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Morpho-phytochemical screening and biological assessments of aerial parts of Iranian populations of wild carrot (*Daucus carota* L. subsp. *carota*)

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This study investigated the morphological and phytochemical characteristics of 118 genotypes of *Daucus carota* L. subsp. *carota*, collected from natural habitats in five populations across West Azerbaijan. The main objectives were to evaluate the variability among these populations and to explore their correlations with the local climatic conditions. The ultimate goal was to identify potential candidates for domestication and contribute to pre-breeding programs. Our findings revealed notable differences in plant height (PH), which ranged from 71.96 cm to 96.08 cm, with the tallest samples originating from the Gharib Hassan population (*DCP5*). The number of nodes on the main branch (NNMMB) was highest in *DCP3* and *DCP5*, with mean values of 6.44 and 6.13, respectively. Essential oil (EO) content varied among the populations, from 0.88 to 1.37 (% V/W), peaking in *DCP3*. Gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS) analyses detected 23 chemical compounds, accounting for 88.49% to 95.5% of the essential oils. The primary compounds included oxygenated sesquiterpenes (47.84% to 76.26%) and hydrocarbon monoterpenes (2.33% to 37.37%). Carotol was the dominant compound in all populations, particularly high in *DCP4* (74.03%) and *DCP5* (73.61%). Based on essential oil composition, populations were classified into distinct chemotypes: *DCP1* as chemotype I (carotol—bornyl acetate), *DCP3* as chemotype II (carotol—α-pinene), and *DCP2*, *DCP4*, and *DCP5* as chemotype III (carotol content of 64.03% to 74.03%). Correlation analysis revealed a significant negative relationship between carotol and several compounds, including daucene (-0.83), β-pinene (-0.65). Among the aerial parts, *DCP3* had the highest total phenolic content (54.81 mg GAE/g DW), while *DCP2* and *DCP3* exhibited high total flavonoid content in seeds (36.07 and 36.22 mg QE/g DW), respectively. Antibacterial activity tests showed that *DCP3* and *DCP5* had notable inhibitory effects against *Escherichia coli* and *Staphylococcus aureus*. Using circular cluster analysis, the genotypes were categorized into three main groups based on the assessed traits, revealing greater variability in phytochemical and morphological characteristics among different populations compared to variations within individuals.

Keywords Carotol, Heretical cluster analysis, Canonical correspondence analysis, DPPH%, Total phenolic contents, Total flavonoid contents, Essential oil, Stepwise regression, Antibacterial activity

Recently, there has been a notable increase in the use of natural drug compounds in the field of medicine. Functional foods and nutraceuticals have garnered significant attention for their beneficial medicinal and nutritional properties. In order to expand the variety of natural compounds available, it is essential to focus on plants that indigenous to their native ecosystems. Iran, with its diverse climate, stands out as one of the most biodiverse regions worldwide, hosting a rich array of biological and genetic diversity[1,2]. Among its many plant families, the Apiaceae commonly known as the carrot or parsley family, contains a wide range of aromatic plants. These plants are notable for containing various compounds in their essential oils[3], which warrant investigation for their potential applications and benefits across multiple domains[4].

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The climate of Iran provides ideal conditions for the growth of the Apiaceae family, supported by the presence of numerous wild plant species[5]. The active compounds found in these plants often exhibit diverse effects, including antioxidant and antibacterial properties[6–8]. These properties are not only beneficial for medicinal use but also play a crucial role in food protection by preventing spoilage and extending shelf life[9].

The Apiaceae family consists of valuable aromatic plants used in many industries including food, perfume, pharmaceuticals, cosmetics, and cosmeceuticals. Some species are popular worldwide as flavorings, seasonings, colorants, and even preservatives[8]. This botanical family includes about 455 genera and 3600–3751 species, most of which are temperate herbs[10].

Daucus carota L., originates from southwestern Asia, specifically the regions of Iran and Afghanistan[11]. Carrots have evidence of cultivation that dates back nearly five thousand years on the Iranian Plateau[12]. The wild carrot plant is primarily valued for its edible roots, which are rich in fiber, minerals, anthocyanins and carotenoids, particularly β-carotene[13]. Also in carrot root have found saponins, flavonoids, and tannins[14]. *D. carota* hole parts have been traditionally used for various purposes, including as aphrodisiacs, diuretics, antidiabetic agents[15].

Essential oils (EOs), which are concentrated liquids containing volatile components with aromatic properties, are extracted from different plant parts. The quality and quantity of essential oil can be influenced by various factors, such as genetics, nutrition, sunlight, temperature, humidity, location, and harvesting time[16,17]. The seeds of Apiaceae plants, such as wild carrots, contain valuable EOs that have applications in the food, pharmaceutical, and spice sectors[18]. A study focusing on the essential compounds present in carrot aerial parts sourced from Turkey highlighted the significance of key compounds like carotol (27%), elemicine (18.1%), and limonene (16%), which exhibit notable antioxidant, antidiabetic, and antidiabetic properties[19]. In samples of inflorescences with seeds collected from Montenegro at various stages of maturity, β-bisabolene (32.3%), 11-α-(H)-himachal-4-en-1-β-ol (27.9%), elemicin (10.1%), and α-longipipene (7.7%) were identified as the predominant components. It was observed that the mature umbels possessed a higher essential oil content (1.06 mL/100 g) compared to the flowering umbels (0.65 mL/100 g)[20]. α-pinene (23.5%) was identified as a major compound in the aerial parts of *Daucus carota* collected from Morocco, with significant antibacterial and antioxidant properties[21]. Geographic variations in Turkey significantly influenced the components, ratios, and yields of *Daucus carota* essential oils. In this study the key constituents were including carotol (1–74.6%), β-bisabolen (0.9–62.4%), 11-α-(H)-himachal-4-en-1-β-ol (0.3–49.4%), and trans-methylisoeugenol (1–45.7%)[22]. The major compounds of aerial parts' essential oil from Algeria were: alismol (15.2%), (E)-β-caryophyllene (10.1%), myrcene (9.6%), α-humulene (9.5%), and β-ionone (5.2%)[23]. Mature umbels with seeds from Italy and Portugal exhibited distinct chemical compositions, with Sardinian samples predominantly containing β-bisabolene (17.6–51.0%) and 11-α-(H)-himachal-4-en-1-β-ol (9.0–21.6%). In this study, Portuguese samples were primarily composed of geranyl acetate (5.2–65.0%) and α-pinene (3.5–37.9%). Notably, the antifungal activities of the essential oils from Sardinian samples were the highest among the tested groups[24].

Although valuable information exists on essential oil samples from wild carrot, no studies have examined the genotypes of its populations in Iran, a region considered a potential origin of *Daucus carota*. Additionally, although *Daucus carota* L. subsp. *carota* is acknowledged for its medicinal properties, data on its morphological and phytochemical variations within Iran are limited. Therefore, this study aims to investigate the essential oil composition, of 118 individuals of *D. carota* collected from five natural habitats. The antioxidant and antibacterial effects were also evaluated within these populations. Multivariate analysis will also be employed to explore these variations and identify potential new chemotypes. A comprehensive understanding of the chemical compounds, morphological traits and bioactive properties, is essential for identifying superior species suitable for use as new germplasm in breeding programs.

Results and discussion

This study investigate the chemical and phytochemical diversity among 118 individuals from five populations of *Daucus carota* L. subsp. *carota*. The collection areas, genotype numbers, soil characteristics, and climatic conditions of five populations are detailed in Table 1. The *Daucus carota* populations (*DCPs*: *DCP1-DCP5*) corresponding to Oshnavieh Road, Qarayi, Mavana, Band, and Gharib Hassan respectively. Specifically, *DCP1* comprises genotypes G1 to G22, *DCP2* includes G23 to G45, *DCP3* spans G46 to G70, *DCP4* covers G71 to G94, and *DCP5* contains G95 to G118. The study site is situated in the West Azerbaijan province of Iran, near to the city of Urmia. Furthermore, detailed information about the study area including geographic maps, depicting slope, elevation, geological characteristics, and the exact locations is illustrated in Fig. 1a-f. The geographic map generated with ArcGIS 10.8 (ESRI) URL: https://support.esri.com/en-us/products/arcmap.

Morphological dimension analysis

Table 2 presents the average morphological characteristics for each population with their respective standard errors. Also different organs from collected samples are illustrated in Fig. 2. Plant height (PH) varied between 71.96 cm and 96.08 cm, with the tallest specimens recorded in the Gharib Hassan population (*DCP5*). The number of nodes on the main branch (NNMMB) was highest in both *DCP3* and *DCP5*, reporting values of 6.44 and 6.13, respectively. Additionally, the number of flowers on the secondary stem (FNMSIN) ranged from 1.17 to 2.19, while the number of nodes on the secondary stem (NNMSIN) spanned from 1.15 to 3.21, with the maximum values observed in *DCP5*. *DCP2* exhibited the highest internode length on the main branch (INNLMB) at 12.37 cm, along with a leaf length (LL) of 12.3 cm and a leaf width (LW) of 6.87 cm. The ratio of leaf length to leaf width (LL/LW) varied from 1.78 to 2.77, with the highest ratio observed in *DCP4*. Furthermore, additional morphological measurements were presented in Table 2. The broad ranges of these measurements highlight substantial morphological diversity among the populations studied. The wild carrot (*Daucus carota* subsp. *carota*) is primarily utilized for its root, which has been the focal point in breeding programs. However, recent attention to the functional aspects of plants has led to an increased exploration of other plant parts for

	Oshnavieh road	Qarayi	Mavana	Band	Gharib Hassan
	**DCP1* (G1-G22)	*DCP2* (G23-G45)	*DCP3* (G46-G70)	*DCP4* (G71-G94)	*DCP5* (G95-G118)
Climatic characterization					
Longitude	37° 21′ 44″	37° 28′ 28″	37° 31′ 42″	37° 28′ 38″	37° 29′ 23″
Latitude	45° 09′ 01″	44° 48′ 05″	44° 47′ 06″	44° 57′ 13″	45° 09′ 17″
Altitude (m)	1361	1883	1803	1587	1318
Annual precipitation (mm)	404	398	400	288	405
Relative Humidity (%)	59	57	57	58	61
Annual Average Temperature (°C)	12	10.5	10	12.3	11.4
Soil characterization					
pH (1:2)H_2O	7.94	8.1	8.07	8.06	8.03
EC (dS m^{-1})	1.04	0.76	0.9	1.06	1.12
O.C. (%)	0.1	1.26	0.9	2.3	0.9
O.M. (%)	0.17	2.17	1.55	3.97	1.55
$CaCO_3$ (%)	16	5	26	17	26
Clay (%)	44	44	56	24	16
Silt (%)	37	30	30	12	57
Sand (%)	19	26	14	64	27
Total N (%)	0.01	0.126	0.09	0.23	0.09
K exchangeable (mg kg^{-1})	42.3	21.7	21.7	9.1	81.5
P exchangeable (mg kg^{-1})	27	9	14	9	47
Fe (mg kg^{-1})	19	7.5	8.4	8.6	11.7
Zn (mg kg^{-1})	0.8	1.1	0.7	0.3	5.1
Cu (mg kg^{-1})	2.4	3	2.4	0.7	6.8
Mn (mg kg^{-1})	6.3	16.7	5.9	11.6	5.8
Ca (Meq l^{-1})	6.4	5.6	6.4	7.2	7.8
Mg (Meq l^{-1})	8.2	3	2.4	4.6	7.6

Table 1. Collection areas, genotypes numbers, geographical, climatic and soil characteristics of Daucus carota populations **Daucus carota* populations (*DCPs*).

supplementary studies. Given that the wild carrot originates from the southwestern regions of Asia like Iran, investigating the populations in these areas can aid in identifying diverse traits, thereby providing essential tools for the breeding of this species. To achieve this objective, it is crucial to maintain a broad range of plant diversity, as this facilitates the selection of suitable candidates for breeding initiatives. The existing diversity within this plant species is important, as it serves as a rich reservoir of genetic resources that can inform effective breeding strategies[25].

Essential oil (EO) content, compositions, chemo-typing and correlation

The essential oils (EOs) displayed a spectrum of colors, ranging from colorless to pale yellow, and each exhibited varying scent intensities. The primary reason for the observed differences in color among the EO samples is likely the alteration of their chemical compounds across the evaluated populations[26,27]. The EO content varied among populations, ranging from 0.88 to 1.37 (% V/W) with the highest amounts was obtained in *DCP3* (Table 3). This region has a relatively high elevation of 1803 m compared to other populations. The region receives an average annual precipitation of 400 mm, experiences a minimum relative humidity level of 57%, and has an average annual temperature of 10 °C. EO yields of *D. carota* subsp. *carota*, derived from the aerial parts, were found to be 0.7% from blooming umbels and 1.0% from ripe umbels (containing mature seeds) in samples collected from Portugal and Baunei, Italy[24]. Additionally, a yield of 0.88% was reported from samples collected in Turkey[22].

According to the GC and GC–MS analyses, a total of 23 distinct compositions were identified, which together accounted for approximately 88.49% to 95.5% of the EOs (Table 3). The main chemical groups identified in the EOs of *Daucus carota* subsp. *carota* included oxygenated sesquiterpenes (47.84 to 76.26%), hydrocarbon monoterpenes (2.33 to 37.37%), sesquiterpene hydrocarbons (2.22 to 5.69%), oxygenated monoterpenes (1.05 to 3.92%), and other compound categories (1.78 to 25.54%). The major compounds found in the EOs of the populations were carotol (46.64 to 74.03%), Daucene (1.38 to 3.92%), limonene (0.34 to 3.72%), and geraniol (0.49 to 2.91%). Notably, the highest concentration of carotol was recorded in the *DCP4* population at 74.03%, followed closely by the *DCP5* population at 73.61%. Carotol emerged as the dominant compound across the populations, specifically noted in *DCP4* and *DCP5*. This finding is significant because carotol is known for its medicinal properties, including antioxidant and anti-inflammatory effects, which may enhance the pharmacological potential of these populations and inform future breeding programs for cultivating desirable traits[28]. The presence of a predominant compound with a high percentage in the EOs of these populations may hold significant value for various pharmaceutical and food industries[29]. The composition of major compounds

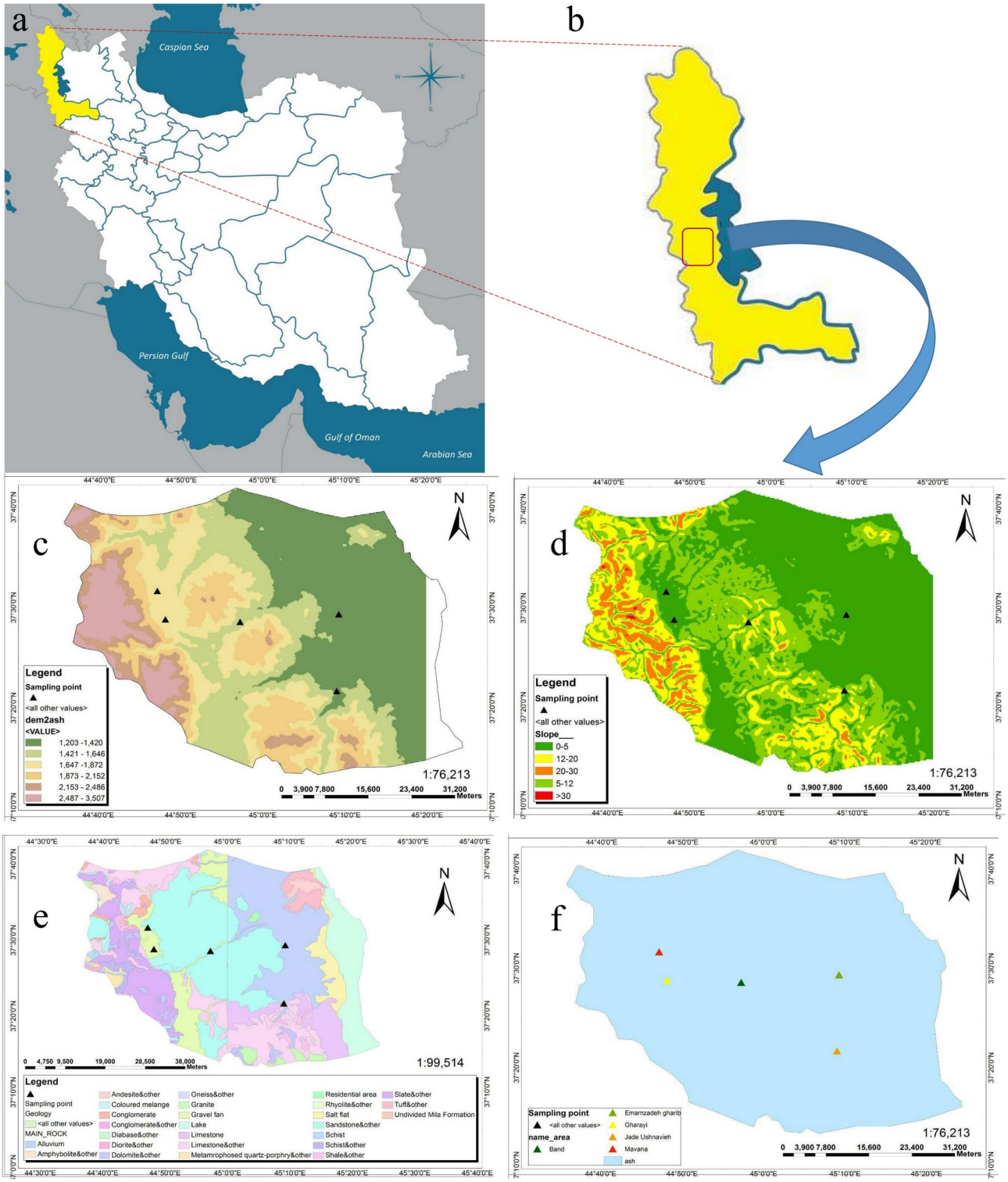

Fig. 1. Detailed information about the study area including a and b) geographic maps c) Digital elevation model (DEM) d) slope, e) geological characteristics, and f) Exact locations of the five populations.

(e.g., carotol) and other constituents varied significantly across the collected samples, a difference likely linked to their diverse geographical origins. This variability highlights a key advantage of plants from natural habitats. It supports the discovery of crucial chemotypes that can be targeted for domestication or breeding programs, serving as a valuable resource for agricultural development. Moreover, the extensive diversity of EO compounds has been documented in prior studies examining various plant species. The composition and quantity of essential oils in plants are influenced by a multitude of factors, including sexual dynamics, seasonal changes, ontogenetic

Morphological traits	abbreviations	units	Oshnavieh road (*DCP1*)	Qarayi (*DCP2*)	Mavana (*DCP3*)	Band (*DCP4*)	Gharib Hassan (*DCP5*)
Plant height	PH	cm	86.27 ± 5.76^{a}	71.96 ± 2.69^{b}	88.76 ± 2.36^{a}	92.75 ± 3.82^{a}	96.08 ± 5.74^{a}
Nodes number on the main branch	NNMMB	-	4.77 ± 0.46^{b}	3.91 ± 0.19^{b}	6.44 ± 0.20^{a}	4.88 ± 0.19^{b}	6.13 ± 0.49^{a}
Flowers number in the secondary stem	FNMSIN	-	2.12 ± 0.24^{a}	1.33 ± 0.15^{bc}	1.17 ± 0.08^{c}	1.93 ± 0.19^{ab}	2.29 ± 0.35^{a}
Nodes number of secondary stem	NNMSIN	-	2.96 ± 0.14^{a}	2.89 ± 0.14^{a}	1.15 ± 0.01^{b}	1.38 ± 0.02^{b}	3.21 ± 0.16^{a}
Internode length in the main branch	INNLMB	cm	10.50 ± 0.58^{b}	12.36 ± 0.39^{a}	8.91 ± 0.35^{c}	11.69 ± 0.38^{ab}	8.82 ± 0.62^{c}
Leaf length	LL	cm	9.41 ± 0.62^{b}	12.30 ± 0.87^{a}	8.67 ± 0.33^{b}	10.58 ± 0.69^{ab}	9.76 ± 0.52^{b}
Leaf width	LW	cm	4.32 ± 0.44^{b}	6.87 ± 0.48^{a}	3.51 ± 0.24^{b}	4.29 ± 0.35^{b}	5.94 ± 0.44^{a}
Leaf index (length/width)	LL /LW	ratio	2.47 ± 0.21^{a}	1.83 ± 0.01^{b}	2.67 ± 0.17^{a}	2.76 ± 0.03^{a}	1.78 ± 0.13^{b}
Petiole length	PL	cm	4.28 ± 0.55^{a}	2.79 ± 0.34^{b}	1.80 ± 0.20^{c}	2.85 ± 0.24^{b}	2.26 ± 0.21^{bc}
Inflorescence length on main branch	INLMB	cm	30.56 ± 1.78^{a}	22.28 ± 1.35^{b}	31.38 ± 1.34^{a}	32.85 ± 2.07^{a}	34.63 ± 2.09^{a}
Main stem Diameter	MSD	cm	0.40 ± 0.04^{a}	0.45 ± 0.03^{a}	0.44 ± 0.02^{a}	0.46 ± 0.04^{a}	0.47 ± 0.03^{a}
Main umbel diameter	MUD	cm	4.85 ± 0.38^{c}	6.02 ± 0.24^{ab}	4.89 ± 0.23^{c}	6.88 ± 0.39^{a}	5.21 ± 0.43^{bc}
Bract length	BL	cm	3.13 ± 0.05^{a}	2.28 ± 0.05^{b}	1.46 ± 0.02^{d}	1.86 ± 0.02^{c}	2.35 ± 0.03^{b}
Bract number	BNM	-	8.59 ± 0.10^{a}	7.82 ± 0.02^{b}	8.60 ± 0.13^{a}	8.01 ± 0.08^{b}	8.67 ± 0.05^{a}
Rays number of umbel	RNM	-	44.33 ± 0.33^{b}	36.22 ± 0.30^{e}	40.92 ± 0.37^{d}	42.75 ± 0.55^{c}	46.89 ± 0.30^{a}
1000 seed weight	W	grams	0.75 ± 0.00^{a}	0.72 ± 0.00^{a}	0.71 ± 0.00^{a}	0.77 ± 0.01^{a}	0.64 ± 0.00^{b}

Table 2. Phenotypical characteristics (means ± standard errors) of D. carota across 118 genotypes from five studied populations

stages, genetic variations, as well as ecological and environmental characteristics[27,30,31]. For the categorization and clustering of five studied populations, the analyzed compounds in a two-way hierarchical clustering analysis were examined. Based on the results, carotol was found to have the most significant impact, followed by two compounds: butrnyl acetate and α-pinene, in clustering the populations. Accordingly, *DCP1* was designated as chemotype I (carotol—butyl acetate), *DCP3* as chemotype II (carotol—α-pinene), and populations *DCP2*, *DCP4*, and *DCP5* were identified as chemotype III (with a very high percentage of carotol ranging from 64.03 to 74.03%) (Fig. 3). The carrot seed EO market size was valued at USD 2.45 billion in 2023 and is estimated to reach USD 3.2 billion by 2030, growing at a CAGR of 5% from 2024 to 2030 (https://www.verifiedmarketreports.com/product/carrot-seed-essential-oil-market/). Carotol, recognized in multiple studies as the primary compound in wild carrots, has drawn considerable attention from the cosmetics and food sectors. This interest stems from efforts to identify high-yield chemotypes with elevated concentrations of this valuable component, positioning it as a strategic target for commercial cultivation and industry applications[32]. In this regard, chemotypes characterized by only a few dominant constituents have attracted the attention of various industries[33]. Two-Way Hierarchical Clustering Analysis serves as a robust method for identifying and classifying these chemotypes, allowing for a deeper understanding of the relationship between different populations and their chemical compositions. By focusing on these specific chemotypes, industries can optimize their products to meet consumer demands and explore new market opportunities[34].

Figure 4 presents a detailed analysis of the correlations between various components of EOs, both quantitatively and visually. In this Figure, larger blue points indicate positive correlations, while larger red points show negative correlations. Specifically, *E*-β-caryophyllene exhibited a strong positive correlation with linalool (0.89), bornyl acetate (0.61), and geraniol (0.79). Conversely, it showed negative correlations with geranyl acetate (-0.63). Additionally, carotol, recognized as a primary constituent of the EO, displayed a significant negative correlation with daucene (-0.83), β-pinene (-0.65), (*E*)-methyl isoeugenol (-0.84), terpin-4-ol (-0.78), and *p*-cymene (-0.78). Further correlations among the various traits are also illustrated in Fig. 4.

Total phenolic content (TPC) and total flavonoid content (TFC)

The analysis of total phenolic content (TPC) and total flavonoid content (TFC) across various locations revealed significant variations ($p < 0.05$). Among the aerial parts (AP), the highest TPC was observed in sample *DCP3* (54.81 mg of gallic acid equivalent (GAE)/g DW) ($p < 0.05$) (Table 4). Conversely, the lowest TPC (10.82 mg GAE/g DW) was measured in Band (*DCP4*). For the seed samples, the highest TPC was noted in Oshnavieh Road (*DCP1*) at 20.26 mg GAE/g DW, whereas Gharib Hassan (*DCP5*) showed the lowest TPC (4.09 mg GAE/g DW). The order of TPC (based on mg GAE/g DW) in aerial parts (AP) from the various populations, ranked from highest to lowest, is as follows: *DCP3* (54.81) > *DCP5* (27.10) > *DCP2* (21.79) > *DCP1* (13.80) > *DCP4* (10.82). In contrast, the lowest TFC was found in Gharib Hassan (*DCP5*), with a value of 26.07 mg QE/g DW. More information about TPC and TFC are shown in Table 4. These results highlight the significant variability in flavonoid content among different populations and plant parts. This difference matches findings from other studies that indicate abiotic stressors, like temperature, drought, salinity, ultraviolet radiation and variations in soil nutrients found in native habitats, can boost phenolic production as a protective response[35]. Phenolic compounds have been shown to possess antimicrobial and antioxidant properties, which play a crucial role in enabling plants to defend against infections caused by pathogens and pathogenic microorganisms. Furthermore, the presence of these compounds in plant tissues provides a protective mechanism against the harmful effects of reactive oxygen species (ROS). This dual functionality supports the overall health and resilience of plants in their environments[36]. In resource-limited environments, plants strengthen their defensive mechanisms

Fig. 2. Different organs from collected samples of *D. carota*.

No	Constitutes	Formula	RI	Oshnavieh road	Qarayi	Mavana	Band	Gharib Hassan	Grand mean	SE	* (CV %)
				DCP1 (G1-G22)	*DCP2* (G23-G45)	*DCP3* (G46-G70)	*DCP4* (G71-G94)	*DCP5* (G95-G118)			
1	α-pinene	$C_{10}H_{16}$	946	1.06	12.16	18.52	2.12	7.82	8.34	3.24	86.96
2	Camphene	$C_{10}H_{16}$	962	0	0.69	1.08	0	0.45	0.44	0.21	104.39
3	Sabinene	$C_{10}H_{16}$	975	0.55	1.61	6.66	0.44	3.53	2.56	1.17	101.92
4	β-pinene	$C_{10}H_{16}$	977	0.38	1.45	5.95	1.13	1.73	2.13	0.98	103.16
5	β -Myrcene	$C_{10}H_{16}$	1005	0	0.5	1.02	0.36	0.44	0.46	0.16	78.97
6	*p*-cymene	$C_{10}H_{14}$	1050	0	0	0.32	0	0	0.06	0.06	223.61
7	**Limonene**	**$C_{10}H_{16}$**	**1053**	**0.34**	**1.74**	**3.72**	**0.75**	**0.79**	**1.49**	**0.63**	**94.15**
8	Linalool	$C_{10}H_{18}O$	1108	0.6	0.26	0.48	0.78	0.18	0.48	0.12	57.76
9	terpinen-4-ol	$C_{10}H_{18}O$	1219	0.41	0.3	0.84	0	0.47	0.4	0.14	75.13
10	α-terpineol	$C_{10}H_{18}O$	1233	0	0	0.33	0.27	0	0.12	0.07	138.07
11	**Geraniol**	**$C_{10}H_{18}O$**	**1268**	**2.91**	**0.49**	**0.57**	**1.53**	**0.87**	**1.27**	**0.45**	**78.65**
12	Bornyl acetate	$C_{12}H_{20}O_2$	1327	23.21	0	0	0	0	4.64	4.46	223.61
13	α-terpinyl acetate	$C_{12}H_{20}O_2$	1375	2.01	0.64	1.4	2	2.81	1.77	0.36	45.55
14	**Daucene**	**$C_{15}H_{24}$**	**1465**	**1.78**	**2.45**	**3.92**	**1.69**	**1.38**	**2.24**	**0.45**	**45.23**
15	geranyl acetate	$C_{12}H_{20}O_2$	1474	0	0.82	0.51	0	0	0.27	0.17	143
16	(E)-β-caryophyllene	$C_{15}H_{24}$	1480	0.58	0.35	0.49	0.55	0.4	0.47	0.04	20.60
17	trans-α-Bergamotene	$C_{15}H_{24}$	1494	0.65	0.28	0.59	0.52	0.44	0.50	0.06	29.03
18	(E)-β-Farnesene	$C_{15}H_{24}$	1519	0.18	0	0.35	0	0	0.11	0.07	148.21
19	ɣ-himachalene	$C_{15}H_{24}$	1525	0	0	0.34	0	0	0.07	0.07	223.61
20	(E)-methyl isoeugenol	$C_{11}H_{14}O_2$	1530	0.32	0.32	0.31	0	0	0.19	0.08	91.31
21	**Carotol**	**$C_{15}H_{26}O$**	**1683**	**56.19**	**64.03**	**46.64**	**74.03**	**73.61**	**62.9**	**5.24**	**18.63**
22	Daucol	$C_{15}H_{26}O_2$	1733	0.51	1.33	0.66	0.88	0.58	0.79	0.15	41.83
23	Bulnesol	$C_{15}H_{26}O$	1771	1.12	1.46	0.54	1.35	0	0.89	0.27	68.59
	Monoterpene hydrocarbons			2.33	18.15	37.37	4.8	14.76	15.48	6.22	89.84
	Oxygenated monoterpenes			3.92	1.05	2.22	2.67	1.52	2.28	0.50	48.81
	Sesquiterpene hydrocarbons			3.19	3.08	5.69	2.76	2.22	3.39	0.60	39.58
	Oxygenated sesquiterpenes			57.82	66.82	47.84	76.26	74.19	64.58	5.66	18.32
	Others			25.54	1.78	2.22	2	2.81	6.87	4.67	152.02
	Total			92.8	90.88	95.34	88.49	95.5			
	Essential oil yield % (V/W)			0.88 ± 0.028	0.88 ± 0.015	1.37 ± 0.071	0.91 ± 0.26	0.97 ± 0.43			

Table 3. Essential oil constituents of D. carota samples collected from five natural habitats. RI: Retention Index. Significant values are in bold.

against environmental stressors while carefully regulating the synthesis of secondary metabolites. This strategic approach reflects their adaptive responses to ecological constraints for survival[37].

Antioxidant activity

The DPPH free radical scavenging activity for aerial parts (AP) was highest (53.25%) in samples from *DCP5*, while Band (*DCP4*) exhibited the lowest activity at 7.55% (Table 4). In the case of seed samples, *DCP2* demonstrated the highest DPPH scavenging activity at 36.71%, whereas *DCP5* had the lowest activity (7.31%). For the Ferric Reducing Antioxidant Power (FRAP) assay, Mavana (*DCP3*) showed the greatest antioxidant capacity for APs, with a measurement of 179.74 µmol Fe(II)/g dry weight (DW). In contrast, Gharib Hassan (*DCP5*) recorded the lowest FRAP value at 21.11 ± 0.34 µmol Fe (II)/g DW. For seed samples, the highest FRAP value was noted at Oshnavieh Road (*DCP1*), reaching 242.15 mg $FeSO_4$/g DW.

Antibacterial activity

The antibacterial activity of extracts derived from various populations of wild carrot was evaluated against two bacterial strains: *Escherichia coli* and *Staphylococcus aureus*. After a 24 h incubation period, the extracts demonstrated significant antibacterial effects against both evaluated strains ($p < 0.01$). The presence of these extracts resulted in the formation of inhibition zones, indicating an effective suppression of bacterial growth. As illustrated in Fig. 5a, the antibacterial effects of all evaluated populations against both bacterial strains showed a statistically significant difference compared to the negative control. However, the extracts exhibited less antibacterial activity than the positive control (Tetracycline 400 mg/ml) (Fig. 5b). For *E. coli*, the order of inhibitory effects was as follows: control$^+$ (3.23) > DCP3 (1.24), DCP2 (1.15) > DCP1 (1.05), DCP4 (0.81) > control$^-$ (0). Similarly, for *S. aureus*, the results showed an order of inhibitory effects as follows: control$^+$ (2.8) > DCP5 (1.82), DCP3 (1.16), DCP1 (1.01) > DCP4 (0.84) > control$^-$ (0). These findings indicate that while the extracts from wild carrot populations were effective in inhibiting bacterial growth, their efficacy was variable

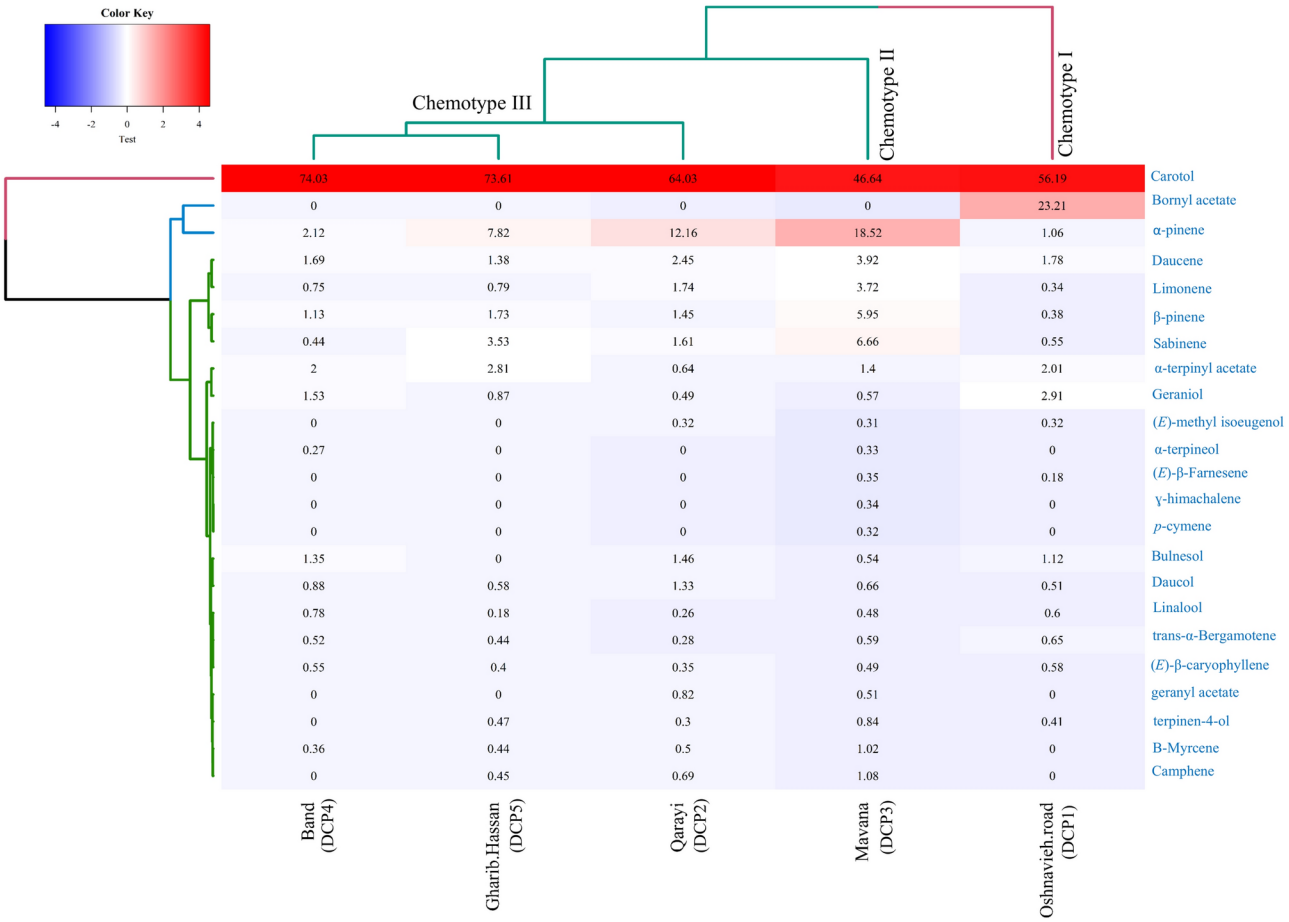

Fig. 3. Two-ways clustering analysis with two distinct dendrograms rows × columns respectively including essential oil constituent's × *Daucus carota* populations (*DCPs*).

among the different populations studied. The variation in inhibition zone sizes observed in the study can be attributed to the differences in bacterial types, specifically *E. coli* and *S. aureus*, as well as the structural variances in their cell membranes[38]. These findings indicate that extracts from wild carrot populations can effectively inhibit bacterial growth, though their effectiveness varies among different populations. This variability is likely due to differences in the chemical composition of the extracts, which may be influenced by environmental conditions and the genetic diversity within each population[39]. Understanding these factors can help optimize the use of wild carrot extracts in antibacterial applications.

Correlations, and multivariate and regression analysis with combined data

The study aimed to examine the relationship between phytochemical traits and their antioxidant and antibacterial properties by using a Mantel Test correlation matrix (Fig. 6). This study systematically evaluated antioxidant and antibacterial activities in wild carrot tissues. Antioxidant capacity was quantified via FRAP (reducing power) and DPPH (radical scavenging) assays in both seeds and aerial parts, while antibacterial efficacy was tested against two Gram-positive and Gram-negative bacterial strains to assess broad-spectrum bioactivity. Additionally, with Mantel Test EO component parameters, morphological traits and phenolic compounds such as AP TFC and TPC, Seed TFC and TPC were assessed. The results indicated that only a limited number of evaluated traits significantly correlated with antioxidant and antibacterial properties at the studied bacterial strains. For antioxidant activity, two key traits including Seed TPC and AP TFC exhibited significant correlations, with Mantel's $P < 0.05$ and Mantel's $r > 0.4$. In relation to *E. coli*, no significant traits with high influence were observed. However, for the strain *S. aureus*, bulnesol from the essential oil showed a significant relationship with antibacterial properties (Mantel's $P < 0.05$, Mantel's $r > 0.4$). Notably, compounds like α-terpinyl acetate and AP TFC demonstrated stronger associations with the antibacterial properties of *S. aureus*. Most traits displayed limited isolated effects, which may be attributed to the synergistic effects of phytochemical compounds. This comprehensive approach sheds light on the intricate relationships between various biological traits and their potential applications in the industry[40].

Correlation analysis is a valuable tool for exploring the relationships between various traits, with significant implications for plant breeding and domestication practices. Figure 6 provides a visual representation of Pearson's correlation coefficients among key essential oil (EO) constituents and other attributes. In this figure, large

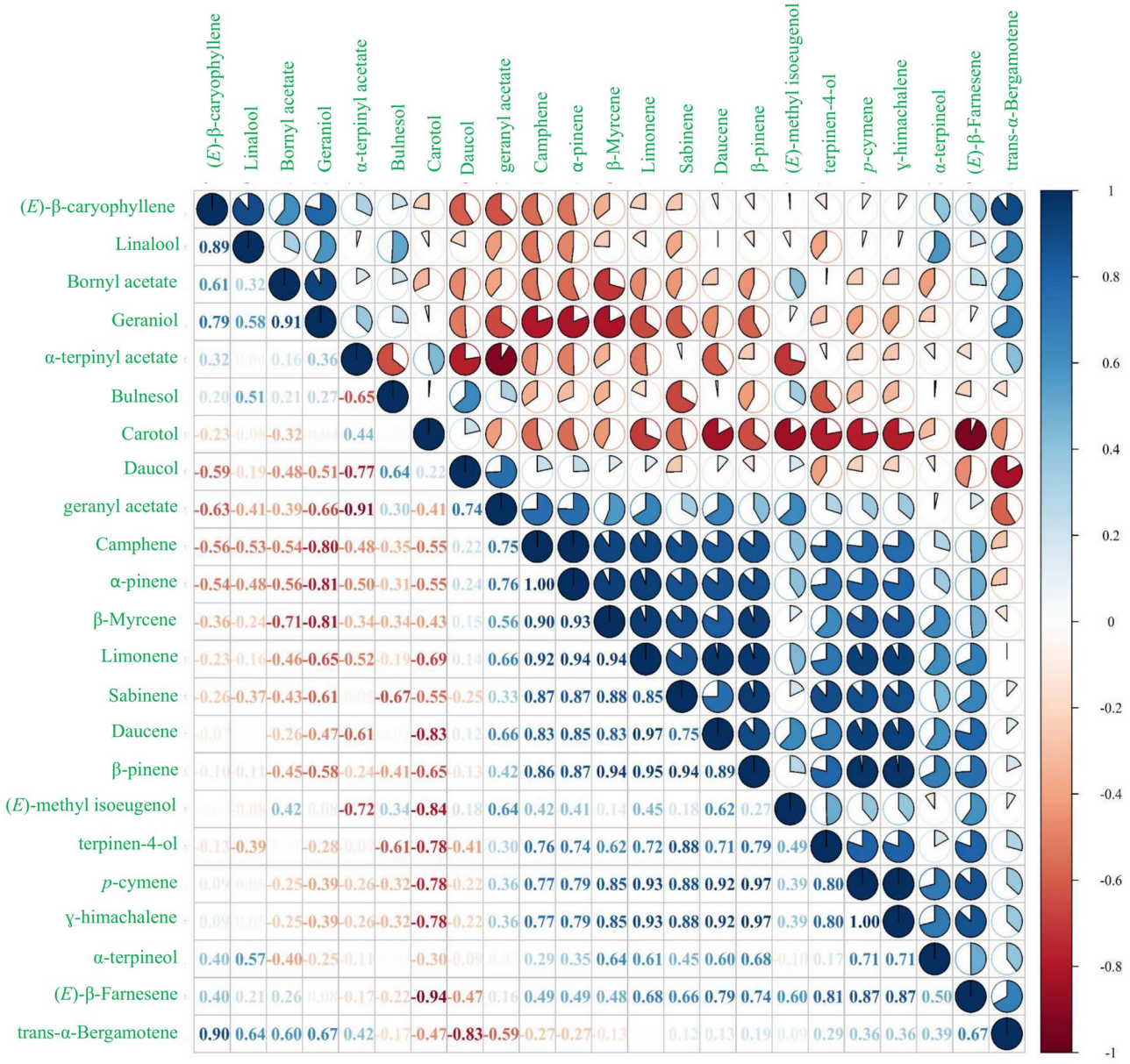

Fig. 4. Simple correlation analysis of 23 phytochemicals in *Daucus carota* Populations (DCPs): Dark blue indicates strong positive correlation (range 0 to + 1) and dark red indicates strong negative correlation (range 0 to -1).

Phytochemical compounds	Oshnavieh road (*DCP1)	Qarayi (DCP2)	Mavana (DCP3)	Band (DCP4)	Gharib Hassan (DCP5)
AP TPC (mg AE/gDW)	13.80 ± 0.10^{d}	21.79 ± 0.34^{c}	54.81 ± 0.57^{a}	10.82 ± 0.26^{e}	27.10 ± 0.24^{b}
Seed TPC	20.26 ± 0.21^{a}	17.93 ± 0.20^{b}	13.12 ± 0.27^{c}	7.22 ± 0.07^{d}	4.09 ± 0.06^{e}
AP TFC (mg Qu/DW)	27.21 ± 0.27^{c}	37.12 ± 0.33^{b}	36.50 ± 0.34^{b}	24.26 ± 0.32^{d}	56.09 ± 0.41^{a}
Seed TFC	26.87 ± 0.44^{bc}	36.07 ± 0.18^{a}	36.22 ± 0.44^{a}	28.04 ± 0.60^{b}	26.07 ± 0.30^{c}
AP DPPH%	11.92 ± 0.25^{d}	25.84 ± 0.21^{b}	24.41 ± 0.36^{c}	7.55 ± 0.07^{e}	53.25 ± 0.91^{a}
Seed DPPH%	20.22 ± 0.36^{b}	36.71 ± 0.34^{a}	18.80 ± 0.19^{c}	10.17 ± 0.18^{d}	7.31 ± 0.10^{e}
AP FRAP	151.36 ± 1.15^{b}	135.96 ± 1.38^{c}	179.74 ± 1.35^{a}	80.45 ± 0.86^{d}	152.11 ± 0.34^{b}
Seed FRAP	242.15 ± 1.93^{a}	179.81 ± 1.32^{c}	173.15 ± 0.80^{d}	187.29 ± 1.85^{b}	29.99 ± 0.40^{e}

Table 4. Total phenolic and flavonoid contents, DPPH free radical scavenging activity, and Ferric Reducing Antioxidant Power (FRAP) of the aerial parts and seed samples from five studied populations. AP: Aerial parts. **Daucus carota* populations (*DCPs*).

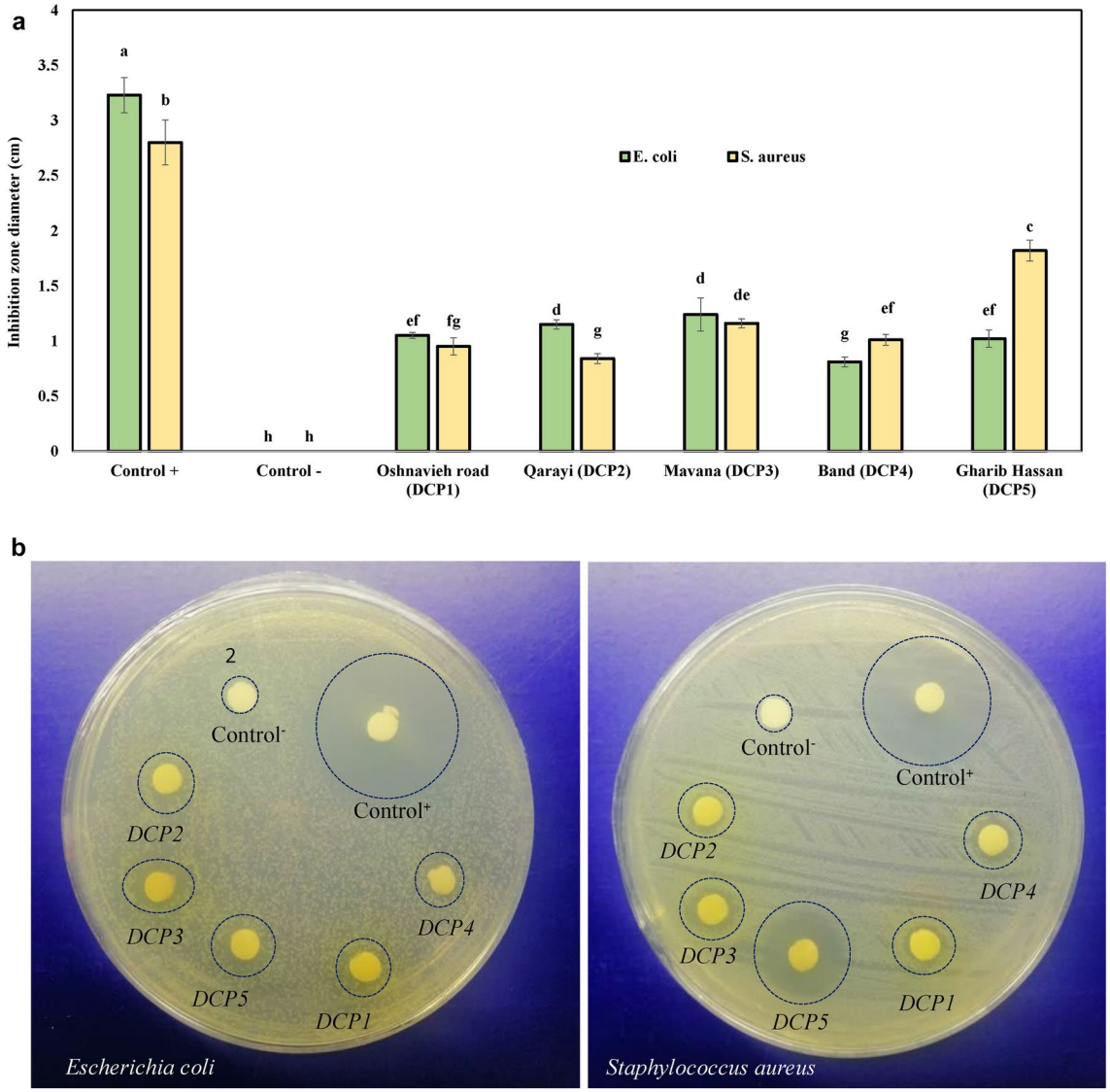

Fig. 5. Antibacterial effects of vegetative extracts from five wild carrot populations (DCPs) against two strains of *E. coli* and *S. aureus*: a) column chart b) Experimental photos: larger inhibition zones indicate a stronger effect of the samples.

dark blue points indicate a strong positive correlation, while large dark red points represent a strong negative correlation. Specifically, the analysis reveals several notable correlations: Bornyl acetate exhibited a negative correlation with MSD ($p < 0.05$); α-pinene showed a positive correlation with AP TPC ($p < 0.05$); Daucene was negatively correlated with FNMSIN ($p < 0.05$); limonene displayed a positive correlation with the ratio LL/LW ($p < 0.05$); β-pinene was positively correlated with AP TPC ($p < 0.01$); linalool was positively associated with both AP TFC ($p < 0.05$) and LL/LW ($p < 0.05$); and geranyl acetate showed a positive correlation with PH ($p < 0.05$). The simultaneous enhancement of one group of metabolites together with another is of significant interest to breeders, especially in the context of medicinal plants, where the goal is often to simultaneously increase all beneficial secondary metabolites. The positive relationship between α-pinene and β-pinene with the total phenolic content (TPC) in the aerial parts suggests a co-regulatory mechanism. This means that these terpenoids might work together to boost antioxidant capacity, potentially by sharing precursor molecules in the phenylpropanoid pathway[41]. This multi-trait breeding approach allows breeders to target multiple desirable attributes, thereby improving the overall quality and efficacy of the plants. As a result, breeders are increasingly focused on strategies that facilitate the parallel breeding of various traits, maximizing both yield and the therapeutic potential of these plants[42]. Additional correlations are illustrated in Fig. 6, where further details can be found.

Canonical Correspondence Analysis (CCA) is a multivariate statistical method utilized to investigate the relationships between two sets of variables. In this study, CCA was applied to analyze the associations between phytochemical and environmental attributes (climatic and soil factors). CCA was done in a sample of 118 genotypes sourced from 5 distinct populations. This type of analysis explains the relationships between

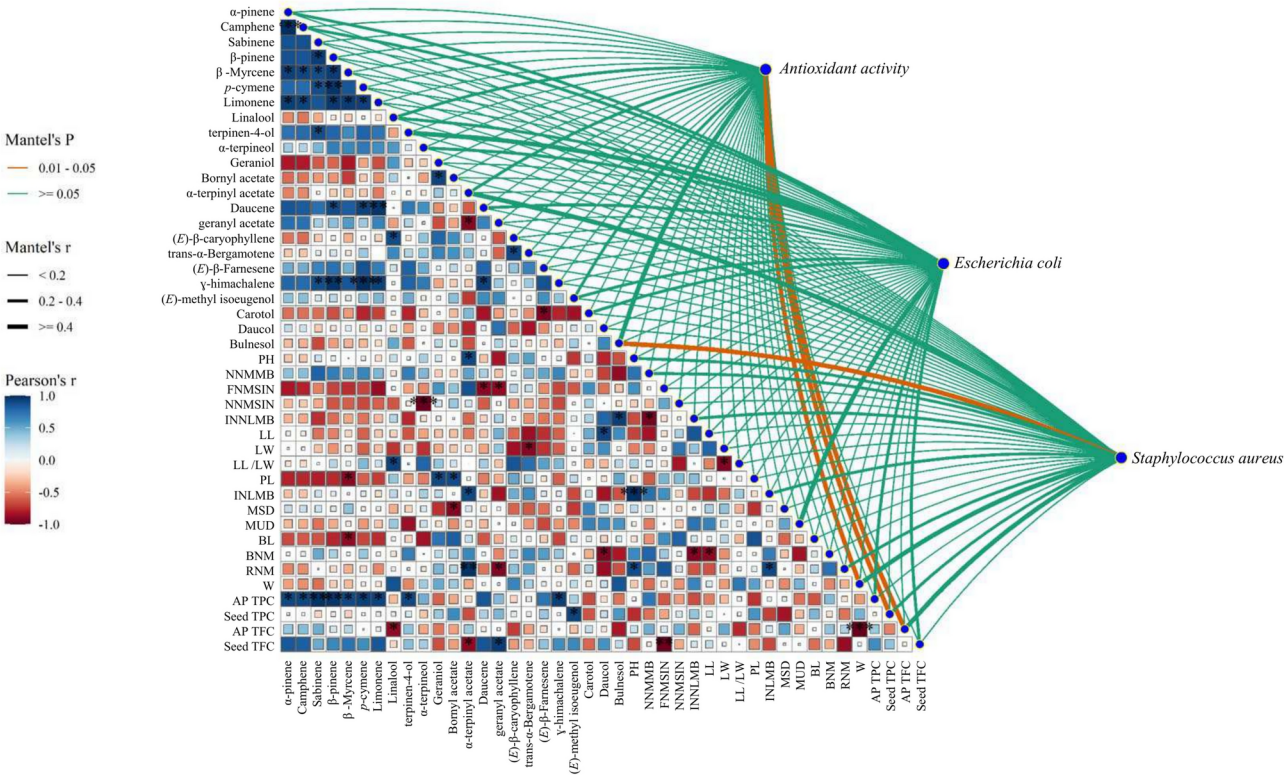

Fig. 6. Combined graph of Mantel test and Pearson correlation heatmap. A combined graph presenting the results of the Mantel test and Pearson correlation heatmap was created to elucidate the relationships among phytochemical and morphological attributes. The Pearson correlation analysis illustrates the correlations among all evaluated traits, with blue indicating positive correlations and red indicating negative correlations. The number of asterisks (*) represents the degree of statistical significance for each correlation. Additionally, the Mantel test was employed to assess the relationship between phytochemical and morphological attributes and their effect on bacterial genera, as well as to explore the association of antioxidant activity with these traits. In this context, a larger Mantel's *r* value and a smaller Mantel's *p*-value signify a stronger interaction between the indicators.

traits by forming Canonical Correspondence (CCs), and the contribution of each canonical correspondence is determined by the Eigenvalues and the percentage of variance obtained. Table 5 illustrates each set of CCs along with their corresponding Eigenvalues and variance percentages. The formation of canonical sets involves the calculation of numerical values for each trait, where traits with high positive values correlate positively and traits with high negative values correlated with each other. This indicates that an increase in any of the related environmental traits is associated with an increase in the corresponding phytochemical values. In CC1, phytochemical components such as α-pinene, sabinene, bornyl acetate, carotol, and AP DPPH, which possess negative values greater than 0.4, showed canonical correlation with negative values of environmental traits such as relative humidity (%), calcium carbonate (%), silt percentage, exchangeable potassium, available phosphorus, zinc, copper, and calcium. This implies that an increase in any of these environmental traits is associated with an increase in the phytochemical traits correlated with them. The first canonical component (CC1), accounting for 63.24% of variance, played a primary role in explaining the variance of phytochemical compounds under different climatic conditions in the studied areas. Furthermore, CC1 displayed strong positive correlations with bulnesol (a promise compound of the *DCP1* population) and clay percentage, alongside a partial but notable relationship with site height, implicating soil and climatic traits in shaping phytochemical variation. Additionally, CC2 explained 21.19% of the variance, where the components carotol (-0.47), α-pinene (-0.57), β-pinene (-0.55), and limonene (-0.65) showed positive correlations with site height (-0.80), pH (-0.56), and clay percentage (-0.77) due to their higher negative values. Also α-pinene and β-pinene exhibited an adverse correlation with annual mean temperature (+0.91), relative humidity (+0.71), electrical conductivity (EC) (+0.65), and magnesium content (+0.83). This suggests that an increase in these environmental traits in the studied areas is associated with a decrease in the aforementioned essential oils. CC3 accounted for 10.53%, while CC4 contributed 5.04% of the variance, representing a minor contribution in explaining the variations of phytochemical components related to environmental conditions. Considering that CC1 and CC2 cumulatively accounted for over 80% of the variance and environmental correlations with phytochemical compounds, a bi-plot of the first two canonical components was constructed. The green vectors represent environmental factors, which indicate a correlation with a phytochemical factor when they point towards it. EO 21, or carotol, positively correlated with phosphorus, exchangeable potassium, and zinc but exhibited a negative correlation with height

Variables	CC 1	CC 2	CC 3	CC 4
Phytochemical Variables				
α-pinene	**-0.41**	**-0.56**	0.01	0.08
Camphene	0.20	0.21	-0.23	-0.33
Sabinene	**-0.49**	**-0.68**	0.08	0.13
β-pinene	**-0.63**	**-0.55**	0.22	-0.26
B-Myrcene	-0.38	-0.31	0.41	-0.11
p-cymene	0.03	**-0.80**	-0.22	0.77
Limonene	-0.18	**-0.65**	-0.05	-0.10
Linalool	0.16	**-0.49**	-0.62	-0.87
terpinen-4-ol	-0.08	0.18	-0.04	-0.17
α-terpineol	-0.10	0.15	0.05	-0.24
Geraniol	-0.04	-1.55	0.29	-0.78
Bornyl acetate	**-0.47**	0.38	0.05	-0.28
α-terpinyl acetate	-0.08	-0.25	-0.02	-0.07
Daucene	-0.33	**-0.65**	0.03	-0.33
geranyl acetate	0.31	**-0.72**	0.61	-0.53
(E)-β-caryophyllene	-0.04	-1.55	0.29	-0.78
trans-α-Bergamotene	0.34	-0.31	0.33	0.31
(E)-β-Farnesene	-0.30	0.25	-0.14	-0.04
ɣ-himachalene	-0.13	0.05	-0.29	0.21
(E)-methyl isoeugenol	0.38	0.17	-0.36	0.16
Carotol	**-0.40**	**-0.47**	-0.16	-0.15
Daucol	0.21	**0.55**	0.20	-0.22
Bulnesol	**0.97**	**0.89**	1.23	-0.06
AP TPC	-0.38	-0.37	0.12	-0.16
Seed TPC	0.21	0.02	0.13	0.19
AP TFC	**-0.55**	0.13	0.05	0.02
Seed TFC	-0.16	0.00	-0.08	0.01
AP DPPH%	**-0.93**	0.13	0.17	0.09
Seed DPPH%	0.08	-0.09	-0.04	0.40
AP FRAP	0.23	-0.19	0.06	0.01
Seed FRAP	0.30	0.08	-0.08	-0.03
Populations				
Oshnavieh road (DCP1)	**0.98**	**0.89**	1.23	-0.06
Qarayi (DCP2)	0.08	-0.33	-0.55	1.74
Mavana (DCP3)	-0.03	**-1.55**	0.30	-0.78
Band (DCP4)	0.38	**0.80**	-1.75	-0.99
Gharib Hassan (DCP5)	**-2.47**	**0.87**	0.50	-0.14
Climatic and soil characterization				
Altitude (m)	0.35	**-0.80**	-0.44	0.37
Annual precipitation (mm)	-0.27	-0.31	0.86	0.46
Relative Humidity (%)	**-0.69**	**0.71**	0.40	-0.19
Annual Average Temperature (°C)	0.14	**0.91**	-0.18	-0.34
pH (1:2) H_2O	-0.19	**-0.56**	-0.67	0.26
EC (dS m^{-1})	-0.36	**0.70**	0.15	-0.68
O.C. (%)	0.00	0.05	-0.98	-0.19
O.M. (%)	0.00	0.05	-0.98	-0.20
$CaCO_3$ (%)	**-0.51**	-0.10	0.32	-0.77
Clay (%)	0.62	**-0.77**	0.30	0.18
Silt (%)	**-0.74**	0.19	0.74	0.21
Sand (%)	0.09	0.48	-0.86	-0.32
Total N (%)	0.00	0.05	-0.98	-0.19
K exchangeable (mg kg^{-1})	**-0.79**	**0.42**	0.62	0.05
P exchangeable (mg kg^{-1})	**-0.74**	**0.47**	0.62	-0.11
Fe (mg kg^{-1})	0.20	**0.54**	0.72	-0.11
Zn (mg kg^{-1})	**-0.95**	0.34	0.34	0.08
Continued				

Variables	CC 1	CC 2	CC 3	CC 4
Cu (mg kg^{-1})	**-0.90**	0.19	0.49	0.23
Mn (mg kg^{-1})	0.29	-0.05	-0.66	0.67
Ca (Meq l^{-1})	**-0.66**	**0.53**	-0.05	-0.64
Mg (Meq l^{-1})	-0.24	**0.83**	0.52	-0.14
Eigenvalue 0.14 0.05 0.02 0.01				
%	63.24	21.19	10.53	5.04

Table 5. The results of the Canonical Correspondence Analysis (CCA), showcasing four sets of canonical correlations (CC 1-4), their corresponding eigenvalues and the variance (%) explained by each correlation among the phytochemical, climatic and soil characterization, in the five studied populations. AP: Aerial parts; **Daucus carota* populations (*DCPs*). Significant values are in bold.

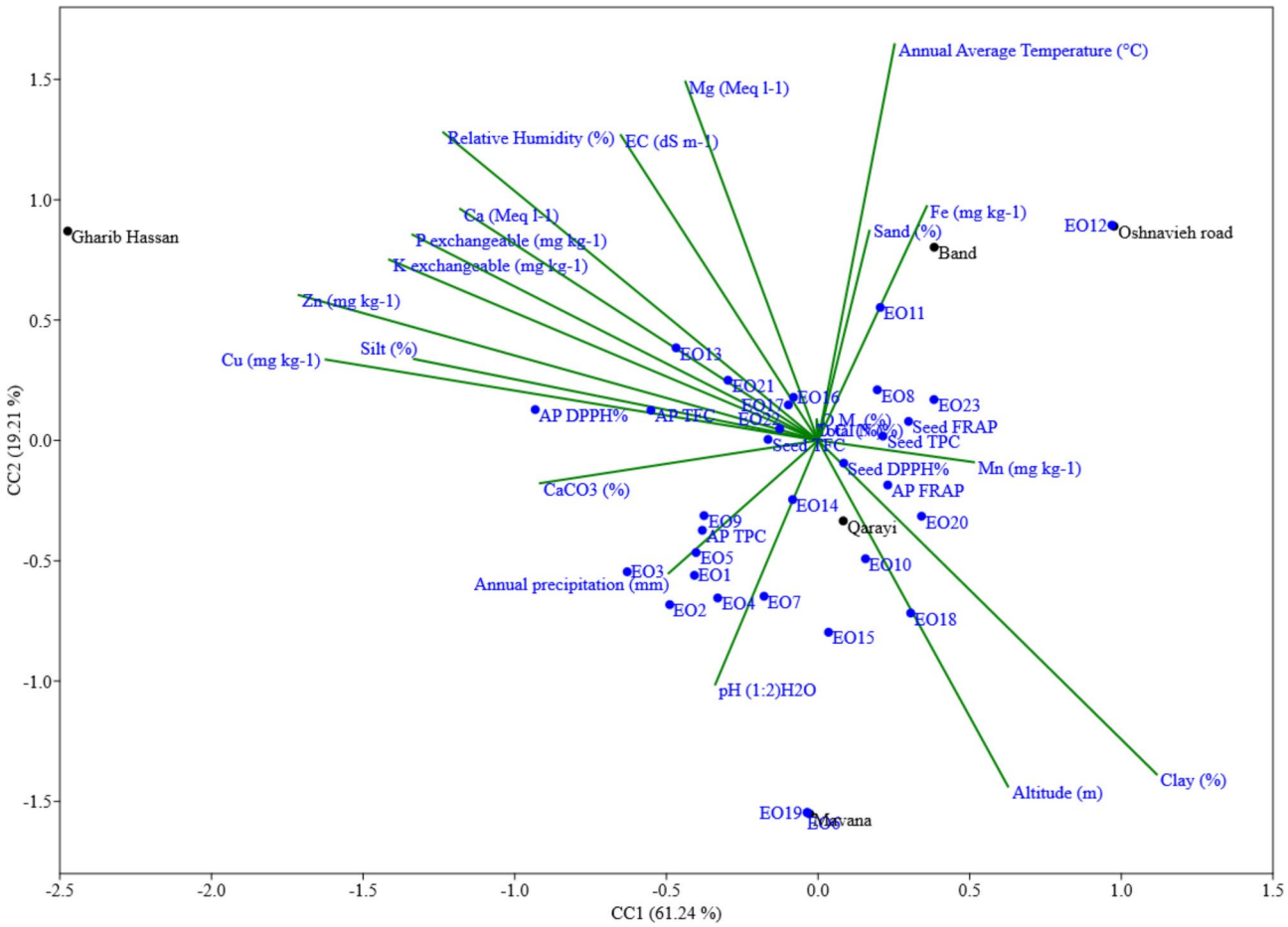

Fig. 7. Canonical correspondence analysis (CCA) among the phytochemical, climatic and soil characterization, in the five studied *Daucus carota* populations (*DCPs*).

and clay percentage. Both AP DPPH and AP TPC positively correlated with clay percentage, zinc, and copper, while showing a negative correlation with soil manganese. Additionally, further details on other environmental correlations with phytochemical components are illustrated in Fig. 7.

To further investigate the diversity of phytochemical and morphological traits among the populations, a cluster analysis was performed using R software with a circular clustering approach (Fig. 8). This analysis categorized the genotypes of all individuals from the five studied populations into three principal groups based on the traits examined. Specifically, within the analysis of 118 *D. carota* genotypes, individuals corresponding to the identified chemotypes were organized into three distinct clusters (Fig. 8). This finding indicates that the genotypes displayed less diversity within populations compared to the diversity between populations. Given

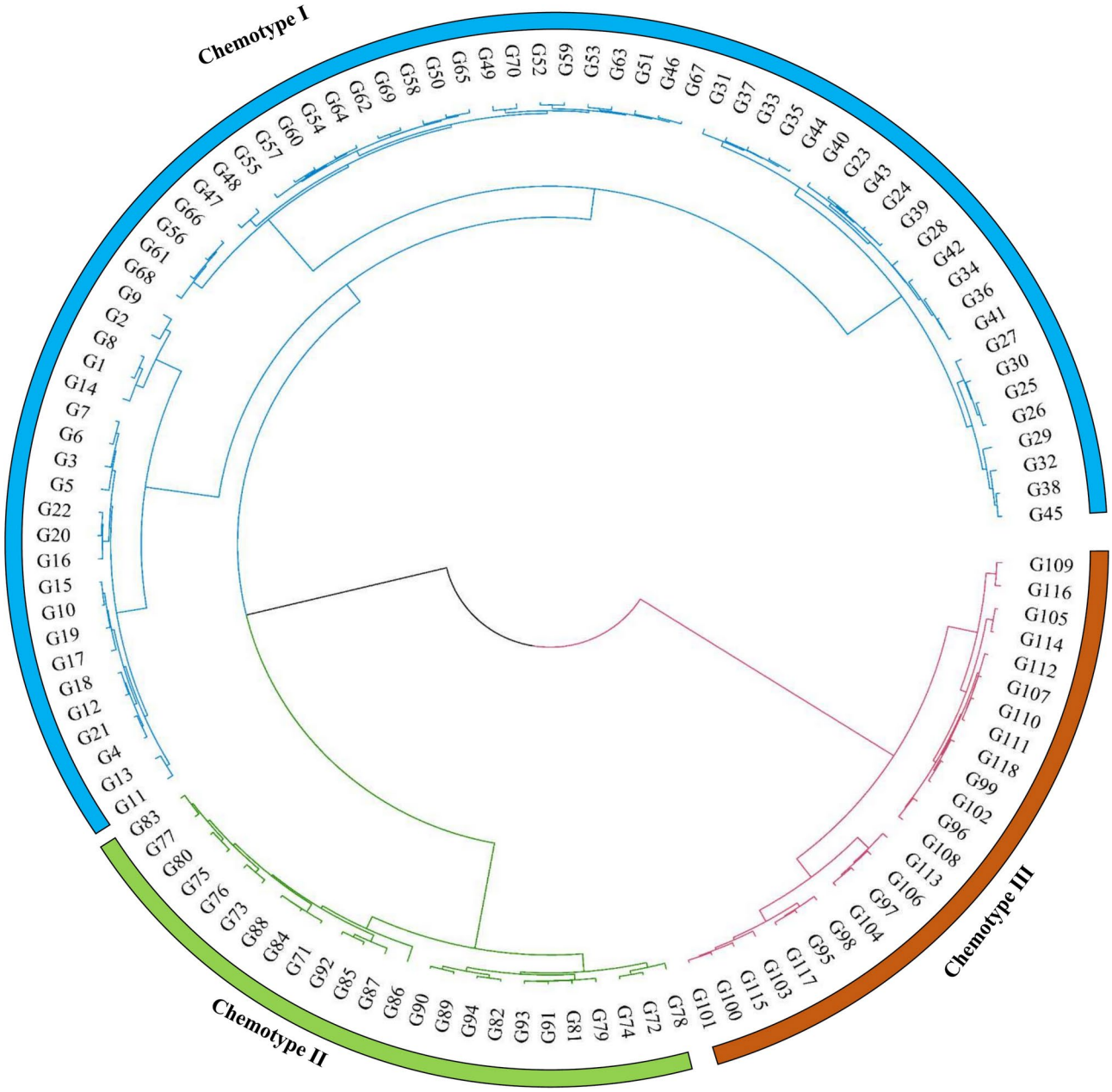

Fig. 8. Circular clustering of all combined attributes among 118 individuals of *Daucus carota*.

that one of the regions is considered a center of origin for wild carrot in Iran, it appears that these wild carrot populations have adapted to their environments over the years. Environmental adaptation, driven by natural selection, has led to uniformity and the development of ecotypes that are well-suited to their surroundings. Wild carrot is self-pollinating; however, pollinators such as insects increase the rate of cross-pollination. This factor, beside natural selection, contributes to genetic exchange within populations and the selection of genotypes that favor adaptation to the natural environment, leading to a remarkable degree of uniformity.

Stepwise regression is a statistical technique employed in linear regression analysis to identify a subset of relevant independent variables for inclusion in a model[31]. This method operates by systematically assessing the statistical significance of each independent variable, thereby determining its contribution to the overall model performance by selecting only those variables that significantly enhance its explanatory power. This analysis was conducted to assess the regression relationship between various phytochemical components and morphological traits. For instance, three morphological factors; BL, W, and RNM exhibited significant negative correlations with the compound α-pinene, with Standardized beta values of -0.502, -0.426, and -0.512, respectively ($p < 0.001$). Additionally, BNM displayed a positive correlation with α-pinene, with a Standardized beta value of 0.210. Therefore, these four morphological traits, with an r value of 0.835 and an r^2 value of 0.697, could significantly serve as morphological markers for the compound α-pinene. Given the presence of BL and W in the formation of linear regressions, these traits can be considered effective morphological markers for predicting phytochemical

characteristics. However, the positive and negative values of Standardized beta indicate the respective negative and positive roles of these traits concerning the associated phytochemical values. Additionally, for the compound carotol, five morphological traits were significantly associated in the linear regression analysis, demonstrating a meaningful relationship with an r value of 0.6 ($p \leq 0.01$). The positive Standardized beta values for three traits NNMSIN, MUD, and RNM were 3.544, 3.778, and 4.878, respectively, indicating a significant positive impact on the regression related to carotol. Conversely, the two traits BNM and MSD exhibited negative correlations in the context of the linear regression associated with Carotol, with beta values of -3.892 and -2.637, respectively, at a significance level of $p \leq 0.01$. The regression models for other phytochemical traits, as detailed in Table 6, underscore the significant role of morphological markers in predicting phytochemical profiles. For instance, traits such as leaf length (BL) and width (W) consistently emerged as strong predictors and could serve as cost-effective alternatives for labor-intensive phytochemical analyses. This approach aligns with marker-assisted selection (MAS) strategies, enabling breeders to identify genotypes with desirable phytochemical traits based on easily measurable morphological characteristics[43]. Integrating stepwise regression into plant breeding pipelines offers a viable and efficient alternative to traditional phytochemical screening methods. By identifying morphological traits that correlate strongly with target compounds, this approach reduces reliance on expensive analytical techniques, accelerating the selection of high-value genotypes. However, the observed trade-offs between specific traits (e.g., positive versus negative beta values) highlight the necessity for balanced selection strategies to mitigate any unintended consequences on plant growth or stress tolerance. Future research should seek to validate these regression models across diverse environments and genetic backgrounds to ensure their robustness. Additionally, exploring the underlying physiological and genetic mechanisms that drive the relationships between these traits and phytochemical profiles could further refine predictive models and enhance their applicability within breeding programs. Overall, this study emphasizes the potential of multivariate analysis, correlation and regression analysis as a powerful tool for linking morphological and phytochemical traits, paving the way for more efficient and targeted efforts in plant domestication.

The analysis of wild carrot populations revealed that the plant serves as a significant source of carotol. This compound, a type of oxygenated sesquiterpene, found in higher percentage in the studied populations. The importance of oxygenated sesquiterpenes has been recognized in various biological activities, including insecticidal, antioxidant, and allelopathic effects[44,45]. Consequently, chemotypes that are rich in these compounds may represent promising candidates for industrial applications targeting these purposes[44,46,47]. This study revealed the high contents of TFC, TPC, and antioxidant activity of the aerial parts (AP) and seed extracts were documented. This suggests their potential application as effective antioxidant agents[9,48]. Therefore, the findings concerning the studied populations indicate that the plant holds considerable potential for using in breeding, facilitating its widespread cultivation. Notably, the *DCP4* and *DCP5* populations contain a high percentage of carotol, exceeding 73%, which can serve as a valuable source of this compound. Carotol is known for its diverse biological properties, including cytotoxicity, antioxidant effects, anti-inflammatory, antimicrobial, and antiviral activities[49–51].

The present study identified three valuable chemotypes, categorized as follows:

(I) Chemotype I: carotol—butrnyl acetate (*DCP1*).
(II) Chemotype II: carotol—α-pinene (*DCP3*).
(III) Chemotype III: carotol, with a high percentage ranging from 64.03 to 74.03% (*DCP2*, *DCP4*, and *DCP5*).

Studies on wild carrot have revealed significant geographic variation in the composition of essential oils across different plant tissues. In aerial parts sourced from Turkey, key compounds such as carotol (27%), elemicin (18.1%), and limonene (16%) were identified as major constituents[19]. Seeds collected from Montenegro at various maturity stages were characterized by high levels of β-bisabolene (32.3%) and 11-α-(H)-himachal-4-en-1-β-ol (27.9%)[20]. In contrast, α-pinene (23.5%) emerged as the dominant compound in aerial parts of Moroccan *D. carota*[21]. Further analyses from Turkey samples highlighted carotol (1–74.6%) and β-bisabolene (0.9–62.4%) as key constituents, underscoring the chemical diversity from Turkey[22]. Algerian aerial parts, were rich in alismol (15.2%), (E)-β-caryophyllene (10.1%), myrcene (9.6%), α-humulene (9.5%), and β-ionone (5.2%)[23] Italian samples exhibited high concentrations of β-bisabolene (17.6–51.0%), while Portuguese samples were primarily composed of geranyl acetate (5.2–65.0%)[24]. When compared to other geographic populations, the chemotypes identified in this study demonstrate both shared and unique traits. For instance, the prominence of carotol across all three chemotypes aligns with its widespread occurrence in *D. carota* from Turkey[22]. However, the specific combinations of carotol with butyl acetate (Chemotype I) or α-pinene (Chemotype II) highlight the influence of localized evolutionary pressures on secondary metabolite biosynthesis.

These chemotypes, exhibiting strong antioxidant and antibacterial activity, hold promise for development as new nutraceuticals and functional foods, contingent upon further supplementary studies. Additionally, to scale up production, domestication and breeding strategies should be considered. This study highlights the significant antibacterial properties of plant extracts from various populations, particularly DCP5 and DCP3, which exhibited enhanced levels of carotol, α-pinene, phenolic, and flavonoid compounds. The significant antibacterial effects of phenolic and flavonoid compounds have been reported in previous studies as well[52,53]. The findings underscore the importance of these phytochemical traits in relation to environmental factors, as revealed by Canonical Correspondence Analysis (CCA) and scatter plots. By identifying the phytochemical characteristics that correlate with these environmental variables, we can establish valuable criteria for optimizing the cultivation and domestication of elite chemotypes of wild carrot. The demonstrated relationships between phytochemical and morphological traits through stepwise regression analysis offer a promising framework for reducing reliance on expensive analytical methods in the selection of plants for domestication and breeding, particularly within the context of marker-assisted selection (MAS)[54]. Moreover the use of CCA, scatter plots, and cluster analysis

#NO	Phytochemical composition	Morphological Marker	*r*	R^2	Standardized beta coefficients	*t* value	*p* value
1	α-pinene	BL	0.627[a]	0.393	-0.502	-9.322	< 0.001
		W	0.706[b]	0.499	-0.426	-7.768	< 0.001
		RNM	0.813[c]	0.661	-0.512	-8.583	< 0.001
		BNM	0.835[d]	0.697	0.210	3.680	< 0.001
2	Camphene	BL	0.583[a]	0.340	-0.457	-8.249	< 0.001
		W	0.691[b]	0.478	-0.470	-8.327	< 0.001
		RNM	0.800[c]	0.639	-0.515	-8.380	< 0.001
3	Sabinene	BL	0.362[a]	0.356	-0.619	-11.933	< 0.001
		W	0.559[b]	0.551	-0.325	-5.983	< 0.001
		BNM	0.645[c]	0.636	0.252	4.606	< 0.001
		MUD	0.673[d]	0.661	-0.342	-4.655	< 0.001
		MSD	0.703[e]	0.690	0.242	3.383	< 0.001
4	β-pinene	BL	0.736[a]	0.541	-0.693	-12.221	< 0.001
		BNM	0.789[b]	0.622	0.305	5.161	< 0.001
		INNLMB	0.804[c]	0.647	-0.208	-3.602	< 0.001
		RNM	0.821[d]	0.673	-0.188	-3.021	0.003
5	β-Myrcene	BL	0.838[a]	0.702	-0.777	-18.420	< 0.001
		W	0.869[b]	0.755	-0.267	-6.193	< 0.001
		RNM	0.889[c]	0.789	-0.264	-5.638	< 0.001
		BNM	0.902[d]	0.814	0.172	3.853	< 0.001
6	*p*-cymene	BL	0.653[a]	0.427	-0.647	-10.103	< 0.001
		BNM	0.721[b]	0.520	0.273	4.252	< 0.001
		LW	0.743[c]	0.551	-0.183	-2.824	0.006
7	Limonene	BL	0.717[a]	0.514	-0.615	-10.783	< 0.001
		RNM	0.761[b]	0.579	-0.422	-6.727	< 0.001
		BNM	0.802[c]	0.643	0.252	4.224	< 0.001
		INNLMB	0.818[d]	0.669	-0.173	-2.968	0.004
8	Linalool	W	0.687[a]	0.472	0.710	14.145	< 0.001
		NNMSIN	0.803[b]	0.644	-0.396	-7.518	< 0.001
		RNM	0.888[c]	0.788	0.279	5.703	< 0.001
		FNMSIN	0.899[d]	0.808	0.139	2.712	0.008
9	terpinen-4-ol	BNM	0.437[a]	0.191	0.309	4.264	< 0.001
		W	0.547[b]	0.300	-0.259	-3.620	< 0.001
		BL	0.607[c]	0.368	-0.272	-3.974	< 0.001
		MUD	0.660[d]	0.435	-0.480	-4.949	< 0.001
		MSD	0.694[e]	0.482	0.301	3.178	0.002
10	α-terpineol	NNMSIN	0.843[a]	0.710	-0.426	-8.837	< 0.001
		BL	0.900[b]	0.809	-0.476	-10.891	< 0.001
		LL:LW	0.921[c]	0.849	0.127	3.787	< 0.001
		PH	0.936[d]	0.875	0.220	6.869	< 0.001
		W	0.942[e]	0.887	0.194	5.152	< 0.001
		INNLMB	0.949f.	0.900	-0.112	-3.419	< 0.001
		LL	0.952[g]	0.906	-0.085	-2.686	0.008
11	Geraniol	BL	0.687[a]	0.472	0.600	13.591	< 0.001
		W	0.803[b]	0.644	0.492	10.899	< 0.001
		RNM	0.888[c]	0.788	0.373	8.117	< 0.001
		LW	0.899[d]	0.808	-0.152	-3.442	< 0.001
12	Bornyl acetate	BL	0.768[a]	0.590	0.868	12.362	< 0.001
		NNMSIN	0.818[b]	0.669	-0.150	-1.958	0.053
		LW	0.837[c]	0.701	-0.146	-2.780	0.006
		W	0.851[d]	0.724	0.234	4.115	< 0.001
		BNM	0.867f.	0.751	0.176	3.486	< 0.001
13	α-terpinyl acetate	RNM	0.876[a]	0.768	0.876	19.591	< 0.001
14	Daucene	BL	0.609[a]	0.371	-0.276	-3.119	0.002
		RNM	0.695[b]	0.483	-0.511	-7.501	< 0.001
		BNM	0.743[c]	0.553	0.257	3.925	< 0.001

Continued

#NO	Phytochemical composition	Morphological Marker	*r*	R^2	Standardized beta coefficients	*t* value	*p* value
		NNMSIN	0.761[d]	0.579	-0.285	-3.423	< 0.001
		NNMMB	0.779[e]	0.607	0.188	2.835	0.005
15	geranyl acetate	RNM	0.812[a]	0.660	-0.857	-16.888	< 0.001
		W	0.856[b]	0.733	-0.278	-5.667	< 0.001
		FNMSIN	0.865[c]	0.749	-0.132	-2.703	0.008
16	(E)-β-caryophyllene	W	0.576[a]	0.331	0.488	8.250	< 0.001
		RNM	0.785[b]	0.616	0.377	6.587	< 0.001
		LW	0.825[c]	0.681	-0.239	-4.577	< 0.001
		NNMSIN	0.836[d]	0.700	-0.434	-5.466	< 0.001
		BL	0.854[e]	0.729	0.351	4.613	< 0.001
		PH	0.871f.	0.759	0.194	3.737	< 0.001
17	trans-α-Bergamotene	LW	0.498[a]	0.248	-0.250	-4.262	< 0.001
		RNM W	0.648[b]	0.420	0.265	3.816	< 0.001
		W	0.728[c]	0.530	0.271	4.139	< 0.001
		BNM	0.770[d]	0.593	0.229	3.913	< 0.001
		NNMSIN	0.789[e]	0.623	-0.467	-5.308	< 0.001
		NNMMB	0.804f.	0.646	0.257	4.405	< 0.001
		BL	0.829[g]	0.688	0.387	4.430	< 0.001
		INLMB	0.843[h]	0.710	0.170	2.890	0.005
18	(E)-β-Farnesene	NNMSIN	0.422[a]	0.178	-0.434	-5.958	< 0.001
		BNM	0.564[b]	0.318	0.264	3.407	< 0.001
		NNMMB	0.604[c]	0.365	0.247	3.287	< 0.001
		MUD	0.637[d]	0.405	-0.211	-2.788	0.006
19	ɣ-himachalene	BL	0.653[a]	0.427	-0.647	-10.103	< 0.001
		BNM	0.721[b]	0.520	0.273	4.252	< 0.001
		LW	0.743[c]	0.551	-0.183	-2.824	< 0.001
20	(E)-methyl isoeugenol	RNM	0.537[a]	0.289	-.624	-8.510	< 0.001
		BL	0.608[b]	0.370	.283	3.861	< 0.001
		MUD	0.653[c]	0.426	-.238	-3.348	0.001
21	Carotol	NNMSIN	0.288[a]	0.083	0.276	3.544	< 0.001
		MUD	0.395[b]	0.156	0.402	3.778	< 0.001
		RNM	0.475[c]	0.225	0.405	4.878	< 0.001
		BNM	0.566[d]	0.321	-0.332	-3.892	< 0.001
		MSD	0.600[e]	0.361	-0.277	-2.637	0.010
22	Daucol	RNM	0.764[a]	0.584	-0.596	-11.470	< 0.001
		BNM	0.826[b]	0.682	-0.284	-5.410	< 0.001
		LW	0.853[c]	0.728	0.212	4.425	< 0.001
		NNMMB	0.865[d]	0.748	-0.149	-3.050	0.003
23	Bulnesol	W	0.742[a]	0.551	0.486	12.575	< 0.001
		RNM	0.835[b]	0.698	-0.253	-5.763	< 0.001
		BNM	0.867[c]	0.752	-0.161	-4.011	< 0.001
		BL	0.895[d]	0.801	0.117	2.801	0.006
		INNLMB	0.908[e]	0.824	0.210	5.017	< 0.001
		PH	0.914f.	0.836	-0.287	-5.847	< 0.001
		MUD	0.926[g]	0.857	0.208	4.689	< 0.001
		PL	0.933[h]	0.871	0.144	3.436	< 0.001
24	AP TPC	BL	0.599[a]	0.359	-.528	-8.584	< 0.001
		W	0.691[b]	0.478	-.286	-4.536	< 0.001
		BNM	0.742[c]	0.551	.328	5.086	< 0.001
		RNM	0.766[d]	0.587	-.266	-3.882	< 0.001
		INNLMB	0.788[e]	0.621	-.204	-3.187	0.002
25	Seed TPC	RNM	0.490[a]	0.240	-0.638	-10.218	< 0.001
		BL	0.729[b]	0.531	0.767	9.167	< 0.001
		NNMSIN	0.764[c]	0.583	-0.309	-3.776	< 0.001
26	AP TFC	W	0.851[a]	0.724	-0.751	-14.421	< 0.001
Continued							

#NO	Phytochemical composition	Morphological Marker	*r*	R^2	Standardized beta coefficients	*t* value	*p* value
		NNMSIN	0.862[b]	0.744	0.206	3.885	< 0.001
		PL	0.872[c]	0.760	-0.141	-2.742	0.007
27	Seed TFC	RNM	0.708[a]	0.501	-0.624	-10.331	< 0.001
		BL	0.778[b]	0.606	-0.335	-5.545	< 0.001
28	AP DPPH%	W	0.849[a]	0.722	-0.736	-14.081	< 0.001
		NNMSIN	0.864[b]	0.746	0.316	4.470	< 0.001
		BL	0.874[c]	0.764	-0.193	-2.902	0.004
29	Seed DPPH%	RNM	0.776[a]	0.602	-.861	-16.616	< 0.001
		BL	0.843[b]	0.711	.339	6.555	< 0.001
30	AP FRAP	RNM	0.527[a]	0.278	-0.560	-7.125	< 0.001
		LW	0.600[b]	0.360	-0.192	-2.597	0.011
		W	0.637[c]	0.405	0.270	3.522	0.001
		BNM	0.668[d]	0.446	0.225	2.869	0.005
31	Seed FRAP	W	0.772[a]	0.596	0.545	8.839	< 0.001
		RNM	0.794[b]	0.631	-0.310	-5.580	< 0.001
		BL	0.818[c]	0.668	0.463	5.955	< 0.001
		NNMSIN	0.848[d]	0.719	-0.359	-4.516	< 0.001

Table 6. The phytochemical constituents related to morphological attributes in Daucus carota populations (DCPs) were analyzed using multiple regression analysis and regression coefficients. AP: Aerial parts; PH: Plant height; NNMMB: Nodes number on the main branch; FNMSIN: Flowers number in the secondary stem; NNMSIN: Nodes number of secondary stem; INNLMB: Internode length in the main branch; LL: Leaf length; LW: Leaf width: LL /LW: Leaf index (length/width); PL: Petiole length INLMB Inflorescence length on main branch; MSD: Main stem Diameter; MUD: Main umbel diameter; BL: Bract length; BNM: Bract number; RNM: Rays number of umbel; W: 1000 seed weight.

provides important insights into the relationships among phytochemical, environmental, and morphological traits within the studied populations. These findings deepen our understanding of the diversity and interactions of these traits in the plant species under investigation[55]. Future research should focus on investigating the specific mechanisms that contribute to the antibacterial activity of the identified compounds, as well as their interactions with various environmental factors. Expanding the scope of this study to encompass a wider range of populations and environmental conditions could further strengthen the reliability of the findings. One limitation of the current study is the potential variability in phytochemical profiles resulting from environmental fluctuations, which underscores the importance of conducting more extensive field trials to validate the results. Overall, these insights enhance our understanding of the diversity and interactions among phytochemical, environmental, and morphological traits in wild carrot species. This knowledge paves the way for more targeted strategies in breeding and cultivation, ultimately contributing to the development of elite chemotypes with desirable characteristics.

Methods

Plant materials, soil samples and climatic characterization

To obtain the plant material, the aerial parts of *Daucus carota* L. subsp. *carota* were collected flowering and fruit set stages from different parts of the West Azerbaijan, Iran. Sampling was done for academic purposes, with the permission of the University and in accordance with relevant institutional, national, and international guidelines and legislation. We confirm that the sampling was conducted in accordance with the IUCN guidelines for research on endangered species and the Convention on International Trade in Endangered Species of Wild Fauna and Flora. We confirm that the national necessary permission has been obtained for the collection of plant materials. The collection areas for five distinct populations (*DCP1-DCP5*), associated with specific geographic locations Oshnavieh Road, Qarayi, Mavana, Band, and Gharib Hassan are presented in Table 1. *DCP1* encompasses genotypes G1 through G22; *DCP2* includes genotypes G23 to G45; *DCP3* comprises genotypes G46 to G70; *DCP4* covers genotypes G71 to G94; and *DCP5* contains genotypes G95 to G118. The species were identified using resources from the Iranian flora by the MF, and a voucher specimen (No. 1533) was labeled and deposited in the herbarium of the Department of Horticultural Science at Urmia University, Iran. The aerial parts of the plants were dried in the shade at room temperature for a period of three weeks. To ascertain the habitats of these species, interviews were conducted with local residents, and relevant literature, including previous articles and books, was reviewed.

To determine the physicochemical properties of the soil in five study areas, soil samples were collected by excavating profiles to a depth of 0 to 30 cm from three different locations within each area. Soil analyses included the determination of soil texture, pH, and electrical conductivity (EC) following the method outlined previously[56]. Organic carbon and cation exchange capacity (CEC)was measured by Nelson and Sommers (1982) method[57], while total nitrogen content was assessed using the Kjeldahl method[58]. Additionally, phosphorus was measured according to Olsen's method[59], and available potassium was analyzed based on the reference[60]. The availability of iron, zinc, copper, and manganese was determined using DTPA extraction with 0.005 molar solution[61]. For

the measurement of calcium and magnesium, the method from Thomas, 1982[62]. Geographical position and elevation data were recorded using a Global Positioning System (GPS) device, and climatic characterization were gathered from the nearest weather station, prioritizing synoptic stations.

Morphological characteristics

The aforementioned traits were employed for the evaluation of the plants morphological characteristics. Plant height, PH; Nodes number on the main branch, NNMMB; Flowers number in the secondary stem, FNMSIN; Nodes number of secondary stem,NNMSIN; Internode length in the main branch, INNLMB; Leaf length, LL; Leaf width, LW; Leaf index (length/width), LL /LW; Petiole length, PL; Inflorescence length on main branch, INLMB; Main stem Diameter, MSD; Main umbel diameter, MUD; Bract length, BL; Bract number, BNM; Rays number of umbel, RNM;1000 seed weight, W (Table 2).

Essential oil isolation, GC/MS analysis, and identification

Essential oil extraction was performed using the water distillation method with a Clavenger apparatus for a duration of three hours. For this means, 35 g of dried plant material was pulverized and combined with 650 ml of water in a specifically designed flask for the apparatus, facilitating the oil extraction process. The volume of the extracted essential oil was directly read from the graduated collector tube. The oils were subsequently stored in sealed glass vials, placed within a dark glass container, and refrigerated for preservation at 4 °C.

The gas chromatography apparatus used was a Shimadzu model A9, manufactured in Japan. It was equipped with a HP-5MS, 30 m × 0.250 mm, 0.25 µm film thickness non-polar fused silica capillary column. The temperature programming commenced at an initial temperature of 60 °C, gradually increasing to a final temperature of 210 °C at a rate of 3 °C per min. Subsequently, the temperature increased to a secondary final temperature of 240 °C over a period of 20 min. The injector and detector chamber temperatures were maintained at 280 °C. Helium gas, with a purity of 99.999%, served as the carrier gas, flowing through the column at a rate of 1 ml/min. For the gas chromatography coupled with mass spectrometry, a Varian 3400 model DB-1 ion trap mass spectrometer was employed. It featured a column of DB-1, 60 m × 0.250 mm, 0.25 µm film thickness. The thermal programming of the column varied from an initial temperature of 50 °C to a final temperature of 280 °C, at an increment of 4 °C per minute. The injector chamber temperature was set to 10 °C higher than the final column temperature. Helium served as the carrier gas, flowing at a rate of 1 ml/min through the column. The scan time was set to one second, with an ionization energy of 70 electron volts and a mass range from 40 to 340. A volume of 0.2 µl from each essential oil sample was injected into the gas chromatography (GC) system, employing a split ratio of 1:60. The percentage composition of the individual compounds present in each essential oil was calculated using the area normalization method, without considering response factors. To assess the retention indices of the compounds, a mixture of normal hydrocarbons ranging from C8 to C22 was injected into the GC under the same conditions as those utilized for the essential oil samples. The identification of the constituents of the essential oils was conducted by analyzing the retention indices, examining the mass spectra of the compounds, and comparing them with standard mass spectra available in computer databases and reputable literature (Shibamato, 1987; Davies, 1990).

Methanolic extracts

The aerial parts and seed samples of various genotypes were extracted separately using an ultrasonic device. One gram of each ground sample was placed in 50-ml Falcon tubes, to which 20 ml of 80% methanol was added. The mixture was then subjected to ultrasonic treatment for 20 min at 40 °C and a frequency of 37 kHz using an Elmasonic E 120 H ultrasonic cleaner (Elma Schmidbauer GmbH, Germany). Following extraction, the resulting mixtures were filtered through Whatman filter paper and stored at 4 °C for further analysis.

Determination of total phenolic and flavonoid content

The measurement of phenolic compounds was conducted using the Folin-Ciocalteu reagent. A volume of 25 µl of the extracted solution was taken and placed in a Falcon tube. Next, 180 µl s of distilled water was added to the extract. Following this, 1200 µl of 10% Folin reagent was added to the mixture, after which 7.5% sodium carbonate was introduced after a 5 min interval. The samples were then allowed to stand in the dark at room temperature for 45 min. Finally, the absorbance was measured at a wavelength of 760 nm using a spectrophotometer (Model: UV2100 PC). Deionized water served as the blank, while gallic acid was used as the standard. A standard curve was constructed based on gallic acid, and the results were reported as mg of gallic acid equivalents per g of dry weight (mg GA E /g DW) of the plant[63]. To measure the total flavonoid content (TFC), a specific volume of each extract (30 µL) was mixed with 150 µL of sodium nitrite. After 5 to 15 min, 300 µL of a 10% aluminum chloride solution was added to the mixture[64]. Then, 1000 µL of 1 M sodium acetate was introduced, and the volume was brought to 5 mL with distilled water. The absorbance of the mixture was measured at a wavelength of 380 nm against the blank. Quercetin was used to construct the standard curve. The TFC of the extracts was reported as mg of quercetin equivalents per gram of dry weight (mg Qu/g DW) of the plant.

DPPH free radical scavenging assay

To measure antioxidant activity using the DPPH method, a specific volume of methanolic extract (30 µL) was placed in a test tube, and then 2000 µL of DPPH solution (prepared at a concentration of 0.002 g in 60 mL of 80% methanol) was added. The resulting solution was mixed and allowed to stand at room temperature for 30 min. Absorbance was then measured at 517 nm using a spectrophotometer. For the blank control, the same procedure was followed, but instead of the extract, 50 µL of 80% ethanol was used[65].

The formula used to calculate the percentage of DPPH inhibition is as follows (Eq. 1):

$$\text{DPPH Inhibition}\% = \frac{(\text{Abs control})\,t = 30\ \text{min} - (\text{Abs sample})\,t = 30\ \text{min}}{(\text{Abs control})\,t = 30\text{min}} \times 100 \quad (1)$$

Here, Abs_{sample} represents the absorbance of the DPPH solution with the extract, while $Abs_{control}$ represents the absorbance of the DPPH solution without the extract (control).

Ferric reducing antioxidant power (FRAP) assay

The extracts of the samples were mixed with 3 mL of freshly prepared FRAP reagent (300 mM sodium acetate buffer at pH 3.6, a solution of 10 mM TPTZ (Tripyridyl-s-triazine), in 40 mM HCl and 20 mM ferric chloride ($FeCl_3$) a ratio of 10:1:1 (v/v/v). The resulting mixture was incubated for 30 min in a water bath at 37 °C. Absorbance was then measured at a wavelength of 593 nm using a spectrophotometer, with a blank control for reference. Iron (II) sulfate was used to construct the standard curve, and the results were expressed as µmol Fe^{2+}/g dry weight (DW) (Zugic et al., 2014).

Antibacterial assays

The antibacterial efficacy of the extracts was evaluated against one Gram-negative bacterial strain, *Escherichia coli*, and one Gram-positive bacterial strain, *Staphylococcus aureus*, using the Kirby-Bauer disc diffusion method[66,67]. To prepare the growth medium, 28 g of nutrient agar (NA) was dissolved in 1000 mL of distilled water, sterilized in an autoclave at 121 °C for 15 min, and then dispensed into Petri dishes. A bacterial colony was aseptically transferred with a loop, suspended in 10 mL of physiological saline, and adjusted to a half McFarland standard (OD600 = 0.08–0.13). Sterile disks were impregnated with 400 µL of the extracts and placed on agar plates inoculated with the bacterial strains. The plates were incubated at 37 °C for 24 h. After incubation, the inhibition zone around the discs, which indicated bacterial growth inhibition, was measured using a digital caliper (Carbon model, China). Tetracycline at a concentration of 400 µg/mL served as the positive control, while distilled water was included as the negative control in the experimental setup.

Statistical analysis

The five populations of *D. carota* were classified and grouped based on ward distances by analyzing the essential oil composition data matrix using hierarchical cluster analysis (HCA) and principal component analysis (PCA) with PAST software (version 4.03). Additionally, canonical corresponding analysis (CCA) was conducted on the morphological, phytochemicals, and essential oil content and composition using the same software. CCA was conducted to assess the significant relationships between environmental characteristics and the phytochemical compounds obtained from five populations. Heat-map cluster, correlation analysis, Mantel Test correlation matrix, circular cluster for combined data of all genotypes were obtained using RStudio version 4.4.1 (URL: https://www.rstudio.com/products/rstudio/download/). In the present study cluster analysis was employed to group chemotypes and identify the phytochemical compounds involved in this classification. The Mantel Test was utilized to determine the phytochemical and morphological traits that influence the antioxidant and antibacterial effects against *E. coli* and *S. aureus*. Furthermore, analysis of variance with completely randomized design (CRD) (ANOVA) and means comparisons with Duncan's Multiple Range test (DMRT) were carried out using SAS software version 9.4. Data analysis for the morphological and phytochemical evaluations and antibacterial properties was conducted using a completely randomized design. Mean comparisons were performed employing Duncan's multiple range test in the MSTATC software version 1.2 (URL: https://www.agroninfo.com/mstat-c/). The correlation between the two data sets was examined through multiple regression analysis, utilizing the "linear regression analysis" and "stepwise method" options in SPSS version 23. Additionally, stepwise regression analysis was used to identify key morphological traits necessary for developing effective regression models for each phytochemical compound.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Leila Dehghani: Investigation, Material collection, Analysis, Drowning and designing of Fig. 2. Mohammad Fattahi: Supervision, Conceptualization, Visualization, Methodology, Data curation, Writing-Review and Editing, Project administration, Sanaz Ashrafi-Saeidlou: Supervision, Editing the manuscript, Methodology, Drowning and designing of Fig. 1.

Declarations

Competing interests

The authors declare no competing interests.

Ethical statement

Plant sampling were comply with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Additional information

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