Diversity in Photosynthetic Pigments and Phytochemical Compounds among Iranian Populations of Inula helenium L.

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Abstract

Elecampane (*Inula helenium* L.) is a valuable medicinal plant known for its well-documented anticancer, cardioprotective, antibacterial, and anti-inflammatory properties. This study aimed to assess various Iranian elecampane populations by collecting 15 populations from their natural habitats and evaluating them in terms of photosynthetic pigments, phenolic content, and essential oil profiles. The populations exhibited variations in chlorophyll (Chl) and carotenoid content, which were positively correlated with soil potassium and phosphorous levels. Content of total phenolic (TPC), total flavonoids (TFC), and proline varied among the populations, with TPC and proline showing a significant correlation with Chl content. The essential oil (EO) content ranged from 0.13 to 0.26%, with the analysis identifying 33 compounds by GC/MS, comprising over 96% of the EO content. The predominant compounds in the EO profiles included β-elemene, tricyclene, α-pinene, isoalantolactone, and santolina triene. Principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) revealed three classes based on the main EO compositions. Populations in habitats with altitudes of 2000-2400 m, pH levels of 6.5-7, and soils with silt/silt loam texture had higher TPC and EO content. Importantly, different populations exhibited essential oils with highly diverse compositions, with some populations containing compounds absent in others. Except for β-elemene, which was present in all populations, other compounds varied in presence and quantity across different populations. This suggests the potential for obtaining diverse and desired chemotypes of Inula helenium through further research.

Introduction

Medicinal plants are regarded as valuable resources for the development of new drugs owing to their substantial capacity for producing secondary metabolites(Velu et al. 2018). The rising global demand for medicinal plants underscores the importance of their production and sustainable harvesting (Van Wyk and Prinsloo 2018). Harvesting these plants inappropriately or unsustainably, particularly in their natural habitats, poses a significant threat. Such practices not only endanger the survival of plant species but also jeopardize regional and global biodiversity (Corlett 2016). Consequently, safeguarding these plants within and beyond their natural habitats is imperative. This necessitates comprehensive assessments of plant species through morphological, ecological, physiological, and phytochemical evaluations, with the ultimate goal of cultivation to reduce reliance on wild harvesting.

Secondary metabolites, while not directly involved in plant growth, development, and reproduction, serve as a crucial component of the plant's defense mechanisms against a wide range of biotic and abiotic stressors. Phenolic compounds, among these secondary metabolites, are of particular significance to human health due to their capacity to neutralize harmful free radicals(Ali-Arab et al. 2022). Additionally, essential oils, as secondary metabolites in medicinal plants, exhibit substantial variation in their composition based on factors such as the plant species, environmental conditions, and extraction methods(Bajalan et al. 2018; Ali-Arab et al. 2022). Consequently, variations in secondary metabolites can be observed among plant populations residing in different habitats (Bajalan et al. 2018). Recent years have seen extensive research on the protection and sustainable utilization of medicinal plants, with the development of viable solutions to ensure their conservation (Shafi et al. 2021).

Elecampane (*Inula helenium*) is a perennial herb belonging to the Asteraceae family and is found in abundant distribution across Asia, Europe, and the Mediterranean region(Seca et al. 2014). The rhizome and root of this plant are utilized for various therapeutic purposes, including antinociceptive, anti-inflammatory, tonic, diuretic, diaphoretic, antibacterial(Mamedov et al. 2015), and antimicrobial(Budán et al. 2021) properties.

The diverse climatic and environmental conditions in Iran, along with significant genetic diversity among plant populations, result in variations in phytochemical compounds found in different populations of plant species within their respective habitats. This diversity has observed in various plant species in Iran, such as *Trachyspermum ammi* L.(Rahimmalek et al. 2017), *Thymus vulgaris* (Tohidi et al. 2017), *Hypericum perforatum* L. (Morshedloo et al. 2015), and *Zataria multiflora* (Niczad et al. 2019).

To assess Iranian elecampane populations, 15 different populations of elecampane were collected from various regions in Iran. These populations were then analyzed for their photosynthetic pigments, phenolic content, proline levels, and essential oil profiles. The goal of this evaluation is to understand the variations and characteristics of elecampane populations in Iran.

Materials and Methods

Plant Materials and Site Description

Fifteen distinct populations of elecampane (*Inula helenium L.*) were collected from their respective natural habitats in Iran (Table 1). The plant specimens were accurately identified by expert botanists at the Institute of Medicinal Plants, ACECR, Karaj, Iran. Detailed chemical and physical analyses of the soil samples from these habitats were carried out and recorded (Table 2). Potassium (K) and phosphorus (P) levels in the soil were measured using the flame photometer and Olsen methods, respectively(Bajalan et al. 2018).

Table 1
The site characteristics of collected elecampane populations.

Population	Region Collected	Site Name	Latitude (N)	Longitude (E)	Elevation (m a.s.l.)	AR (mm)	AMT (°C)
P1	Hamedan-Hamedan	Haydareh	34.8199°	48.3471°	2138	370	10.9
P2	Zanjan-Zanjan	Mary-1	36.9975°	48.4733°	2236	303	10.4
P3	Ardabil-Meshkin Shahr	Park Jangali	38.3861°	47.6894°	1547	415	11.8
P4	Ardabil-Meshkin Shahr	Khorshid	38.2102°	47.5837°	1306	387	10.8
P5	Ardabil-Meshkin Shahr	Koyej	38.2356°	47.4055°	1248	386	10.9
P6	Zanjan-Soltanieh	AmirAbad-2	36.4358°	48.7920°	2236	370	12.5
P7	Zanjan-Soltanieh	AmirAbad-1	36.4288°	48.7458°	1984	360	12.5
P8	Zanjan-Zanjan	Golujeh-1	37.0097°	48.3094°	2015	295	10.9
P9	Zanjan-Zanjan	Zaker	36.6455°	48.7204°	2324	310	11.4
P10	Zanjan-Zanjan	Mary2	36.7544°	48.5842°	2288	302	10.6
P11	Zanjan-Zanjan	Golujeh-2	37.5588°	48.6241°	2460	330	11.8
P12	Zanjan-Zanjan	Golujeh-3	37.2057°	48.9588°	2118	325	10.4
P13	East Azarbayjan-Mianeh	khanegah	37.7089°	47.7615°	2575	330	13.4
P14	Zanjan-Zanjan	Khanchaei	36.6830°	48.5087°	2257	304	10.5
P15	Zanjan-zanjan	Emam Kandi	36.7030°	48.8077°	1956	293	10.2

AR: annual rainfall, AMT: annual mean temperature, m a.s.l: Metres above sea level

Table 2
Soil characteristics of the studies sites of elecampane populations

Population	Soil Texture	pH	EC ($ds\ m^{-1}$)	Organic Carbon (%)	K (ppm)	P (ppm)
P1	Silty Clay	6.15	1.17	0.81	67.2	5.61
P2	Sandy Clay	6.21	2.15	0.73	100.5	7.34
P3	Loam	6.32	1.78	1.32	187.4	10.22
P4	Silty Loam	6.23	1.32	1.93	205.4	9.53
P5	Sandy Loam	4.78	2.43	1.36	265.8	11.23
P6	Silt	6.81	2.31	2.35	345.5	14.27
P7	Silty Loam	6.38	2.16	2.18	257.4	13.23
P8	Sandy Loam	6.51	2.78	1.35	276.8	8.87
P9	Silty Clay Loam	6.42	2.54	1.74	282.4	10.12
P10	Sandy Loam	6.32	2.62	3.05	218.2	8.17
P11	Sandy	6.52	2.43	2.41	198.4	11.12
P12	Loam	6.37	2.16	2.35	265.7	9.13
P13	Clay Loam	6.32	2.97	2.65	354.5	13.4
P14	Sandy Clay	6.63	2.38	2.31	312.7	12.4
P15	Silty Clay	6.58	2.43	2.17	307.8	12.9

Meteorological data for the habitats were obtained from the nearest meteorological station. Plant aerial parts were sampled during the flowering stage in September, and rhizomes were harvested in October for phytochemical analysis.

Chlorophyll and carotenoids assay

Fresh leaves (0.5 g) were crushed using liquid nitrogen, and 20 ml of 80% acetone was added. The mixture was then centrifuged at 6000 rpm for 10 minutes. The supernatant was transferred to a glass flask, and absorbance values were measured with a spectrophotometer at wavelengths of 470, 645, and 663 nm. Chlorophyll (Chl) a and b as well as carotenoids content were determined using the following equations (Arnon 1967):

- Chl a = (12.7×A663–2.69×A645) ×V / (1000×W)
- Chl b = (22.9×A645–4.68×A663) ×V / (1000×W)
- Total Chl = (20.2×A645–8.02×A663) ×V / (1000×W)
- Carotenoids = 100×A470–1.82× (mg Chl a) – 85.02× (mg Chl b) / 198

In these equations, V represents the volume of the purified solution, A is the absorption at wavelengths of 663, 645, and 470 nm, and W is the sample weight in grams.

Measurement of total phenolic content

To measurement total phenolic content (TPC), samples (200 mg) were dissolved in 8 ml of 80% ethanol and then centrifuged at 1300 rpm for 15 minutes. Following this, 2.5 ml of 0.2 N Folin-Ciocalteu reagent was added. After 5 minutes, 2 ml of a 75 g L-1 sodium carbonate solution was added. After 2 hours, the absorbance of the mixture was read at a wavelength of 760 nm using a spectrophotometer (JENWAY 6305, JENWAY, UK). Gallic acid (GA) was used as a standard to construct a calibration curve, and the TPC was determined as milligrams of gallic acid (GA) per gram of dry weight (DW).

Measurement of total flavonoid content

The total flavonoid content (TFC) was measured using an aluminum chloride reagent. To a 0.5 ml extract, 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride solution, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water were added. After 30 minutes at room temperature, the absorption of the mixture was read at 415 nm. Quercetin was used as a standard to create the calibration curve, and the flavonoids content was determined as milligrams of quercetin (QE) per gram of DW(Chang et al. 2002).

Measurement of proline Content

Proline content in plant tissue was determined by mixing 0.1 g of fresh leaves with 10 ml of 3% w/v sulfosalicylic acid and centrifuging for 20 minutes at 4000 rpm. This was followed by mixing with 2 ml of ninhydrin acid and 2 ml of glacial acetic acid. Simultaneously, 2 ml of standard proline solutions (0, 4, 8, 12, 16, 20 mg proline) were mixed with 2 ml of ninhydrin acid and 2 ml of acetic acid. All samples were placed in a hot water bath for 60 minutes and then fully cooled on ice. To the solution, 4 ml of toluene was added. Using proline standards, a regression equation was established from the standard curve using a spectrophotometer at a wavelength of 520 nm (Bates et al. 1973).

Essential Oil content and composition

Essential oils (EO) were extracted from 100 grams of plant material of each population using a Clevenger-type apparatus with water distillation for 3 hours. The extracted essential oils were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) to identify and quantify their components. A TRACE MS gas chromatograph with an MS-HP-5 column (30 m×0.25 mm, 0.25 µm film thickness) coupled to a mass detector (HP-6973) was used. Helium gas was used as the carrier gas at a flow rate of 1.1 mL/min. The oven temperature was initially set at 60°C and gradually increased to 250°C at a rate of 5°C/min. The injection temperature was 250°C, and 1 µL of essential oil samples were injected with a split ratio of 1:50. MS spectra were acquired by electron ionization at 70 eV. Kovats retention indices (KI) were calculated for identified compounds using a homologous series of n-alkanes injected under the same conditions as the essential oil samples. Compounds were identified based on software matching with the Wiley 7 N library and direct comparison of retention indices and MS fragmentation patterns with those of standard compounds(Adams 2001) .

Statistical analysis

Statistical analysis was conducted in a completely randomized design with 5 replicates for 15 populations using SAS software version 9.2. Mean data were compared using the Duncan multiple range test. Agglomerative Hierarchical Clustering (AHC) based on the Ward variance method and Principal Component Analysis (PCA) were performed using XLSTAT software.

Results

The study evaluated various physiological and phytochemical traits in different populations of elecampane (*Inula helenium*). While there were no significant differences observed between the two years of evaluation, the study did find significant differences among the populations for the traits examined. The key findings are summarized as follows:

Chlorophyll and carotenoid

Chl a content varied among populations, ranging from 0.60 mg g^{-1} in population P1 to 2.19 mg g^{-1} in population P6. The highest Chl b content was recorded in population P3, followed by populations P6, P5, and P13. Population P6 exhibited a remarkable approximately three-fold increase in total Chl content, with a maximum value of 3.2 mg g^{-1}, in contrast to the minimum of 1.06 mg g^{-1} observed in population P1 (Table 3). Population P14 displayed the highest carotenoid content at 0.75 mg g^{-1}, which was approximately three times higher than the carotenoid content in population P1, measured at 0.24 mg g^{-1} (Table 3).

Table 3
Chlorophyll (Chl) and carotenoid contents in different elecampane populations

Population	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
P1	0.60 ± 0.11k	0.39 ± 0.03h	1.06 ± 0.05l	0.24 ± 0.03g
P2	0.99 ± 0.08j	0.78 ± 0.12b-d	1.81 ± 0.14ij	0.33 ± 0.09f
P3	1.41 ± 0.04fg	0.91 ± 0.06a	2.38 ± 0.1g	0.316 ± 0.09f
P4	1.56 ± 0.07de	0.73 ± 0.04c-e	2.28 ± 0.08de	0.53 ± 0.03d
P5	1.87 ± 0.07b	0.85 ± 0.05b	2.73 ± 0.05b	0.64 ± 0.06bc
P6	2.19 ± 0.07a	0.90 ± 0.05a	3.12 ± 0.11a	0.70 ± 0.03ab
P7	1.17 ± 0.04i	0.60 ± 0.07f	1.74 ± 0.06J	0.43 ± 0.04e
P8	1.27 ± 0.06h	0.69 ± 0.05d-f	2.01 ± 0.04gh	0.54 ± 0.05d
P9	1.76 ± 0.12c	0.76 ± 0.05b-d	2.57 ± 0.13c	0.66 ± 0.03bc
P10	1.06 ± 0.09j	0.50 ± 0.04g	1.52 ± 0.09k	0.63 ± 0.04bc
P11	1.33 ± 0.03gh	0.62 ± 0.06ef	1.91 ± 0.12hi	0.64 ± 0.03bc
P12	1.27 ± 0.02h	0.79 ± 0.04b-d	2.10 ± 0.02fg	0.61 ± 0.07c
P13	1.44 ± 0.05ef	0.83 ± 0.14a-c	2.25 ± 0.25d-f	0.71 ± 0.01ab
P14	1.52 ± 0.08de	0.76 ± 0.07b-d	2.31 ± 0.15de	0.75 ± 0.06a
P15	1.33 ± 0.03gh	0.73 ± 0.07c-e	2.16 ± 0.04fg	0.64 ± 0.02bc

The means (± standard deviation) with the same letters in each column indicates no significant difference between treatments at the 5% level of probability.

The TPC and TFC

The study found significant differences ($P \leq 0.05$) in TPC and TFC among the populations of elecampane. TPC ranged from 53.86 mg of gallic acid (GA) per gram of dry weight (DW) in population P1 to 100.27 mg GA g^{-1} DW in population P6 (Table 4). TPC was positively correlated with Chl a, Chl b, total Chl, carotenoid, and proline, while it had a negative correlation with EO (essential oil) content (Table 5). The TFC ranged from 7.25 mg of quercetin equivalents (QE) per gram of DW in population P10 to 11.33 mg QE g^{-1} DW in population P2 (Table 4). TFC had a significant and positive correlation with Chl b and carotenoid but showed a negative correlation with proline (Table 5).

Table 4
The content of total phenolic (TPC), total flavonoid (TFC), proline, and essential oil (EO) of elecampane populations

Population	TPC (mg GA g^{-1} DW)	TFC (mg QE g^{-1} DW)	Proline (µg g^{-1} FW)	EO (%)
P1	53.86 ± 3.26h	9.42 ± 0.16cd	6.05 ± 0.07i	0.21 ± 0.01b-d
P2	58.37 ± 7.15gh	11.33 ± 0.78a	7.295 ± 0.55g	0.21 ± 0.01a-d
P3	59.34 ± 1.43fg	9.42 ± 0.84cd	6.68 ± 0.51h	0.21 ± 0.01a-d
P4	63.63 ± 3.49g	9.92 ± 0.12bc	7.15 ± 0.05g	0.22 ± 0.02a-d
P5	87.33 ± 6.09b	10.49 ± 0.69b	9.11 ± 0.56bc	0.24 ± 0.02b
P6	100.27 ± 8.19a	9.46 ± 0.2cd	10.06 ± 0.07a	0.18 ± 0.04de
P7	69.33 ± 1.58ef	7.58 ± 1gh	8.11 ± 0.59f	0.20 ± 0.03b-e
P8	73.01 ± 1.01de	8.09 ± 0.08fg	8.34 ± 0.1ef	0.19 ± 0.04c-e
P9	76.81 ± 2.92cd	9.11 ± 0.84d	9.21 ± 0.09b	0.17 ± 0.03d-f
P10	67.93 ± 1.62ef	7.25 ± 0.82h	8.60 ± 0.15d-f	0.23 ± 0.03b
P11	70.11 ± 3.73e	8.33 ± 0.06ef	9.21 ± 0.13b	0.26 ± 0.03a
P12	78.32 ± 2.18cd	8.09 ± 0.54fg	8.70 ± 0.21c-e	0.22 ± 0.05a-d
P13	82.44 ± 4.37bc	8.99 ± 0.19de	9.39 ± 0.6b	0.17 ± 0.03d-f
P14	88.00 ± 1.09b	10.37 ± 0.45b	9.08 ± 0.44b-d	0.13 ± 0.03f
P15	72.22 ± 5.33de	9.48 ± 0.35cd	8.43 ± 0.24ef	0.16 ± 0.03ef

The means (± standard deviation) with the same letters in each column indicates no significant difference between treatments at the 5% level of probability.

Table 5
Pearson correlation among biochemical traits of elecampane populations

Trait	Chl a	Chl b	Total Chl	Carotenoid	TPC	TFC	Proline	EO
Chl a	1							
Chl b	0.672**	1						
Total Chl	0.939**	0.833**	1					
Carotenoid	0.580**	0.246*	0.505**	1				
TPC	0.733**	0.390**	0.675**	0.799**	1			
TFC	0.183	0.348**	0.289**	-0.158	0.012	1		
Proline	0.625**	0.540**	0.540**	0.845**	0.809**	-0.207*	1	
EO	-0.165	-0.172	-0.211*	-0.234*	-0.317**	-0.085	-0.179	1

Proline and the EO

Proline and EO content also exhibited significant differences among the populations. Population P6 had the highest concentration of proline at 10.06 µg g-1 fresh weight (FW), followed by populations P13, P9, and P11 (Table 4). Proline content positively correlated with Chl, carotenoid, and TPC (Table 5). A two-fold difference in EO content was observed between populations P11 (0.26%) and P14 (0.13%) (Table 4). There was a significant negative correlation between EO and total Chl, carotenoid, and TPC (Table 5).

The EO composition

Gas chromatography-mass spectrometry (GC/MS) analyses revealed the presence of 33 compounds in the EO of elecampane populations (Tables 6 and 7). The main compounds identified in the EO were β-Elemene, tricyclene, α-Pinene, isoalantolactone, and santolina Triene. β-Elemene was the only compound consistently observed in the EO of all populations over two years. Its percentage varied between 1.75% in population P9 to 34.9% in population P1 in the first year and between 2.54% in population P12 to 32.16% in population P1 in the second year.

Table 6
Essential oil components of different elecampane populations (P) in the first year (2019)

Compounds	RT	KI	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P1
Santolina triene	5.12	908						18.28	28.72	11.61		19.23	24.73	4.23	21
Tricyclene	5.53	926		27.32	1.97	1.64					11.53	5.02	5.17		
α-Thujene	5.64	930			9.21										
α-Pinene	5.85	939			22.29	2.05	7.15	15.83	13.63	2.84	4.76	15.37	15	1.61	23
Sabinene	6.91	971			2.41								1.21		1.0
Terpineol	12.77	1133			16.78										
Bicyclo (5.3.1) undec-1-en-9-one	14.26	1164					11.23	7.6	2.72			4.01		7.85	
Benzene,1(1,5-dimethyl-4-hexenyl)	14.27	1168					2.27								
Isoalantolactone	15.32	1187				53.48	27.93	31.79	32.63	58.82	78.81	20.46	5.51	82.72	23
Verbanol	15.66	1197	13.93	12.15	2.74										
Dodecane	15.77	1200	2.38			15.14									
Tridecane	20.19	1300				6.16						7.5			
Bromoacetyl bromide	21.25	1307					5.42	2.31	2.8	3.19			5		3.1
β-Elemene	21.77	1389	34.9	32.29	16.72	4.99	10.8	8.8	12.38	6.49	1.75	10.43	20.56	1.44	15
Neryl acetate	22.81	1359					10.99					4.53			
Benzene,1-(1,5-dimethyl-4-hexenyl)	24.37	13.95													
Geranyl acetone	26.85	1455	4.56	9.8	3.16								2.79		
Geranyl propanoate	27.82	14.77											3.56		
β-Selinene	28.37	1490					4.17					1.8	2.51		1.4
Neryl Isobutanoate	28.43	14.9			3.02			5.79		12.59	3.08			1.68	9.7
Valancene	28.66	1496	7.41	4.08	6.36		3.06								
α-Selinene	28.74	1498	9.58	4.63	6.78							2.51	2.25		
Pentadecane	28.82	1500	2.8	9.71		6.7		5.06							
Bicyclogermacrene	28.83	1500						3.59	2.41	3.43					
Thujyl alcohol	31.34	1721					6.73								
Caryophyllene oxide	32.16	1583	4.76		2.85		2.58						5.2		
Hexadecane	32.89	1600	2.61		5.66	2.47	2.52					2.42			
Humulene epoxide	33.2	16.08	6.15												
α-Cadinol	34.93	1654													
Muurolene(14-oxy-α)	39.22	1768	1.87										1.46		
Muurolene (14, oxy-α)	39.22	1768	1.13				2.86								
Muurolene(14-hydroxy-α)	39.64	1779	2.83										2.13		
Octadecane	40.4	1800	1.52			0.74	2.26								
Total (%)			96.53	99.98	99.95	93.37	99.97	99.99	95.29	98.97	99.93	93.28	97.08	99.53	99

Table 7
Essential oil components of different elecampane populations (P) in the second year (2020)

Compounds	RT	KI	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13
Santolinatriene	5.12	908	2.4					20.54	26.5	13.2	6.09	22.04	23.87	3.98	18.76
Tricyclene	5.53	926		24.59		5.4					10.12	7.6	7.65		
α-Thujene	5.64	930			7.81										
α-Pinene	5.85	939			25.34	2.1	6.13	13.27	15.4	4.32	5..87	16.2	13.4	3.24	27.12
Sabinene	6.91	971	2.11		1.21								1.87		1.24
Terpineol	12.77	1133			15.4										
Bicyclo (5.3.1) undec-1-en-9-one	14.26	1164					10.65	8.64	1.87			3.22		6.76	
Benzene,1(1,5-dimethyl-4-hexenyl)	14.27	1168													
Isoalantolactone	15.32	1187				54.7	24.12	34.2	30.1	50.45	74.12	21.85	4.32	75.1	20.65
Dodecane	15.66	1197	3.24			13.5									
Verbanol	15.77	1200	12.71	13.41	5.71									4.12	
Tridecane	20.19	1300				7.2						5.34			
Bromoacetyl bromide	21.25	1307					7.54		2.8	4.15			2.65		3.12
β-Elemene	21.77	1389	32.16	30.72	9.13	3.21	9.74	6.12	13.5	7.32	3.72	9.54	23.1	2.54	14.12
Neryl acetate	22.81	1359					7.67					1.65			
Benzene,1-(1,5-dimethyl-4-hexenyl)	24.37	13.95													
Geranyl acetone	26.85	1455	3.12	8.71	4.14										
Geranyl propanoate	27.82	14.77													
β-Selinene	28.37	1490					8.12					2.87	5.43		0.98
Neryl Isobutanoate	28.43	14.9			3.13	0.6		2.14		11.87	4.32			2.65	9.79
Valancene	28.66	1496	8.22	6.74	6.41	2.1	4.22	4.01	3.24						
α-Selinene	28.74	1498	7.32		8.21					3.54		3.24	3.13		2.14
Pentadecane	28.82	1500		7.32		5.43									
Bicyclogermacrene	28.83	1500						9.7	4.21	3.01					
Thujyl alcohol	31.34	1721					6.32								
Caryophyllene oxide	32.16	1583	3.73		4.63		4.34					4.3	5.76		
Hexadecane	32.89	1600	3.27	6.51	8.21	4.2	3.16				0.86				
Humulene epoxide	33.2	16.08	7.31												
α-Cadinol	34.93	1654													
Muurolene(14-oxy-α)	39.22	1768	4.55										1.56		
Muurolene(14,oxy-α)	39.22	1768	3.4				4.13								
Muurolene(14-hydroxy-α)	39.64	1779	2.83										2.13		
Octadecane	40.4	1800	3.13			1.3	2.12						3.12		
Total (%)			99.5	98	99.33	99.74	98.26	98.62	97.62	97.86	99.23	97.85	97.99	98.4	97.92

The composition of essential oils varied significantly among populations, with some compounds such as isoalantolactone constituting over 70% of the EO in specific populations (P9 and P12), while other populations like P2 and P3 lacked it entirely. This high variation in phytochemical composition is a valuable

finding (Tables 6 and 7).

Multivariate analysis

Principal Component Analysis (PCA) results indicated that the primary variability in biochemical traits and main EO components was explained by axes 1 and 2 (F1 and F2). Total phenolic content (TPC) showed different variation patterns compared to other traits. Essential oils exhibited a distinctly different trend among populations. Chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, proline, and TPC displayed similar variation patterns (Fig. 1a). Isoalantolactone was the main distinguishing component in populations P3 and P5 (Fig. 1b). Tricyclene and β-Elemene were key agents that differentiated populations P1, P2, and P15 from others. Both α-Pinene and santolina Triene were the distinguished components in populations P11, P6, P8, P14, and P10. β-Elemene was slightly identified as a specific compound for P9. Population P7 was not significantly distinguished by any EO compounds.

Agglomerative Hierarchical Clustering (AHC) analysis revealed three different clusters based on biochemical traits among elecampane populations. Cluster 1 included populations P6, P5, P14, P13, P9, and P12. Cluster 2 consisted of populations P8, P15, P11, P7, and P10. Cluster 3 comprised populations P1, P4, P2, and P3 (Fig. 2a). In terms of the main EO composition, cluster 1 included populations P3, P4, P5, and P13, while cluster 2 consisted of populations P6, P11, P8, P7, and P10. Cluster 3 included populations P2, P15, P1, P12, P9, and P14 (Fig. 2b).

Discussion

This study revealed variations in the levels of photosynthetic pigments among different populations, with P6 exhibiting the highest concentrations of chlorophyll (Chl) and carotenoids, as indicated in Table 3. This disparity could be attributed to the comparatively higher potassium (345.5 ppm) and phosphorus (P) (14.2 ppm) content within the P6 habitat when compared to other environments, as shown in Table 2. Previous research has documented the positive influence of potassium and phosphorus on augmenting photosynthetic pigment levels in various plant species, such as *Brassica oleracea* (Zhang et al. 2017) and Spinacia oleracea L. (Nemadodzi et al. 2017). Furthermore, the electrical conductivity (EC) and pH levels of these habitats may significantly impact the quantities of chlorophyll and carotenoids (Table 2). The elevated presence of photosynthetic pigments in P6 could be linked to its relatively higher pH of 6.8 in comparison to other habitats. Shareef et al., (2018) demonstrated that a decrease in pH resulted in diminished nitrogen and chlorophyll content in corn. A similar pattern is observed in the case of P1, where the chlorophyll and carotenoid contents are at a minimum, coinciding with lower pH, potassium, and phosphorus levels in comparison to other habitats.

Phenolic compounds represent a group of valuable phytochemicals found in medicinal plants, known for their diverse health benefits, including anti-cancer, antioxidant, cardioprotective, anti-bacterial, and anti-inflammatory properties (Tungmunnithum et al. 2018). In our study, we observed a significant correlation between Total Phenolic Content (TPC) and photosynthetic pigments (Table 5), consistent with findings by(Shariffa et al., 2021). However, P6 exhibited the highest TPC among the studied populations (Table 4). This could be attributed to the enhanced potential of its habitat or population in producing phenolic compounds, similar to carotenoids and chlorophyll. It's interesting to highlight that while a previous study demonstrated a positive correlation between TPC and TFC (Khosropour et al. 2021), our study yielded different results, as we found no significant correlation between TFC and TPC, as depicted in Table 5. Similar to Total Phenolic Content (TPC), the presence of proline in P6 exceeded that in other populations (Table 4). Proline, being an amino acid, can experience an increase in concentration due to improved soil nutrient availability (Pavlíková et al. 2017). Furthermore, Principal Component Analysis (PCA) affirmed that chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and TPC were the pivotal traits distinguishing P6 from the other populations (Fig. 1a and b).

The content of Essential Oils (EO) exhibited variations across different populations, ranging from 0.13–0.26% (Table 4), surpassing the 0.07% reported by Jaimand, (2001) for elecampane roots. Notably, in our study, P14 displayed the lowest EO content, which correlated with the habitat having a lower temperature than the other habitats. In general, the production of secondary metabolites is closely linked to primary metabolites. Alterations in photosynthesis and the accumulation of osmolytes can influence EO production. In this study, we observed a negative correlation between EO and photosynthetic pigments, a finding consistent with Parvanak, (2019) report, as illustrated in Table 5. It's crucial to recognize the impact of environmental factors on plant metabolism. A study byMollaei et al., (2020) demonstrated that sunlight, temperature, and rainfall play a significant role in altering the biosynthesis of both primary and secondary metabolites in plants. Plants, in response to environmental changes, adjust their production of Essential Oils (EO) as a strategy to enhance their antioxidant capacity and, consequently, their chances of survival. This adaptive mechanism is discussed in the study by Taati et al., (2022). Moreover, Ali-Arab et al., (2022) emphasized that environmental factors, particularly altitude and soil characteristics, stand as primary determinants affecting the content and composition of EO in thyme populations. These findings underscore the dynamic interplay between plants and their surroundings, highlighting the adaptive nature of plant metabolism and secondary metabolite production. In the course of this research, it was observed that while β-elemene was consistently present in the essential oil of all populations, the presence and quantity of other compounds exhibited significant variation. Notably, some of these compounds were detected in certain populations but were notably absent in others (Tables 6 and 7).

Over the span of two years of investigation, Isoalantolactone, for instance, was absent in P1, P2, and P3 populations. However, this compound dominated in P4, P5, P6, P7, P8, P9, P10, and P12 populations. Similarly, β-elemene emerged as the primary compound in P1 and P2 populations over the two years of study, while being found in comparatively smaller quantities in other populations (Tables 6 and 7). The presence or absence of these secondary metabolites in plants is predominantly regulated by genetic factors. Consequently, the observed diversity and disparities among populations concerning essential oil compounds can be attributed to genetic variations, potentially indicating the existence of distinct chemotypes within these populations. Therefore, this study unveils, or at the very least identifies, two distinct chemotypes of Isoalantolactone and β-Elemene within these plant populations.

The results of the Principal Component Analysis (PCA) shed light on an intriguing observation: the composition of EO displayed a distinct pattern compared to other biochemical attributes (Fig. 1a and 1b). It is noteworthy to mention that there is limited available information regarding the composition of EO in elecampane roots. However, Stojanović-Radić et al., (2020) reported that alantolactone and isoalantolactone were the predominant constituents, accounting

for more than 90% of the total EO. In our present study, a different composition profile emerged, with β-Elemene, tricyclene, α-Pinene, isoalantolactone, and santolina Triene being the primary components across the majority of populations. The composition appears to diverge from that reported by Stojanović-Radić et al. Moreover, the Agglomerative Hierarchical Clustering (AHC) analysis for EO compositions revealed the existence of three distinct clusters for both biochemical attributes (Fig. 2a) and the primary EO compounds (Fig. 2b). This clustering reinforces the notion that EO composition represents a unique and differentiating feature among these populations.

Conclusion

In general, these findings emphasize the considerable variation in total phenolic, total flavonoid, proline, as well as essential oil contents and composition across different elecampane populations. The variations in these compounds may be linked to genetic diversity and environmental factors within these populations. The PCA and AHC analyses provide insights into the relationships and groupings of populations based on their biochemical traits and EO compositions, which can be valuable for further research and potential applications of elecampane. Notably, it is evident that populations residing in habitats with altitudes ranging from 2000 to 2400 meters above sea level, characterized by soil with silt or silt loam texture and a pH level of 6.5 to 7, exhibited significantly higher content of essential oils and phenolic compounds. One particularly intriguing finding of this study is the distinct dissimilarity in essential oil compound types observed in populations across two consecutive years. In some cases, compounds that were the main constituents in certain populations were entirely absent in others, underscoring the presence of genetic variations and the existence of specific chemotypes within these populations. Over the course of the two-year study, isoalantolactone emerged as the primary and dominant compound in populations P4, P5, P6, P7, P8, P9, P10, and P12, while β-Elemene took precedence in populations P1 and P2. This distinction allows for the identification and introduction of at least two distinct chemotypes, specifically isoalantolactone and β-elemene, within these populations. These findings hold substantial value for elecampane breeding and domestication programs. However, further comprehensive studies are required to delve deeper into these intricate mechanisms and their implications.

Declarations

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Authors' contributions I/we declare that the contribution of particular Authors to the publication is as follows: Developing the concept, the methods and the assumptions: Hassanali Naghdi Badi, Sepideh Kalateh Jari, Ali Mehrafarin and Nader Moradi; conducting the research: Nader Moradi, Hassanali Naghdi Badi, Sepideh Kalateh Jari and Elham Danaee; statistical analyzes of data: Nader Moradi, Ali Mehrafarin and Hassanali Naghdi Badi; All authors contributed to the drafts and gave final approval for publication.

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References

1. Adams RP (2001) Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Allured publishing corporation
2. Ali-Arab H, Bahadori F, Mirza M, et al (2022) Variability in essential oil composition and phenolic acid profile of Thymus daenensis Celak. populations from Iran. Ind Crops Prod 178:114345
3. Arnon AN (1967) Method of extraction of chlorophyll in the plants. Agron J 23:112–121
4. Bajalan I, Rouzbahani R, Ghasemi Pirbalouti A, Maggi F (2018) Quali-quantitative variation of essential oil from Iranian rosemary (Rosmarinus officinalis L.) accessions according to environmental factors. Journal of Essential Oil Research 30:16–24
5. Bates LS, Waldren RP a, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207
6. Budán F, Nan M, Kocsis B, Laczkó-Zöld E (2021) Antimicrobial activity and potential secondary signal transduction mechanisms of elecampane (Inula helenium L.) root extract. Plant Cell Biotechnol Mol Biol 22:86–92
7. Chang C-C, Yang M-H, Wen H-M, Chern J-C (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 10:
8. Corlett RT (2016) Plant diversity in a changing world: status, trends, and conservation needs. Plant Divers 38:10–16
9. Jaimand K (2001) Chemical Constitutes of the Essential Oil of Elecampane (Inula helenium L.). Iranian Journal of Medicinal and Aromatic Plants Research 8:135–148
10. Khosropour E, Weisany W, Tahir NA, Hakimi L (2021) Vermicompost and biochar can alleviate cadmium stress through minimizing its uptake and optimizing biochemical properties in Berberis integerrima bunge. Environmental Science and Pollution Research 1–11
11. Mamedov N, Mehdiyeva NP, Craker LE (2015) Medicinal plants used in traditional medicine of the Caucasus and North America. Journal of medicinally active plants 4:42–66
12. Mollaei S, Ebadi M, Hazrati S, et al (2020) Essential oil variation and antioxidant capacity of Mentha pulegium populations and their relation to ecological factors. Biochem Syst Ecol 91:104084

13. Morshedloo MR, Ebadi A, Maggi F, et al (2015) Chemical characterization of the essential oil compositions from Iranian populations of Hypericum perforatum L. Ind Crops Prod 76:565–573
14. Nemadodzi LE, Araya H, Nkomo M, et al (2017) Nitrogen, phosphorus, and potassium effects on the physiology and biomass yield of baby spinach (Spinacia oleracea L.). J Plant Nutr 40:2033–2044
15. Niczad A, Sharafzadeh S, Alizadeh A, et al (2019) Variability in essential oil constituent, phenolic content, antioxidant and antimicrobial activities of different ecotypes of Zataria multiflora Boiss. from Iran. Journal of Essential Oil Bearing Plants 22:1435–1449
16. Parvanak K (2019) Investigate the effects of drought stress on some physiological parameters, percentage and yield of essential oil of Salvia sahendica. Environmental Stresses in Crop Sciences 12:1237–1249
17. Pavlíková D, Zemanova V, Břendová K, et al (2017) Effect of biochar application on the content of nutrients (Ca, Fe, K, Mg, Na, P) and amino acids in subsequently growing spinach and mustard. Plant Soil Environ 63:322–327
18. Rahimmalek M, Heidari EF, Ehtemam MH, Mohammadi S (2017) Essential oil variation in Iranian Ajowan (Trachyspermum ammi (L.) Sprague) populations collected from different geographical regions in relation to climatic factors. Ind Crops Prod 95:591–598
19. Seca AML, Grigore A, Pinto DCGA, Silva AMS (2014) The genus Inula and their metabolites: From ethnopharmacological to medicinal uses. J Ethnopharmacol 154:286–310
20. Shafi A, Hassan F, Zahoor I, et al (2021) Biodiversity, management and sustainable use of medicinal and aromatic plant resources. Medicinal and Aromatic Plants: Healthcare and Industrial Applications 85–111
21. Shareef RS, Mamat AS, Al-Shaheen MR, Ismaaiel OH (2018) The effect of soil pH and cadmium on nitrogen, phosphorus and chlorophyll contents in corn (Zea mays L). J Adv Res Appl Chem Eng 3:12–17
22. Shariffa AHM, Baharina S, Abdul R, et al ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND CHLOROPHYLL CONTENT OF KENINGAU GROWN CUCUMIS SATIVUS L. AT TWO GROWTH STAGES
23. Stojanović-Radić Z, Dimitrijević M, Genčić M, et al (2020) Anticandidal activity of Inula helenium root essential oil: Synergistic potential, anti-virulence efficacy and mechanism of action. Ind Crops Prod 149:112373
24. Taati S, Pilehvar B, Mirazadi Z (2022) Essential Oil, Phenol and Flavonoid Contents in Leaves and Fruits of Prunus scoparia (Spach) CK Schneid. Populations. Journal of Medicinal plants and By-product 11:201–209
25. Tohidi B, Rahimmalek M, Arzani A (2017) Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of Thymus species collected from different regions of Iran. Food Chem 220:153–161
26. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A (2018) Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines 5:93
27. Van Wyk AS, Prinsloo G (2018) Medicinal plant harvesting, sustainability and cultivation in South Africa. Biol Conserv 227:335–342
28. Velu G, Palanichamy V, Rajan AP (2018) Phytochemical and pharmacological importance of plant secondary metabolites in modern medicine. Bioorganic phase in natural food: an overview 135–156
29. Zhang J, Wang Y, Wang P, et al (2017) Effect of different levels of nitrogen, phosphorus, and potassium on root activity and chlorophyll content in leaves of Brassica oleracea seedlings grown in vegetable nursery substrate. Hortic Environ Biotechnol 58:5–11

Figures

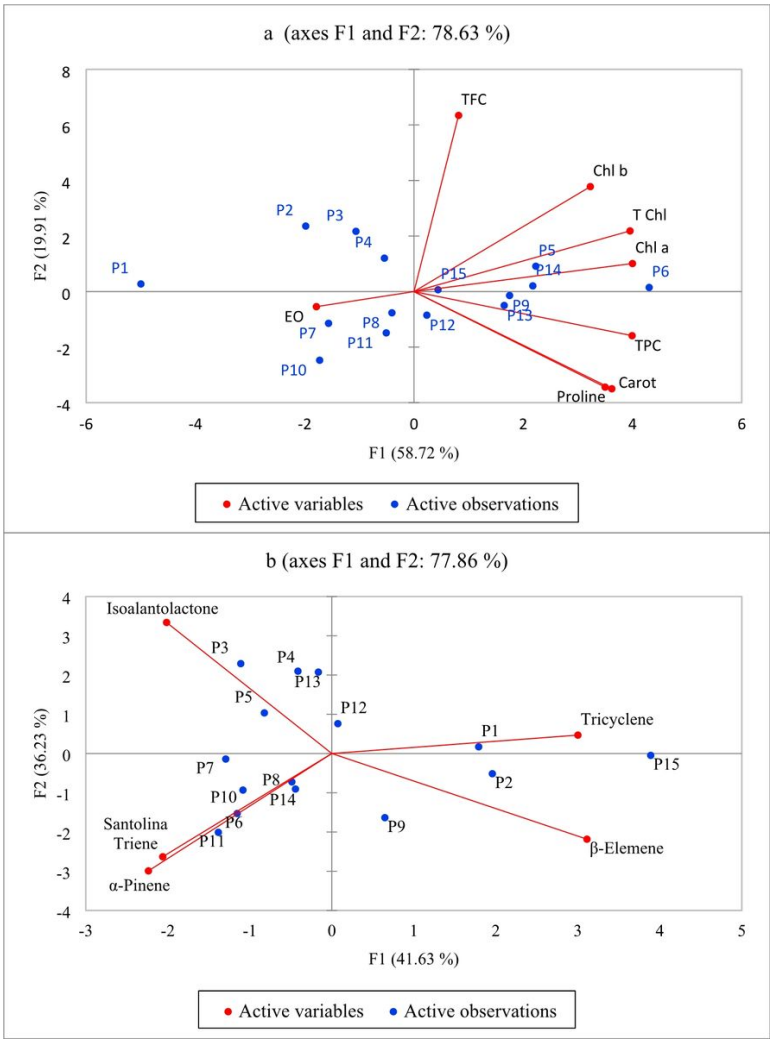

Figure 1

Principal component analysis (PCA) analysis for biochemical traits (a) and main essential oil compounds (b) of elecampane populations

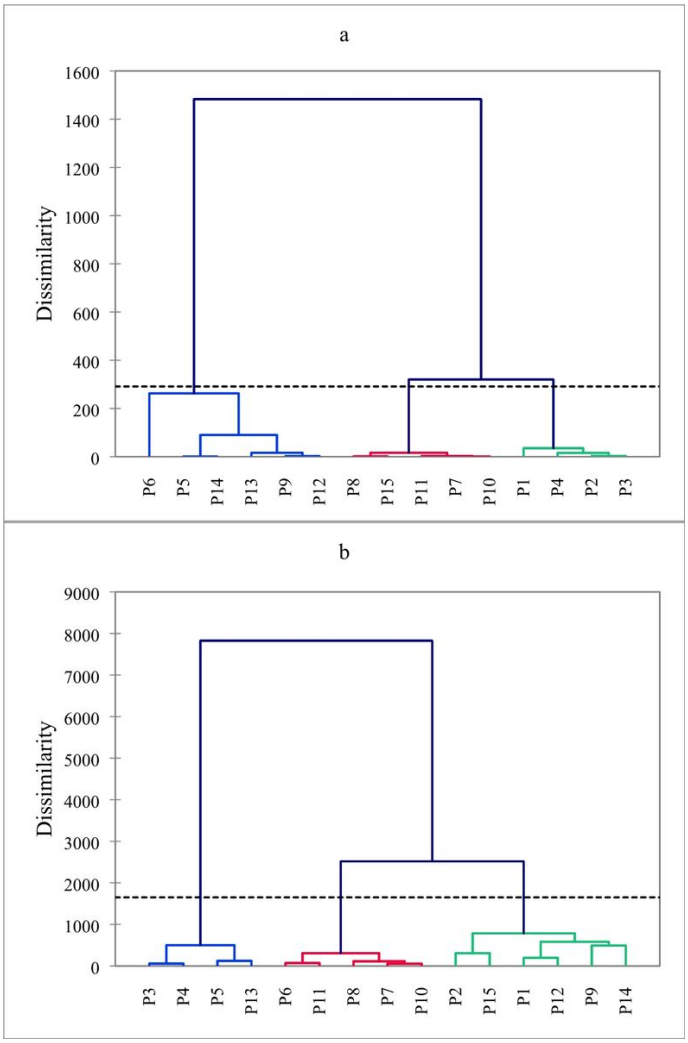

Figure 2

The dendrogram of Agglomerative Hierarchical Clustering (AHC) for biochemical traits (a) and main essential oil compounds (b) of elecampane populations