 2020. Advances and challenges in medicinal plant breeding. Plant Science 298: 110573.

Zlatic NM and Stankovic MS, 2017. Variability of secondary metabolites of the species *Cichorium intybus* L. from different habitats. Plants 6(3): 1-9.

Zuccarini P, 2009. Camphor: risks and benefits of a widely used natural product. Journal of Applied Sciences and Environmental Management 13(2): 69-74.

ارزیابی صفات مورفولوژیکی و ترکیبات شیمیایی گیاه دارویی کاسنی (*Cichorium intybus*) در مرکز ایران (استان مرکزی)

گلپر رحمتی و منصور قربانپور*

گروه گیاهان دارویی و معطر، دانشکده کشاورزی و منابع طبیعی، دانشگاه اراک، اراک
مسئول مکاتبه؛ Email: m-ghorbanpour@araku.ac.ir

چکیده

گیاه کاسنی به دلیل داشتن ترکیبات زیست فعال ارزشمند مانند اسیدهای فنولیک، ترپنوئیدها، ویتامین‌ها و اینولین، دارای طیف استفاده وسیعی در صنایع دارویی و غذایی می‌باشد. حضور و میزان این ترکیبات متأثر از عوامل ژنتیکی و محیطی مختلف و روش فرآوری است. در این پژوهش جمعیت‌های وحشی مختلف از گیاه کاسنی واقع در مرکز کشور (استان مرکزی) با استفاده از صفات مورفولوژیکی و بیوشیمیایی مورد ارزیابی قرار گرفت. استخراج اسانس با روش تقطیر با آب و دستگاه کلونجر انجام شد. برای شناسایی و جداسازی ترکیبات موجود در اسانس از دستگاه GC و GC/MS و برای تعیین میزان اینولین از دستگاه اسپکتروفتومتر استفاده شد. نتایج نشان داد که تنوع بالا و معنی‌دار بین جمعیت‌ها از نظر صفات مختلف وجود دارد. محتوی و ترکیبات اسانس نیز در جمعیت‌های مورد مطالعه دارای تنوع قابل ملاحظه‌ای بود. ترکیبات اصلی اسانس شامل کامازولن، ۱و۸-سینئول، کارواکرول، کومینیک آلدهید، تیمول، سینامیک آلدهید، کامفور، بورنئول، لینالول و کارون بودند. تجزیه کلاستر بر اساس صفات مورفولوژیکی و ترکیبات شیمیایی اسانس، جمعیت‌ها را در دو گروه مختلف تفکیک کرد. در نتیجه وجود تنوع قابل توجه در صفات مورفولوژیکی و ترکیبات شیمیایی در بین جمعیت‌های کاسنی می‌تواند در برنامه‌های اصلاحی این گیاه مفید واقع شود.

واژه‌های کلیدی: اسانس؛ ترکیبات شیمیایی؛ تنوع ژنتیکی؛ کاسنی