Chemical and Biological Technologies in Agriculture

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Characterization of essential oil profiles, triterpenic acids, and biological assay in aerial parts of various *Thymus persicus* Jalas (Ronniger ex Rech.f.) populations

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Abstract

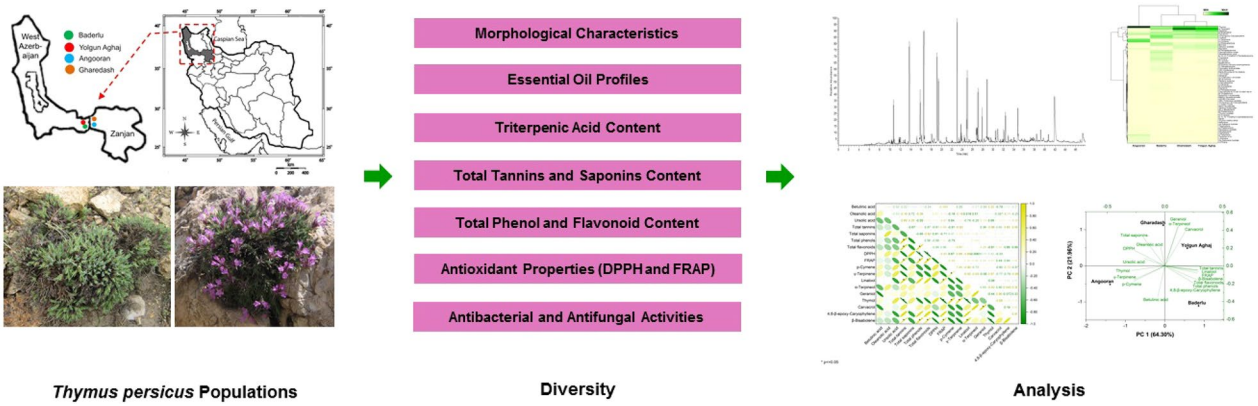
Thymus persicus is a valuable natural source of anticancer triterpenic acids (TAs) such as betulinic acid (BA), oleanolic acid (OA), and ursolic acid (UA), which is growing wild in the northwest of Iran. In the present study, variability in morphological characteristics, phytochemical composition, and biological activity among *T. persicus* populations (*TPPs*) were investigated. The plants were phenotypically different with the highest variations in some morphological traits. In total, sixty-seven compounds representing 97.2—99.9% of the essential oils were identified. Thymol (8.1—43.9%), α-terpineol (1.8—34.2%), and *p*-cymene (0.4—13.4%) were the major components of the studied oils. The content of BA, OA, and UA was ranged as 530.55 ± 13.04—856.89 ± 6.76, 419.35 ± 11.44—584.43 ± 12.67, and 941.66 ± 11.49—1070.82 ± 10.14 mg 100 g^{-1} DW in the studied *TPPs*, respectively. The highest total phenol content (87.26 ± 4.35 mg GAE g^{-1} DW), total flavonoid content (72.34 ± 2.63 mg QE g^{-1} DW), and antioxidant property (IC_{50} = 64.28 ± 4.57 µg ml^{-1} and 61.68 ± 1.10 µmol Fe^{+2} g^{-1} DW) were recorded in *TPP1* (Baderlu). The essential oil of the *TPP3* (Angooran) showed the lowest minimal inhibitory concentration (MIC) against the bacteria (0.005—0.080 mg ml^{-1}) and fungi (0.077—0.100 mg ml^{-1}) among the studied *TPPs*. Multiple regression analysis showed an associated correlation among morphological, phytochemical characteristics, and biological activities. Canonical correspondence analysis also determined relationship between phytochemical traits and environmental factors. These findings contain valuable data for the conservation and sustainable exploitation of this valuable medicinal plant.

Keywords Chemotype, Essential oil, Germplasm, Lamiaceae, Morphology, Thyme, Triterpenoids

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Graphical Abstract



Introduction

Medicinal and aromatic plants (MAPs) are shown to possess a high broad diversity among the widespread worldwide plants. The MAPs have come to the consideration of many countries as a natural chemical wealth [35, 64, 69]. Unluckily, the increased demand for consumption of MAPs puts this valuable asset at risk of degradation. Evidently, about eight percent of the world's plants (more than 34,000 plant species involved, by considering their medication) are in a hazardous unstable situation that may enhance their erosion and destruction. Thus, a critical focus on the protection and sustainable exploitation of the plant genetic resources is urgently needed [38]. The plants are heterogeneous based on the morphological and phytochemical traits in different climates and natural habitats [63]. Thereby, for the principle and industrial use of MAPs, it is necessary to evaluate the identity and the nature of them from a variety of genetic, morphological, chemical, and manufacturing perspectives [16, 55, 56].

Due to climate diversity, Iran has a vast and distinctive biodiversity, especially for MAPs. Seven thousand five hundred plant species, of which 1700 are MAPs are represented in the Flora of Iran [42]. Of course, any efforts to characterize the morphological and phytochemical diversity of each medicinal plant can lead to the introduction of vulnerable species in the agricultural systems and for the production of new pharmaceutical products as well. The high quality and content of natural products has attracted their application in food and pharmaceutical industries, and their high potential in the agricultural sector as a fungicide, insecticide and herbicide has also been presented [27, 77].

Thymus sp. L. is one of the most important genera of the Lamiaceae family and consists of over 300 species of herbaceous annuals and perennials that are widely distributed throughout the world, especially in the Mediterranean region [59]. *Thymus* species are known to contain a different class of compounds such as essential oils (EOs), phenolics *i.e.* tannins [61, 71], saponins [62], and triterpenes [66]. The essential oils and crude extracts of *Thymus* species are extensively used in the perfumery, food, cosmetic, and pharmaceutical industries [37, 72]. Antiseptic, antioxidative, carminative, bacterial impressionability, antimicrobial, and insecticidal properties of *Thymus* species have been extensively reported [46, 72].

The genus *Thymus* is represented in Iran by fourteen species, of which four as *T. persicus* (Ronniger ex Rech. f.) Jalas, *T. daenensis* Celak, *T. caramanicus* Jalas, and *T. trautvetteri* Klokov & Desj.-Shost are endemic [54]. *Thymus persicus*, commonly known as "*Avishan-e-Irani*" is one of the valuable and rare medicinal species which is grown in the restricted region of northwest of Iran. The chemical composition and antibacterial activity of the plant EOs have previously been reported [14, 65]. The aerial parts of *T. persicus* are also interesting as a source of the three well-known triterpenic acids (TAs) namely, betulinic acid (3β-hydroxy-20(29)-lupaene-28-oic acid, BA), oleanolic acid (3β-hydroxyolean-12-en-28-oic acid, OA), and ursolic acid (3β-hydroxy-12-ursen-28-ic acid, UA) [13, 52]. A wide range of biological activities of TAs including anti-inflammatory and antioxidant, anti-HIV, antifungal, antibacterial, immunomodulatory, antidiabetic, and anticancer activities have been characterized [1, 51]. Various *Thymus* species have been studied worldwide for their total phenolic content (TPC) and total flavonoid content (TFC). Antioxidant activities of different *Thymus* species have also been reported [73]. Based on the attractive metabolic profile of *T. persicus* and likely

the high demand for the plant materials, there is an immediate need to protect and exploit this plant in the agricultural system. In vitro propagation, conservation, and cell suspension culture of the plant have been previously reported [11–13].

Morphological characterization, as well as the phytochemical assessment, are the main steps in the description and classification of germplasm [9]. Cluster analysis allows analyzing both quantitative and qualitative traits simultaneously and has been employed to assess similarities among genotypes in plant breeding programs. As it could be ascertained, *T. persicus* populations (*TPPs*) have not been studied yet. Therefore, the present study aimed to characterize the morphological and phytochemical diversity, and biological activities among *TPPs* grown in Iran and to detect the connection between the two sets of data by multiple regression analysis. According to the variation of the diversification of *T. persicus* in morphological and phytochemical traits, as well as antioxidant, antifungal, and antibacterial activities, this study probed the advantages of the characteristics in terms of elucidating of outstanding traits to manipulate in breeding programs, defining as main selection criteria for the high TAs content and desired essential oil chemotype. These findings can be interestingly considered by breeders and farmers for the commercial exploitation.

Materials and methods

Plant materials and chemicals

Aerial parts of the thirty individual plants of *T. persicus* representing of *TPPs* were collected from four different localities (Baderlu, Yolgun Aghaj, Angooran, and Gharedash) in the Northwest Provinces of Iran (Table 1, Fig. 1). The individuals were selected from the same age plants. The distance between the sampled individuals and populations in each collection site was at least 100 and 2000 m, respectively. The plant samples were botanically identified by Prof. Ali Sonboli and voucher specimens have been deposited at the Herbarium of Medicinal Plants and Drugs Research Institute (MPH-2232, MPH-2233, MPH-2234, and MPH-2235), Shahid Beheshti University, Tehran, Iran (Table 1).

Standards, reagents, streptomycin, fluconazole, and chemical compounds were supplied from Sigma-Aldrich company (USA). Hydrochloric acid, sulphuric acid, diethyl ether, acetic acid, ethanol, acetone, methanol, dimethylsulfoxide (DMSO), HPLC grade methanol, and phosphoric acid of analytical grade were purchased from Merck Corporation (Germany). Authentic of some essential oil components used as standards for GC-FID analyses were purchased from Merck.

Morphological analysis

Morphological characteristics were measured on thirty samples. Names and morphological traits of each population are listed in Table 2. All the traits except the number of branches, calyx nervure, nodes per shoot, leaves per stem, flowers per inflorescence, inflorescences per plant, seed per inflorescence, and non-numeric morphological characteristics were measured using a ruler and digital caliper.

Essential oil isolation and analysis

The EOs were isolated from the air-dried aerial parts (100 g) of *TPPs* by hydro-distillation using a Clevenger apparatus recommended by the British Pharmacopeia [22] for 3 h. The content of EOs (mg 100 g $^{-1}$) was calculated just after isolation, and based on triplicate isolation runs. The isolated oils were then dried over anhydrous sodium sulfate (Na_2SO_4) and were kept in the freezer (–20 °C) until analysis. The oil content (%) was calculated as follows formula [73]:

$$\text{Essential oil content (\%)} = \left[\text{mass of oil obtained (g)} / \text{ mass of dry matter (g)}\right] \times 100.$$

Table 1 Localities and climatic condition of the studied *Thymus persicus* populations (*TPPs*) from Iran

No	Population name	Code	Collection site	Voucher number	Climate conditions				
					Altitude (m)	Longitude	Latitude	MAT (°C)	MAP (mm)
1	Baderlu	*TPP1*	Takab, West Azerbayjan	MPH-2232	2374	47°14′	36°28′	9.1	352
2	Yolgun Aghaj	*TPP2*	Takab, West Azerbayjan	MPH-2233	2450	47°13′	36°27′	9.2	390
3	Angooran	*TPP3*	Angooran, Dandy road, Zanjan	MPH-2234	2272	47°26′	36°36′	9.0	340
4	Gharedash	*TPP4*	Gharadash Dandy road, Zanjan	MPH-2235	2522	47°25′	36°45′	8.7	380

MAT mean annual temperature, *MAP* mean annual precipitation

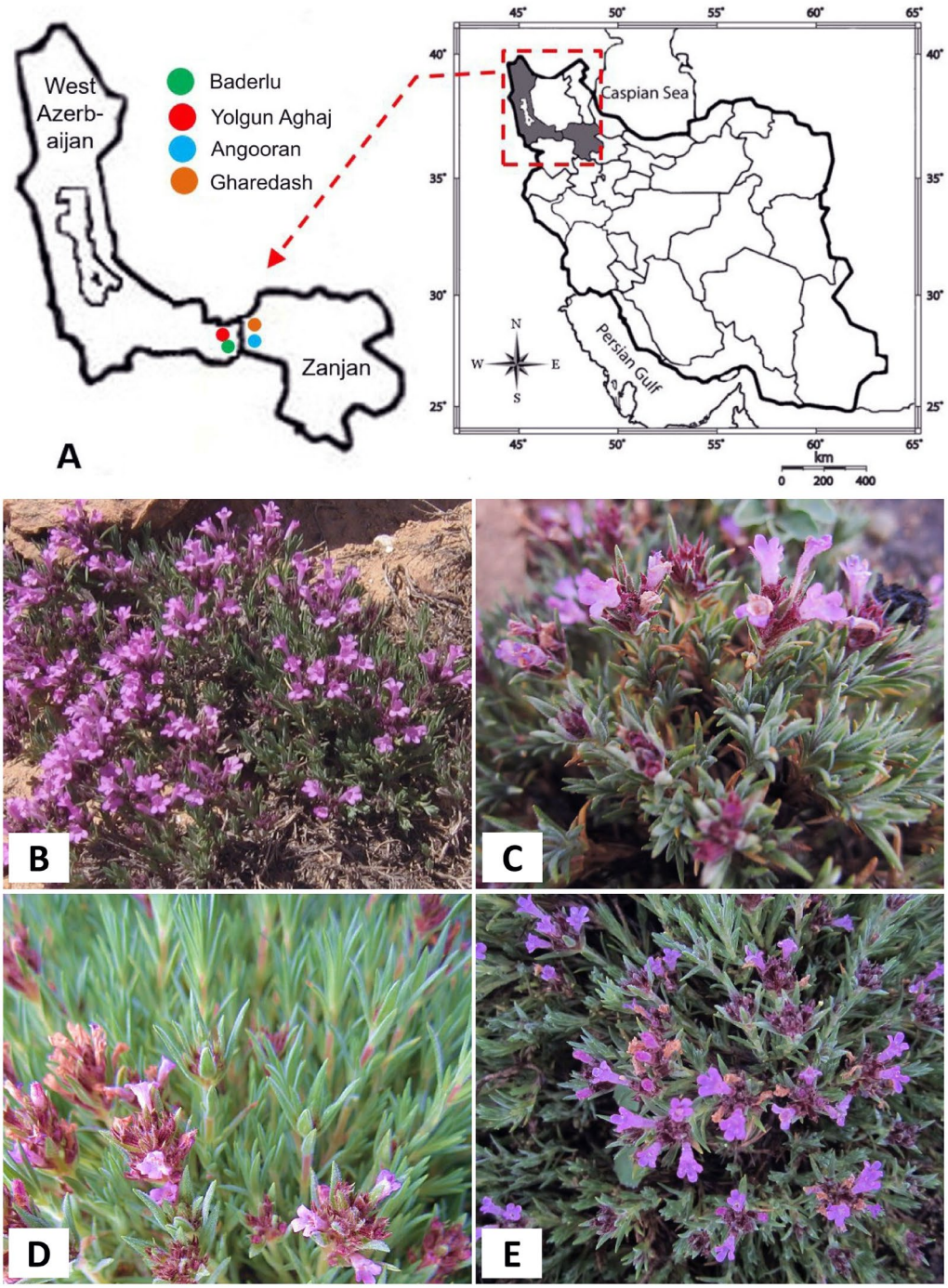


Fig. 1 Geographical distribution of the studied populations of *Thymus persicus* (**A**). Wild growing plants in their natural habitats including Baderlu (**B**), Yolgun Aghaj (**C**), Angooran (**D**), and Gharadash (**E**)

The EOs samples were analyzed by gas chromatography-flame ionization detector (GC-FID) and GC-mass spectrometry (GC–MS), and the chemical constituents were then identified. Analytical conditions were: helium as carrier gas (flow rate, 1.1 ml/min) with ionization voltage of 70 eV, injector temperature 250 °C, detector temperature 300 °C, split ratio (1:50), oven temperature program: 60–250 °C at the rate of 4 °C/min and then held for 5 min. The analysis was performed on fused silica capillary DB-5 column (30.0 m × 0.25 mm,

Table 2 Means comparison of morphological characteristics of the studied *Thymus persicus* populations (*TPPs*)

Parameters	Unit	Code	Mean ± SE			
			Baderlu (*TPP1*)	Yolgun Aghaj (*TPP2*)	Angooran (*TPP3*)	Gharadash (*TPP4*)
Plant height	cm	PH	7.7 ± 1.0^{bc}	7.5 ± 1.1^{c}	7.9 ± 1.1^{b}	9.2 ± 0.9^{a}
Canopy diameter	cm	CanD	34.5 ± 7.3^{ab}	35.0 ± 1.0^{a}	28.9 ± 5.0^{c}	32.4 ± 4.1^{b}
Collar diameter	mm	ColD	20.1 ± 5.9^{a}	19.8 ± 0.9^{b}	17.2 ± 3.1^{c}	18.9 ± 1.0^{bc}
Number of branches	–	NB	6.4 ± 2.2^{b}	6.7 ± 1.2^{ab}	5.8 ± 1.1^{c}	6.2 ± 3.1^{bc}
Branches length	cm	BrL	14.8 ± 0.7^{b}	14.2 ± 1.1^{b}	14.5 ± 2.1^{b}	15.0 ± 3.7^{a}
Dry matter weight	g/plant	DMW	209.4 ± 19.8^{ab}	187.5 ± 10.9^{c}	235.5 ± 20.0^{a}	200.9 ± 17.1^{b}
Root weight	g/plant	RW	123.4 ± 17.0^{b}	102.6 ± 1.7^{b}	151.1 ± 40.5^{a}	90.6 ± 13.5^{c}
Root length	mm	RL	6.1 ± 0.1^{a}	5.9 ± 1.4^{ab}	4.1 ± 0.5^{c}	5.1 ± 0.3^{bc}
Stem length	cm	StL	5.1 ± 0.8^{c}	5.2 ± 0.2^{bc}	5.5 ± 0.8^{b}	6.0 ± 0.6^{a}
Stem diameter	mm	StD	1.0 ± 0.3^{c}	1.0 ± 0.0^{c}	1.2 ± 0.1^{b}	2.0 ± 0.1^{a}
Stem color[a]	–	StCl	1.4 ± 0.5^{b}	1.2 ± 0.4^{bc}	1.0 ± 0.5^{c}	3.8 ± 0.3^{a}
Stem coat[b]	–	StCo	2.3 ± 0.0^{ab}	2.1 ± 0.9^{b}	2.2 ± 0.3^{ab}	3.7 ± 0.1^{a}
Stem glands[c]	–	StG	0.1 ± 0.7^{b}	0.4 ± 0.8^{b}	3.0 ± 0.1^{a}	0.6 ± 0.4^{b}
Flower stem length	mm	FSL	4.6 ± 0.3^{b}	4.7 ± 0.2^{a}	4.4 ± 0.3^{b}	4.5 ± 0.1^{b}
Peduncle length	mm	PL	1.5 ± 0.5^{b}	1.5 ± 0.2^{b}	1.8 ± 0.1^{a}	1.7 ± 0.6^{ab}
Peduncle color[d]	–	PCl	3.0 ± 1.2^{b}	3.0 ± 0.5^{bc}	2.5 ± 1.0^{c}	3.9 ± 1.0^{a}
Number of calyx nervure	–	NCN	9.5 ± 0.5^{b}	9.7 ± 0.0^{a}	8.8 ± 0.5^{c}	8.3 ± 0.3^{c}
Internode length	mm	IntL	7.7 ± 1.5^{c}	8.1 ± 1.9^{b}	9.8 ± 0.0^{ab}	10.1 ± 4.0^{a}
Number of nodes per shoot	–	NNS	8.7 ± 1.1^{b}	8.4 ± 1.0^{b}	9.1 ± 0.9^{ab}	9.2 ± 1.1^{a}
Leaf length	cm	LL	11.1 ± 0.9^{a}	10.0 ± 1.0^{b}	10.3 ± 1.0^{b}	11.8 ± 0.5^{a}
Leaf width	cm	LW	0.6 ± 0.2^{d}	0.7 ± 0.2^{c}	0.8 ± 0.1^{b}	0.9 ± 0.4^{a}
Leaf index (length/width)	ratio	LLWR	18.7^{a}	15.0^{b}	13.7^{c}	13.8^{c}
Number of leaves per stem	–	NLS	21.3 ± 1.8^{a}	17.0 ± 0.0^{b}	17.1 ± 2.0^{b}	15.7 ± 1.0^{c}
Leaf color[e]	–	LCl	2.9 ± 0.7^{b}	2.9 ± 0.0^{b}	2.3 ± 0.4^{c}	3.6 ± 0.2^{a}
Leaf coat[f]	–	LCo	1.6 ± 0.1^{c}	1.8 ± 0.1^{bc}	1.6 ± 1.0^{ab}	2.1 ± 0.2^{a}
Leaf gland spots[g]	–	LGS	0.7 ± 0.6^{d}	1.6 ± 0.9^{c}	2.3 ± 0.5^{b}	3.5 ± 0.0^{a}
Flower length	mm	FL	8.9 ± 1.3^{b}	9.0 ± 0.9^{ab}	9.3 ± 1.1^{a}	9.2 ± 1.0^{ab}
Number of flowers per inflorescence	–	NFI	8.5 ± 0.3^{ab}	8.8 ± 0.3^{ab}	9.0 ± 0.6^{a}	8.1 ± 0.0^{b}
Number of inflorescences per plant	–	NIP	159.1 ± 12.0^{a}	128.2 ± 6.5^{c}	143.9 ± 11.1^{b}	140.1 ± 10.8^{b}
Inflorescence length	mm	InfL	9.1 ± 0.8^{bc}	9.3 ± 0.9^{b}	8.2 ± 0.4^{c}	10.2 ± 1.0^{a}
Bract length	mm	BL	3.0 ± 0.0^{b}	3.0 ± 0.5^{b}	3.5 ± 0.2^{a}	3.1 ± 0.6^{ab}
Bract width	mm	BW	0.5 ± 0.0^{b}	0.5 ± 0.2^{b}	0.5 ± 0.0^{b}	0.6 ± 0.1^{a}
Bract index (length/width)	ratio	BLWR	6.0^{b}	6.0^{b}	7.0^{a}	5.2^{c}
Bracteole length	mm	BolL	3.5 ± 0.7^{a}	3.5 ± 0.3^{ab}	3.2 ± 0.5^{b}	3.1 ± 0.0^{b}
Bracteole width	mm	BolW	0.9 ± 0.0^{b}	0.9 ± 0.0^{b}	1.1 ± 0.2^{a}	1.1 ± 0.1^{a}
Calyx length	mm	CaL	3.9 ± 0.4^{ab}	3.7 ± 1.2^{b}	4.8 ± 0.7^{a}	4.3 ± 0.9^{ab}
Calyx width	mm	CaW	1.5 ± 0.9^{b}	1.5 ± 0.5^{b}	1.5 ± 0.2^{b}	1.6 ± 0.3^{a}
Calyx color[h]	–	CaCl	2.5 ± 0.7^{ab}	2.1 ± 0.0^{b}	3.9 ± 0.5^{a}	3.9 ± 1.1^{a}
Calyx coat[i]	–	CaCo	2.8 ± 0.8^{b}	1.0 ± 0.3^{bc}	1.9 ± 0.1^{c}	3.1 ± 0.5^{a}
Corolla length	mm	CoL	7.5 ± 0.3^{b}	7.7 ± 0.8^{ab}	7.8 ± 1.1^{ab}	7.9 ± 0.6^{a}
Corolla width	mm	CoW	1.4 ± 0.4^{b}	1.4 ± 0.2^{b}	1.4 ± 0.7^{b}	1.6 ± 1.1^{a}
Corolla color[j]	–	CoCl	3.0 ± 1.0^{c}	2.9 ± 0.4^{d}	3.6 ± 0.2^{b}	3.9 ± 0.1^{a}
Corolla coat[k]	–	CoCo	2.1 ± 0.7^{b}	2.1 ± 0.2^{b}	2.3 ± 0.0^{ab}	2.9 ± 1.1^{a}
Anther length	mm	AL	0.5 ± 0.1^{a}	0.4 ± 0.1^{b}	0.4 ± 0.0^{b}	0.5 ± 0.0^{a}
Pistil length	mm	PisL	5.3 ± 0.5^{a}	5.1 ± 0.0^{ab}	5.2 ± 0.2^{ab}	5.0 ± 1.1^{b}
Stamina length	mm	StaL	2.0 ± 0.9^{b}	2.1 ± 0.2^{ab}	2.1 ± 0.1^{ab}	2.3 ± 0.5^{a}
Seed length	mm	SL	1.8 ± 0.4^{b}	1.7 ± 0.8^{b}	2.2 ± 0.1^{ab}	2.4 ± 0.9^{a}
Seed width	mm	SW	0.9 ± 0.5^{c}	0.9 ± 0.2^{c}	1.0 ± 0.6^{b}	1.5 ± 0.0^{a}
Number of seed per inflorescence	–	NSI	3.9 ± 0.9^{a}	3.9 ± 0.5^{a}	3.8 ± 0.4^{ab}	3.5 ± 0.0^{b}

a: 2 Light green, 3 Green, 4 Purplish green. *b*, *f*, *l*, *k*: 1 Glabrous, 2 Tomentose, 3 Strigose, 4 Densely hispid. *c*: 1 No gland, 2 Yellow gland, 3 Violet gland. *d*: 2 Light Green, 3 Green, 4 Purplish green. *e*: 2 Greenish, 3 Green, 4 Grayish green. *g*: 1 No gland spots, 2 Light yellow gland spots, 3 Yellow gland spots, 4 Light purple gland spots. *h*: 2 Light green, 3 Light purple, 4 Violet purplish. *j*: 2 Light pink, 3 Pink, 4 Light violet

The data represents mean ± standard error (SE) of replicates ($n = 30$). Different letters mean significant difference at 95% (Tukey test—$p < 0.05$) (Tukey's pairwise comparison test; $p < 0.05$)

0.25 µm). To identify the constituents of the EOs, their mass spectra were compared with those of authentic standards from the internal reference mass spectra library [3]. From the GC data, the retention indices of constituents were calculated against those of n-alkanes (C_6 to C_{24}) and the EOs on a DB-5 column under the same chromatographic conditions.

Extraction of triterpene acids and HPLC analysis

Betulinic acid, OA, and UA were extracted from the aerial parts of *TPPs* following the method reported [11], with some modifications. Dried Powder of plant material (each 1.0 g) was mixed with 40 ml methanol and extracted by sonication (150 W,28 kHz) for 40 min. The obtained methanolic mixture was centrifuged at 10,000 rpm for 10 min. The supernatant was pooled and concentrated in a rotary evaporator at 40 °C (Heidolph Instruments GmbH, Schwabach Germany). The methanolic extract was further separated into organic (30 ml ethyl acetate) and aqueous (30 ml double distilled water) layers. The ethyl acetate phase was collected and evaporated in a rotary evaporator at 40 °C. The dry ethyl acetate extract was dissolved in HPLC grade methanol (10 ml), filtered through a Millipore filter (0.45 mm), and used for analysis. The HPLC instrument properties and proportion of solvents used for TA analysis were according to the method of Bakhtiar et al. [13]. The analysis was performed using HPLC equipped with a 2800 Smartline photodiode array (PDA) detector with a C_{18} analytical column (250×4.6 mm, 3.5 µm and a UV detector (Waters 2487. The following gradient system was used with methanol-phosphoric acid–water (87:0.05:12.95, v/v/v. The flow was maintained at 0.5 ml/min and column temperature at 25 °C; sample injection was 20 µl. Calibration curves were constructed by injecting separately standard solutions at the seven concentrations of 2, 10, 50, 100, 200, 500, and 1000 ppm. All injections were performed in triplicates. Absorbance was recorded at 210 nm wavelength. System suitability tests were performed by checking the linearity, precision, and recovery of three triterpene acids in the quantification experiment. The calibration curves were prepared by linear regression by a graph informing the area ratio of an external standard.

Determination of total tannins and total saponins content

Total tannin content (TTC) was performed according to Abdouli et al. [2] with some modifications. For instance, powdered dried aerial parts of *TPPs* (500 mg) were mixed with 5 ml diethyl ether containing 1% acetic acid and were then centrifuged at 2,500 rpm for 5 min. After removing the supernatant, re-extraction was performed with 5 ml of acetone (70%) and shaking for 1 h. The TTC was calculated as the difference in TPC based on the Folin–Ciocalteu method before and after the treatment with polyvinylpyrrolidone (PVP) 4000.

Total saponin content (TSC) of the studied samples was determined as described previously [4] with some modifications. The samples were extracted in a microwave system (Milestone ETHOS UP, Italy). Initially, powdered dried sample (500 mg) was extracted using a microwave-assisted extraction method subjected to irradiation (5 min), 575-Watt microwave power, and 1:10 g ml^{-1} solid-to-solvent ratio (500 mg sample, 5 ml ethanol). The mixture was then centrifuged at 2000 rpm for 10 min and dried under reduced pressure in a rotary evaporator. After that, 50 µl extract was mixed with 200 ml methanol, 100 µl vanillin-ethanol (10:90 w/v), and 300 µl sulphuric acid (70%) and heated at 100° C for 5 min. The absorption was read at a wavelength of 540 nm by the spectrophotometer (Bio-Tek Instruments, Inc., USA). The results were expressed as mg diosgenin equivalent per gram dry weight basis (mg DE g^{-1} DW). Calibration curves ($y=0.0015x+0.0045$, $R^2=0.9999$) were plotted using several concentrations of diosgenin (100–500 mg ml^{-1}). The TSC was determined as follows: [the volume of extraction solvent (ml)×the concentration measured from diosgenin standard curve (mg ml^{-1}]/the dry weight of the sample (g).

Determination of total phenol and total flavonoid content

The total phenolic content (TPC) of the samples was determined by the method of Singleton [70] using gallic acid (GA) as the standard. Briefly, plant extracts (25 µl), Folin–Ciocalteu's reagent (125 µl), and sodium carbonate (100 µl, 7.0%) were mixed and incubated for 30 min at room temperature. Absorbance was measured at 765 nm against methanol as a blank. Data expressed as mg GA equivalents per g of dry matter (mg GAE g^{-1} DW). The extraction was conducted in triplicate. The linearity range of the calibration curve was 10 to 1000 µg ml^{-1} ($y=0.0038x+0.1579$, $R^2=0.9938$).

The total flavonoid content (TFC) was determined using the method of Chang et al. [25] with some modifications. In summary, 20 µl solution of the sample, 80 µl of distilled water, 6 µl (0.5 M) sodium nitrite ($NaNO_2$), 6 µl (0.3 M) aluminum chloride hexahydrate ($AlCl_3.6H_2O$), and 80 µl (1.0 M) sodium hydroxide (NaOH) were pipetted to plate, respectively. The mixture was allowed to stand for 10 min at room temperature, and absorbance was determined at 510 nm versus the prepared water blank. The average of three readings was used and then expressed as quercetin equivalents (QE) on g dry weight basis (mg QE g^{-1} DW). The linearity range of the calibration curve was 10 to 1000 µg ml^{-1} ($y=0.0005x+0.1270$, $R^2=0.9888$). The assay for each sample was conducted in triplicate.

Assay of antioxidant properties

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was determined based on the method of Blois [20] with some modifications. Briefly, 200 µl of methanolic extract (1 mg ml^{-1}) of the plant sample were serially diluted in a 96 well plate and 400 µl of DPPH solution (0.1 mM) was added to each well containing the diluted samples. The negative control was prepared by mixing the DPPH working solution (2 ml) with methanol (1 ml). The solutions were incubated at room temperature for 60 min in the dark. The absorbance values were recorded at 515 nm. DPPH assay was carried out in triplicate for each sample. The inhibition percentage of anti-oxidative activity was determined using the equation: DPPH clearance = $A_{control}-A_{sample})/A_{control}\times 100\%$. The DPPH radical scavenging activity of butylated hydroxytoluene (BHT) was also assayed for comparison. The concentration providing 50% inhibition (IC_{50}) was calculated using a calibration curve in the linear range by plotting the extract concentration vs. the corresponding scavenging effect. IC_{50} value, representing the amount of extract which scavenged 50% of the DPPH radical, was calculated from percent scavenging versus concentration curve. A higher concentration to reduce 50% of DPPH solution showed lower antioxidant activity. Results were expressed as IC_{50} µg ml^{-1}.

The ferric reducing-antioxidant assay (FRAP) solutions were prepared as described previously [17]. The reagent was prepared by mixing acetate buffer (20 ml, 300 mmol l^{-1}, pH 3.6), 10 mmol l^{-1} TPTZ solution (2.5 ml) in 40 mmol l^{-1} hydrochloric acid and 20 mmol l^{-1} iron (III) chloride ($FeCl_3$) solution (2.5 ml) in proportions of 10:1:1 (v/v), respectively. The mixture was allowed to react for 30 min at temperature of 37 °C. The absorbance of the mixture was then read at 593 nm. Ascorbic acid was used as the standard curve. The standard curve was constructed using iron (II) sulfate ($FeSO_4$) solution (0.5–10 mg ml^{-1}). The regression equation was obtained: $y=0.0035x-0.0030$, $R^2=0.9991$. The results were expressed as µmol of Fe^{+2} per gram dry plant weight (µmol Fe^{+2} g^{-1} DW).

Absorbance was measured using a spectrophotometer (Bio-Tek Instruments, Inc., USA). All the analyses were run in three replicates and the results were expressed as mean ± standard deviation (SD).

Antimicrobial assay

Four strains of bacteria and fungi were obtained from ATCC (American Type Culture Collection, Rockville, MD, USA). The two gram-positive strains: *Staphylococcus aureus* (ATCC 33591) and *Pseudomonas aeruginosa* (ATCC 27853) and two gram-negative strains: *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). The following four fungal strains including *Candida albicans* (ATCC 90028), *C. glabatra* (ATCC 90030), *C. krusei* (ATCC 6258), and *C. parapsilosis* (ATCC 22019) were used for the antifungal assay.

The bacterial strains were preserved at − 80 °C, subcultured on Mueller–Hinton agar (MHA) medium, and maintained at 4 °C, and grown at 37 °C when required. Stock fungal strains were subcultured on Sabouraud Dextrose Agar (SDA; Merck, Germany) and were maintained at 4 °C until testing was performed. Minimal inhibitory concentration (MIC) was defined as the concentration of test compound at which no macroscopic sign of cellular growth was detected in comparison to the control without compound. Minimal concentrations of bactericidal (MBC) and fungicidal (MFC) were defined as the concentration of compound at which no macroscopic sign of cellular growth was detected compared to the control upon subculturing. The MIC, MBC, and MFC were determined by microdilution method in 96 well microtitre plates described previously [26, 29]. The microbial suspensions of each bacterial and fungal strain were produced from freshly cultured cells in sterile saline that had been adjusted to 1.0×10^5 CFU/well. The EOs were dissolved in 5% DMSO solution that contained 0.10% Tween 80 (v/v) and added appropriate medium with bacterial and fungal inoculum. Essential oils were added in Tryptic Soy Broth (TSB) medium for bacteria, Sabouraud Dextrose Broth (SDB) medium for fungi. The microplates were incubated for 24 h and 48 h at 37 °C for bacteria and fungi, respectively. The MIC of samples was determined following the addition of 40 µl P-Iodonitrotetrazolium violet (INT) 0.2 mg/ml (Sigma I8377) and 30 min of incubation at 37 °C. The lowest concentration with no visible growth was defined as the MBC and MFC, indicating 99.5% killing of the original inoculum. Streptomycin and fluconazole were used as positive controls for bacteria and fungi, respectively. Sterilized distilled water containing 0.1% Tween 80 and 5% DMSO was used as negative control. The experiments were performed in triplicate.

Statistical analysis

Experiments were conducted in triplicates. Analysis of variance for morphological traits, correlation, cluster analysis, and principal components analysis were applied using the SPSS software Version 23. (SPSS Inc., Chicago, IL, USA) and Origin 2021. The significant difference between means were found using Tukey's multiple range test ($p<0.05$) and each of the values was expressed as Mean ± standard error (SE). Pearson product-moment correlation coefficient (r) was used for

Table 3 Codes non-numeric morphological characteristics of the studied *Thymus persicus* populations (*TPPs*)

Parameters	Code	Baderlu (*TPP1*)	Yolgun Aghaj (*TPP2*)	Angooran (*TPP3*)	Gharadash (*TPP4*)
Stem color	StCl	Light green	Light green	Purplish green	Purplish green
Stem coat	StCo	Tomentose	Tomentose	Tomentose	Densely hispid
Stem glands	StG	No gland	No gland	Yellow gland	No gland
Peduncle color	PC	Purplish green	Green	Green	Purplish green
Leaf color	LC	Green ash	Green ash	Greenish	Grayish green
Leaf coat	LO	Tomentose	Tomentose	Tomentose	Strigose
Leaf gland spots	LGS	No gland spots	No gland spots	Yellow gland spots	Light purple gland spots
Calyx color	CaCl	Violet purplish	Green	Light purple	Violet purplish
Calyx coat	CaCo	Tomentose	Tomentose	Tomentose	Strigose
Corolla color	CoCl	Pink	Ligth pink	Ligth violet	Ligth violet
Corolla coat	CoCo	Tomentose	Tomentose	Tomentose	Strigose

total characteristics ($\alpha = 0.01$ and 0.05). The correlation between two sets of data was performed by multiple regression analysis, using a "linear regression analysis" "stepwise" option of SPSS version 23.

Principle component analysis (PCA) was used to illuminate marked changes in the morphological data set. The PCA outcome was used to build biplots to portray the distribution and connection of *TPPs* concerning GC–MS and HPLC–PDA data. Biplots help with identifying clusters of metabolites that may be associated with the performance or regulations of the plant genotypes. Canonical correspondence analysis (CCA) was assessed using PAST software.

Results

Morphological traits

The morphological characteristics were significantly different between the *TPPs* (Tables 2 and 3). Among the studied traits, dry matter weight varied ranged from 187.5 (*TPP2*) and 235.5 g (*TPP3*). The plant height varied from 7.5 (*TPP2*) to 9.2 cm (*TPP4*). The leaf length ranged between 10 (*TPP2*) and 11.8 cm (*TPP4*), while leaf width varied from 0.6 (*TPP1*) to 0.9 cm (*TPP4*). The number of inflorescences per plant ranged from 128.2 (*TPP2*) to 159.1 (*TPP1*). Also, the calyx color varied from green (*TPP2*) to purple in the rest of the populations. Spearman's correlation coefficient was positively high if $0.68 < r < 0.97$. The results demonstrated a positive and negative correlation ($p < 0.01$, $p < 0.05$) between the morphological characteristics. Stem diameter, color, and coat, plant height, bract width, corolla width and coat, seed width, and number of seed per inflorescence had the highest positive and negative correlations with the studied morphological traits (Table 4). Understanding the relationship between the morphological traits helps to select suitable options for breeding programs [32].

The cluster analysis of morphological data based on UPGMA split populations into two distinct clusters, the first branch was divided into two sub-branches that the first one comprising *TPP1* (Baderlu), and the second one included *TPP2* (Yolgun Aghaj) and *TPP4* (Gharedash). The last completely separated branch was the *TPP3* (Angooran) (Fig. 2).

Essential oil content and composition

The highest yield of essential oils (w/w%) was recorded in *TPP3* (1.2), *TPP4* (0.85), *TPP2* (0.14), and *TPP1* (0.11), respectively (Table 5). In total, 44, 39, 29, and 26 components were identified in *TPP1*, *TPP2*, *TPP3,* and *TPP4* representing 97.2, 99.4, 97.2, and 99.9% of the total oils, respectively. Thymol (43.9%), followed by *p*-cymene (13.4%) and γ-terpinene (11.1%) were the major compounds identified in the *TPP3*. 4,8-β-*epoxy*-Caryophyllene (10.7%), α-terpineol (9.5%), and linalool (8.6%) were characterized in the *TPP1* as the main essential oil constituents, while α-terpineol (34.2%), thymol (17.7%) and geraniol (10.7%) were the main compounds in the *TPP4* oil. The major components of the *TPP2* were α-terpineol (23.3%), thymol (13.4%), and geraniol (12.8%) (Table 5). GC–MS chromatograms of the EOs from all *TPPs* are shown in Fig. 3.

The oils were found rich in oxygenated monoterpenes (41.2–78.8%), followed by monoterpene hydrocarbons (4.7–35.2%), sesquiterpene hydrocarbons (2.6–7.3%), and oxygenated sesquiterpenes (0.3–23.3%). Most of the essential oil samples were rich in terpenes, with the majority of monoterpenes followed by sesquiterpenes and diterpenes (Table 5).

Heatmap analysis classified *TPPs* into two main groups based on their essential oil compositions (Fig. 4). The group 1 consisted of the *TPP2*, *TPP4,* and *TPP1* and

Table 4 Correlation matrix among the studied characteristics of *Thymus persicus* populations (*TPPs*)

	PH	CanD	ColD	NB	BrL	DMW	RW	RL	StL	StD	StCl	StCo	StG	FSL	PL	PCl	NCN	IntL	NNS	LL	LW	LLWR	NLS	LCl
PH	1																							
CanD	− 0.26	1																						
ColD	0.43	0.76	1																					
NB	− 0.37	0.96*	0.66	1																				
BrL	0.92	− 0.09	0.56	− 0.26	1																			
DMW	− 0.03	− 0.87	− 0.81	− 0.92	− 0.02	1																		
RW	− 0.48	− 0.65	− 0.91	− 0.65	− 0.45	0.89	1																	
RL	− 0.31	0.98*	0.72	0.91	− 0.09	− 0.77	− 0.54	1																
StL	0.95*	− 0.46	0.19	− 0.47	0.77	0.08	− 0.36	− 0.54	1															
StD	0.99**	− 0.27	0.41	− 0.31	0.88	− 0.07	− 0.51	− 0.34	0.97*	1														
StCl	0.96*	0.03	0.67	− 0.05	0.92	− 0.31	− 0.71	− 0.03	0.85	0.95*	1													
StCo	0.98*	− 0.08	0.59	− 0.17	0.94	− 0.19	− 0.62	− 0.14	0.89	0.97*	0.99**	1												
StG	− 0.04	− 0.95	− 0.92	− 0.85	− 0.23	0.85	0.77	− 0.93	0.21	− 0.02	− 0.32	− 0.22	1											
FSL	− 0.45	0.95	0.56	0.99**	− 0.37	− 0.86	− 0.55	0.91	− 0.57	− 0.43	− 0.18	− 0.29	0.80	1										
PL	0.50	− 0.94*	− 0.57	0.30	0.73	0.42	− 0.98*	0.69	0.51	0.23	0.33	0.85	− 0.09	0.56	1									
PCl	0.82	0.34	0.86	0.26	0.82	− 0.57	0.88	− 0.26	0.66	0.82	0.95*	0.91	− 0.59	0.13	− 0.08	1								
NCN	− 0.90	0.65	− 0.00	0.69	− 0.77	− 0.36	0.09	0.68	− 0.95*	− 0.90	− 0.74	− 0.81	− 0.39	0.78	− 0.82	− 0.50	1							
IntL	0.76	− 0.78	− 0.23	− 0.74	0.53	0.42	0.03	− 0.84	0.91	0.79	0.56	0.63	0.60	− 0.81	0.92	0.29	− 0.94	1						
NNS	0.78	− 0.77	− 0.17	− 0.85	0.71	0.60	0.16	− 0.75	0.82	0.76	0.57	0.66	0.52	− 0.91	0.87	0.29	− 0.96*	0.89	1					
LL	0.83	0.04	0.64	− 0.15	0.95	− 0.08	− 0.46	0.07	0.63	0.78	0.86	0.88	− 0.37	− 0.25	0.14	0.81	− 0.64	0.35	0.60	1				
LW	0.82	− 0.58	− 0.02	− 0.51	0.56	0.14	− 0.24	− 0.68	0.96*	0.87	0.69	0.74	0.40	− 0.60	0.77	0.49	− 0.90	− 0.96*	0.77	0.38	1			
LLWR	− 0.46	0.61	0.31	0.44	− 0.10	− 0.16	0.06	0.74	− 0.71	− 0.54	− 0.31	− 0.35	− 0.58	0.48	− 0.74	− 0.13	0.63	− 0.84	− 0.50	0.10	− 0.88	1		
NLS	− 0.55	0.38	0.04	0.21	− 0.19	0.11	0.34	0.54	− 0.75	− 0.63	− 0.48	− 0.49	− 0.33	0.28	− 0.56	− 0.36	0.60	− 0.76	− 0.40	− 0.01	− 0.88	0.96*	1	
LCl	0.73	0.47	0.92	0.39	0.76	− 0.67	− 0.92	0.40	0.55	0.72	0.90	0.84	− 0.70	0.28	− 0.22	0.99*	− 0.37	0.15	0.16	0.77	0.36	− 0.02	− 0.27	1
LCo	0.82	0.16	0.67	0.18	0.67	− 0.55	− 0.85	0.03	0.78	0.86	0.91	0.87	− 0.36	0.05	0.12	0.92	− 0.57	0.50	0.32	0.59	0.71	− 0.49	− 0.69	0.88
LGS	0.86	− 0.49	0.09	− 0.44	0.61	0.05	− 0.34	− 0.60	− 0.97*	0.91	0.76	0.79	0.30	− 0.53	0.71	0.58	− 0.90	0.93	0.74	0.44	0.99**	− 0.85	0.88–	0.46
FL	0.50	− 0.91	− 0.54	− 0.82	0.23	0.59	0.30	− 0.96*	0.72	0.54	0.25	0.34	0.82	− 0.85	0.97*	− 0.03	− 0.79	0.94	0.79	0.04	0.85	− 0.87	− 0.73	0.17–
NFI	− 0.79	− 0.33	− 0.86	− 0.18	− 0.90	0.43	0.75	− 0.32	− 0.60	− 0.75	− 0.91	− 0.88	0.62	− 0.07	0.11	− 0.95	0.48	− 0.20	− 0.34	− 0.93	− 0.33	− 0.10	0.09	− 0.94
NIP	− 0.27	0.34	0.20	0.12	0.11	0.13	0.22	0.50	− 0.52	− 0.37	− 0.20	− 0.21	− 0.39	0.15	− 0.46	− 0.12	0.35	− 0.60	− 0.17	0.29	− 0.72	0.93	0.95*	− 0.05
InfL	0.68	0.52	0.93	0.46	0.69	− 0.74	− 0.96*	0.44	0.51	0.68	0.86	0.80	− 0.72	0.35	− 0.27	0.97*	− 0.30	0.11	0.08	0.69	0.35	− 0.05	− 0.30	0.99**
BL	0.05	− 0.97*	− 0.88	− 0.91	− 0.13	0.89	0.76	− 0.95*	0.28	0.06	− 0.25	− 0.14	0.99**	− 0.87	0.89	− 0.53	− 0.47	0.65	0.61	− 0.26	0.43	− 057	− 0.31	− 0.64
BW	0.98*	− 0.07	0.58	− 0.13	0.90	− 0.24	− 0.66	− 0.15	0.91	0.98*	0.99**	0.99**	− 0.21	− 0.26	0.33	0.91	− 0.80	0.65	0.63	0.82	0.77	− 0.43	− 0.59	0.85
BLWR	− 0.63	− 0.58	− 0.96*	− 0.50	− 0.69	0.74	0.94	− 0.52	− 0.44	− 0.63	− 0.83	− 0.76	0.79	− 0.38	0.35	− 0.96*	0.24	− 0.02	− 0.04	− 0.72	− 0.24	− 0.08	0.18	− 0.99**
BolL	− 0.84	0.73	0.13	0.74	− 0.65	− 0.41	0.02	0.78	− 0.94	− 0.85	− 0.65	− 0.72	− 0.51	0.84	− 0.89	− 0.39	0.98*	− 0.99*	− 0.94	− 0.50	− 0.94	0.75	0.69	− 0.25
BolW	0.71	− 0.85	− 0.32	− 0.84	0.52	0.56	0.17	− 0.90	0.86	0.73	0.48	0.57	0.67	− 0.89	0.96*	0.20	− 0.94	0.99*	0.94	0.35	0.90	− 0.77	− 0.65	0.05
CaL	0.34	− 0.98*	− 0.67	− 0.99**	0.24	0.90	0.64	− 0.94	0.50	0.33	0.05	0.17	0.87	− 0.99**	0.95	− 0.26	− 0.70	0.77	0.85	0.12	0.55	− 0.50	− 0.27	− 0.39
CaW	0.98	− 0.07	0.58	− 0.13	0.90	− 0.24	− 0.66	− 0.15	0.91	0.98*	0.99**	0.99**	− 0.21	− 0.26	0.33	0.91	− 0.80	0.65	0.63	0.82	0.77	− 0.43	− 0.57	0.85
CaCl	0.72	− 0.85	0.31	− 0.88	0.59	0.63	0.22	− 0.86	0.83	0.72	0.49	0.58	0.64	− 0.94	0.95	0.19	− 0.95	0.95	0.98*	0.45	0.83	− 0.64	− 0.52	0.05
CaCo	0.70	− 0.10	0.43	− 0.33	0.91	0.19	− 0.17	− 0.02	0.50	0.62	0.68	0.71	− 0.21	− 0.41	0.21	0.58	− 0.60	0.31	0.66	0.95	0.24	0.23	0.20	0.53

Table 4 (continued)

	PH	CanD	ColD	NB	BrL	DMW	RW	RL	StL	StD	StCl	StCo	StG	FSL	PL	PCl	NCN	IntL	NNS	LL	LW	LLWR	NLS	LCl
CoL	0.72	− 0.56	− 0.08	− 0.45	0.40	0.10	− 0.23	− 0.69	0.89	0.78	0.59	0.63	0.44	− 0.53	0.75	0.40	− 0.81	0.92	0.66	0.22	0.98*	− 0.95	− 0.95*	0.28
CoW	0.98*	− 0.07	0.58	− 0.13	0.90	− 0.24	− 0.66	− 0.15	0.91	0.98*	0.99**	0.99**	− 0.21	− 0.26	0.33	0.91	− 0.80	0.65	0.63	0.82	0.77	− 0.43	− 0.57	0.85
CoCl	0.87	− 0.70	− 0.06	− 0.73	0.72	0.40	− 0.05	− 0.73	0.95	0.88	0.69	0.77	0.45	− 0.81	0.86	0.44	− 0.99**	0.96*	0.96*	0.58	0.91	− 0.68	− 0.63	0.31
CoCo	0.99**	− 0.18	0.49	− 0.23	0.89	− 0.15	− 0.58	− 0.25	0.95	0.99**	0.97*	0.99*	− 0.11	− 0.35	0.43	0.87	− 0.86	0.73	0.70	0.80	0.83	− 0.49	− 0.61	0.78
AL	0.65	0.31	0.72	0.08	0.83	− 0.18	− 0.43	0.38	0.29	0.48	0.66	0.65	− 0.58	0.00	− 0.19	0.69	− 0.31	− 0.02	0.31	0.92	0.00	0.47	0.34	0.71
PisL	− 0.69	0.01	− 0.40	− 0.10	− 0.42	0.47	0.71	0.17	− 0.77	− 0.76	− 0.73	− 0.70	0.11	0.00	− 0.26	0.71	0.54	− 0.59	− 0.28	− 0.29	− 0.80	0.74	0.90	− 0.66
StaL	0.91	− 0.23	0.36	− 0.19	0.69	− 0.21	− 0.59	− 0.35	0.95*	0.95	0.88	0.89	0.01	− 0.31	0.49	0.77	− 0.81	0.80	0.61	0.55	0.92	− 0.72	− 0.83	0.68
SL	0.87	− 0.70	− 0.07	− 0.74	0.73	0.42	− 0.03	− 0.73	0.94	0.87	0.69	0.77	0.45	− 0.82	0.86	0.43	− 0.99**	0.95*	0.97*	0.60	0.90	− 0.65	− 0.60	0.30
SW	0.99**	− 0.23	0.44	− 0.28	0.89	− 0.10	− 0.54	− 0.31	0.96*	0.99**	0.96*	0.98*	− 0.05	− 0.40	0.48	0.84	− 0.89	0.77	0.74	0.79	0.85	− 0.52	− 0.63	0.75
NSI	− 0.99**	0.32	− 0.36	0.36	− 0.87	0.02	0.47	0.39	− 0.98*	− 0.99**	− 0.93	− 0.96*	− 0.04	0.48	− 0.56	− 0.78	0.92	− 0.82	− 0.79	− 0.76	− 0.89	0.57	0.65	− 0.69

	LCo	LGS	FL	NFI	NIP	InfL	BL	BW	BLWR	BolL	BolW	CaL	CaW	CaCl	CaCo	CoL	CoW	CoCl	CoCo	AL	PisL	StaL	SL	SW	NSI
LCo	1																								
LGS	0.78	1																							
FL	0.23	0.79	1																						
NFI	− 0.76	− 0.42	0.14	1																					
NIP	− 0.50	− 0.70	− 0.65	− 0.18	1																				
InfL	0.89	0.44	− 0.20	− 0.90	− 0.11	1																			
BL	− 0.33	0.34	0.84	0.54	− 0.34	− 0.68	1																		
BW	0.92	0.83	0.36	− 0.85	− 0.30	0.81	− 0.14	1																	
BLWR	− 0.83	− 0.35	0.30	0.92	− 0.01	− 0.99**	0.74	− 0.77	1																
BolL	− 0.53	− 0.92	− 0.89	0.33	0.48	− 0.20	− 0.58	− 0.73	0.12	1															
BolW	0.37	0.85	0.95	− 0.15	− 0.49	0.00	0.73	0.58	0.08	− 0.98*	1														
CaL	− 0.15	0.47	0.86	0.20	− 0.18	− 0.46	0.92	0.14	0.50	− 0.76	0.86	1													
CaW	0.92	0.83	0.36	− 0.85	− 0.30	0.81	− 0.14	0.99**	− 0.77	− 0.73	0.58	0.14	1												
CaCl	0.30	0.79	0.89	− 0.20	− 0.33	− 0.02	0.72	0.57	0.08	− 0.96	0.98	0.89	0.57	1											
CaCo	0.31	0.29	0.06	− 0.78	0.48	0.44	− 0.09	0.63	− 0.49	− 0.46	0.36	0.29	0.63	0.50	1										
CoL	0.68	0.97*	0.85	− 0.20	− 0.83	0.28	0.45	0.68	− 0.17	− 0.88	0.84	0.50	0.68	0.75	0.06	1									
CoW	0.92	0.83	0.36	− 0.85	− 0.30	0.81	− 0.14	0.99**	− 0.77	− 0.73	0.58	0.14	0.99**	0.57	0.63	0.68	1								
CoCl	0.54	0.90	0.84	− 0.41	− 0.40	0.25	0.53	0.76	− 0.18	− 0.99**	0.96*	0.74	0.76	0.96*	0.55	0.83	0.76	1							
CoCo	0.89	0.88	0.46	− 0.80	− 0.34	0.75	− 0.03	0.99**	− 0.69	− 0.80	0.66	0.24	0.99**	0.65	0.63	0.74	0.99**	0.83	1						
AL	0.37	0.07	− 0.32	− 0.88	0.60	0.63	− 0.48	0.58	− 0.70	− 0.14	0.00	− 0.12	0.58	0.12	0.91	− 0.17	0.58	0.24	0.53	1					
PisL	− 0.93	− 0.84	− 0.42	0.46	0.79	− 0.69	0.11	− 0.77	0.59	0.56	− 0.45	0.05	− 0.77	− 0.33	0.00	− 0.83	− 0.77	− 0.54	− 0.77	0.00	1				
StaL	0.92	0.96*	0.58	− 0.61	− 0.63	0.68	0.06	0.93	− 0.59	− 0.80	0.69	0.23	0.93	0.62	0.33	0.89	0.93	0.80	0.94	0.23	− 0.92	1			
SL	0.52	0.89	0.83	− 0.41	− 0.37	0.23	0.53	0.76	− 0.17	− 0.99**	0.96*	0.75	0.76	0.97*	0.57	0.81	0.76	0.99**	0.82	0.26	− 0.51	0.78	1		
SW	0.87	0.90	0.51	− 0.77	− 0.36	0.71	0.02	0.99*	− 0.65	− 0.83	0.70	0.30	0.99*	0.69	0.62	0.76	0.99*	0.86	0.99**	0.50	− 0.76	0.94	0.85	1	
NSI	− 0.84	− 0.92	− 0.58	0.72	0.39	− 0.64	− 0.11	− 0.97*	0.58	0.88	− 0.77	− 0.37	− 0.97*	− 0.75	− 0.61	− 0.80	− 0.97*	− 0.90	− 0.99*	− 0.46	0.75	− 0.94	− 0.89	− 0.99**	1

*, ** Correlation significant at $p < 0.05$ and $p < 0.01$, respectively

group 2 included *TPP3*. The group 1 was further divided into two sub-clusters.

Variability in phytochemical compounds

The phytochemical traits were significantly different among the studied *TPPs* (Table 6). Maximum contents (mg 100 g $^{-1}$ DW) of BA (856.89 ± 6.76), OA (584.43 ± 12.67), and UA (1070.82 ± 10.14) were determined in the aerial parts of *TPP1*, *TPP4* and *TPP3*, respectively (Table 6). Calibration curves for the standards illustrated good linearity at examined concentrations (2 to 1000 mg l^{-1}), with correlation coefficients (R^2) of 0.9991, 0.9994, and 0.9994 for BA, OA, and UA, respectively. Important differences were found in the correlations among the studied TAs. Total tannins content ranged from 246.32 ± 6.87 mg 100 g^{-1} DW in Angooran (*TPP3*) to 690.13 ± 9.38 mg 100 g^{-1} DW in Baderlu (*TPP1*). The highest TSC (36.78 ± 1.85 mg DE g^{-1} DW) was observed in Gharadash (*TPP4*), while the lowest value (18.46 ± 1.22 mg DE g^{-1} DW) was found in Baderlu (*TPP1*). The TPC in the extracts of the studied samples ranged from 24.31 ± 1.26 to 87.26 ± 4.35 mg GAE g^{-1} DW in *TPP3* and *TPP1*, respectively. The highest TFC (mg RE g^{-1} DW) was found in *TPP1* (72.34 ± 2.63), while the lowest content (21.12 ± 1.08) was determined in *TPP3* (Table 6).

Antioxidant properties

IC_{50} in *TPPs* ranged from 209.73 ± 4.32–64.28 ± 4.57 µg ml^{-1} for *TPP3* and *TPP1*, respectively. The antioxidant power varied from 34.11 ± 1.75–61.68 ± 1.10 µmol Fe^{+2} g^{-1} DW. This value in the studied samples was in the order of *TPP3* < *TPP4* < *TPP2* < *TPP1*.

According to PCA analysis, the studied populations were grouped into four different classes. The first and second PCA for the phytochemical compounds yielded 64.30% and 21.96% of the total variance, respectively (Fig. 5). Along axis 1 of the graph, *TPP2* was grouped on the positive region and contributed to carvacrol, α-terpineol, and geraniol. The *TPP4* was negatively correlated with TSC, OA, UA, and DPPH. Along axis 2 of the graph, *TPP1* formed a separate group on the positive region of the PC2 axis and were associated with TPC, TFC, TTC, FRAP, linalool, β-bisabolene, and 4,8-β-*epoxy*-caryophyllene. The highest BA, thymol, *p*-cymene, and γ-terpinene were found in *TPP3* that formed a group in the negative section of the PC2 axis (Fig. 5).

Antimicrobial activity

The studied EOs of *TPPs* showed a significant antibacterial activity against gram-positive and gram-negative bacteria (Table 7). The MIC for *TPPs* was ranged as 0.005–1.190 mg ml^{-1}, while the MBC was varied from 0.010 to 2.416 mg ml^{-1}. The essential oil of Angooran population (*TPP3*) had the strongest antibacterial activity. The highest MIC values in *TPP3* ranged from 0.005 to 0.080 mg ml^{-1}, and the MBC values varied from 0.010 to 0.160 mg ml^{-1}, depending on the bacteria tested.

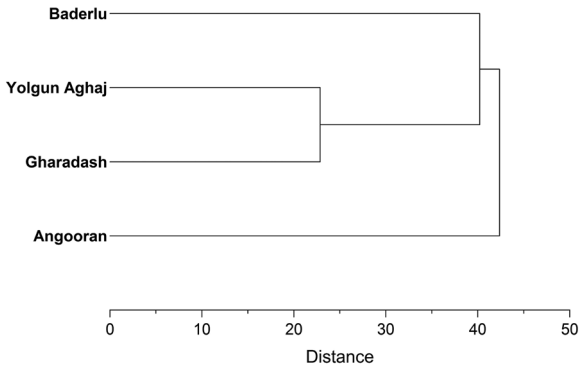


Fig. 2 Dendrogram of four *Thymus persicus* populations based on the morphological characteristic

Among all tested EOs, generally Angooran population (*TPP3*) proved to be the most efficacious against all fungi at the lowest concentration applied (MIC 0.077 mg ml^{-1} and MFC 0.154 mg ml^{-1} against *C. albicans*; MIC 0.100 mg ml^{-1} and MFC 0.201 mg ml^{-1} against *C. glabrata*; MIC 0.083 mg ml^{-1} and MFC 0.167 mg ml^{-1} against *C. krusei*; MIC 0.080 mg ml^{-1} and MFC 0.165 mg ml^{-1} against *C. parapsilosis*) (Table 8).

The EOs from Baderlu population (*TPP1*) exhibited a weaker antibacterial (MIC range: 0.780–1.190 mg ml^{-1}; MBC range: 0.156–2.416 mg ml^{-1}) and antifungal (MIC range: 0.250–0.500 mg ml^{-1}; MFC range: 0.500–1.000 mg ml^{-1}) activity against the tested strains. The antimicrobial potential of the EOs tested can be ordered as *TPP3* > *TPP4* > *TPP2* > *TPP1*. The EOs exhibited different antifungal activities with respect to the geographical region of the plant origin.

Association between phenotypical and phytochemical data

The results of the correlation analysis between the chemical compounds are presented in Fig. 6. The correlation matrix showed the relationships among TTC and TPC (r = 0.95), TFC (r = 0.97), FRAP (r = 0.99), linalool (r = 0.97), thymol (r = –0.98), and β-bisabolene (r = 0.99). A positive correlation of TPC was recognized between the TFC (r = 0.98), FRAP (r = 0.97), and β-bisabolene (r = 0.98). Significant positive correlations between *p*-cymene and γ-terpinene (r = 1.00), as well as thymol (r = 0.97) were observed. The "*r*" value for FRAP and

Table 5 Chemical variability in the essential oils of four *Thymus persicus* populations (*TPPs*)

No	Compounds	CRI	LRI	Content (%)			
				I		***II***	***III***
				TPP2	***TPP4***	***TPP1***	***TPP3***
1	α-Pinene	0928	0924	0.3	–	–	1.2
2	Camphene	0944	0936	0.5	0.6	–	0.4
3	Sabinene	0965	0958	0.8	0.4	–	–
4	1-Octen-3-one	0972	0972	–	–	0.4	–
5	β-Pinene	0982	0974	–	–	3.7	0.6
6	2,3-Dehydro-1,8-cineol	0994	0988	0.5	0.4	0.4	–
7	Myrcene	0990	0988	2.9	2.6	–	2.1
8	α-Phellandrene	1008	1002	–	–	–	0.4
9	δ-3-Carene	1014	1008	–	–	–	0.2
10	α-Terpinene	1020	1014	–	–	–	3.6
11	*p*-Cymene	1026	1020	0.6	0.4	0.5	13.4
12	Limonene	1032	1024	0.4	0.5	0.5	1.7
13	β-Phellandrene	1034	1025	0.1	–	–	–
14	1,8-Cineol	1035	1026	0.4	0.3	0.6	–
15	γ-Terpinene	1063	1054	0.5	0.3	–	11.1
16	*cis*-Sabinene hydrate	1070	1065	0.3	–	0.3	1.5
17	Terpinolene	1091	1086	–	–	–	0.5
18	Linalool	1100	1095	5.9	5.3	8.6	0.6
19	*cis*-Sabiene hydrate	1102	1098	–	–	–	0.5
20	Camphor	1150	1141	0.1	–	0.7	–
21	Borneol	1174	1165	3.6	2.7	4.5	0.9
22	Terpinen-4-ol	1183	1174	0.5	0.3	0.7	1.5
23	α-Terpineol	1199	1186	23.3	34.2	9.5	1.8
24	*n*-Decanal	1203	1201	–	–	0.6	0.2
25	Thymol methyl ether	1236	1232	0.4	–	–	0.4
26	Neral	1242	1235	0.4	–	–	–
27	Geraniol	1254	1249	12.8	10.7	0.8	–
28	Geranial	1270	1264	0.6	0.3	0.4	–
29	Thymol	1291	1289	13.4	17.7	8.1	43.9
30	Carvacrol	1301	1298	7.2	6.9	5.9	5.2
31	Thymol acetate	1353	1349	–	–	–	0.1
32	γ-Nonalactone	1357	1358	–	–	0.5	–
33	Geranyl acetate	1383	1379	3.7	3.2	–	–
34	4,8-β-*epoxy*-Caryophyllene	1438	1423	5.1	3.7	10.7	0.2
35	Geranyl acetone	1453	1451	–	–	0.5	–
36	(2*E*)-Dodecenal	1468	1464	0.5	–	1.6	–
37	β-Acoradiene	1472	1469	0.3	–	0.8	–
38	*Iso*bornyl *n*-butanoate	1475	1473	–	–	0.3	–
39	(*E*)-β-Ionone	1493	1487	–	–	0.6	–
40	*trans*-Muurola-4(14),5diene	1498	1493	0.3	0.3	0.4	–
41	β-Bisabolene	1517	1505	5.7	4.3	6.1	2.6
42	β-Thujaplicinol	1538	1536	–	–	0.3	–
43	(*E*)-γ-Bisabolene	1549	1549	0.2	–	–	–
44	Geranyl butanoate	1569	1562	0.2	–	–	–
45	2-Tetradecanone	1598	1597	–	–	1.2	–
46	Spathulenol	1599	1577	0.8	0.5	1.2	–
47	Neryl isovalerate	1602	1581	0.2	–	–	–

Table 5 (continued)

No	Compounds	CRI	LRI	Content (%)			
				I		*II*	*III*
				TPP2	*TPP4*	*TPP1*	*TPP3*
48	Caryophyllene oxide	1604	1582	1.3	1.0	4.6	0.1
49	8-Pentadecanone	1651	1648	1.2	0.7	2.9	0.3
50	Caryophylla-4(12),8(13)-dien-5a-ol	1652	1639	–	–	0.3	–
51	14-Hydroxy-(β)-Caryophyllene	1678	1666	–	–	0.4	–
52	γ-Dodecalactone	1682	1676	2.5	1.7	5.2	0.8
53	2-Pentadecanone	1697	1697	–	–	0.3	–
54	(2*E*)-Tridecenol acetate	1714	1703	–	–	0.4	–
55	(2*Z*,6*Z*)-Farnesol	1753	1742	–	–	0.4	–
56	8-Hydroxy-dihydro-eremophilone	1761	1756	–	–	1.0	–
57	*Z*-Lanceol	1773	1760	1.0	–	1.7	–
58	6,10,14-Trimethyl-2-Pentadecanone	1845		–	–	2.5	–
59	Hexadecanal	1816	1815	0.2	0.5	–	0.7
60	6,10,14-Trimethyl-2-pentadecanone	1847	1847	0.4	–	–	–
61	Methyl hexadecanoate	1926	1921	–	–	0.4	–
62	Hexadecanoic acid	1964	1959	–	–	2.8	–
63	Hexadecyl acetate	2014	2003	0.3	0.4	–	0.7
64	Citronellyl anthranilate	2183	2180	–	–	1.6	–
65	Tricosane	2308	2300	–	–	3.6	–
	Monoterpene hydrocarbons			6.1	4.8	4.7	35.2
	Oxygenated monoterpenes			69.4	78.8	41.2	56.3
	Sesquiterpene hydrocarbons			6.5	4.6	7.3	2.6
	Oxygenated sesquiterpenes			12.3	8.4	23.3	0.3
	Others			5.1	3.3	22.0	2.8
	Total identified			99.4	99.9	98.5	97.2
	Essential oil content (w/w%)			0.14	0.85	0.11	1.2

CRI calculated retention index, *LRI* literature RI, retention indices determined in the present work relative to *n*-alkanes C_6–C_{24} on DB-5 Column

linalool ($r=0.99$), β-bisabolene ($r=0.99$), TPC ($r=0.97$), and TFC ($r=0.96$) was positive and high, indicating a notable association among these compounds with antioxidant activity in the plant.

The correlation ($p<0.01$, $p<0.05$) between phenotypical and phytochemical data was significant. In particular, factors relating to leaves and flowers, plant height, number of inflorescences per plant, dry matter weight, and number of flowers per inflorescence showed an association with BA, while leaf length and bract length correlated with OA. Similar to BA, UA showed an association with the number of inflorescences per plant and dry matter weight. The TSC had a positive correlation ($\beta=0.965$) with dry matter weight, while had a negative correlation with flower stem length ($\beta=-0.966$). In the EOs, α-terpineol correlated with leaf coat, internode length, and number of flowers per inflorescence. Furthermore, two variables, including the number of flowers per inflorescence and the number of inflorescences per plant showed an association with γ-terpinene. 4,8-β-*epoxy*-Caryophyllene is associated with the number of inflorescences per plant, root length, and number of flowers per inflorescence (Table 9).

Discussion

Phenotypic traits are influenced by genetic factors and environmental conditions, which is very important to investigate these traits as primary studies in introducing plants to breeding and cultivation systems. For this purpose, the diversity of morphological traits has been considered in many medicinal and aromatic plants so far [32, 40]. In this research, morphological and phytochemical traits showed statistically significant variation among the studied populations in each parameter measured. Fattahi et al. [31] obtained similar results about morphological and chemical correlation in the study of *Salvia reuterana* Boiss. wild populations. Méndez-Tovar et al. [50] found that the morphological characteristics of *T. mastichina* (L.) L., including the number of flowers per inflorescence, number of inflorescences per plant, bract length,

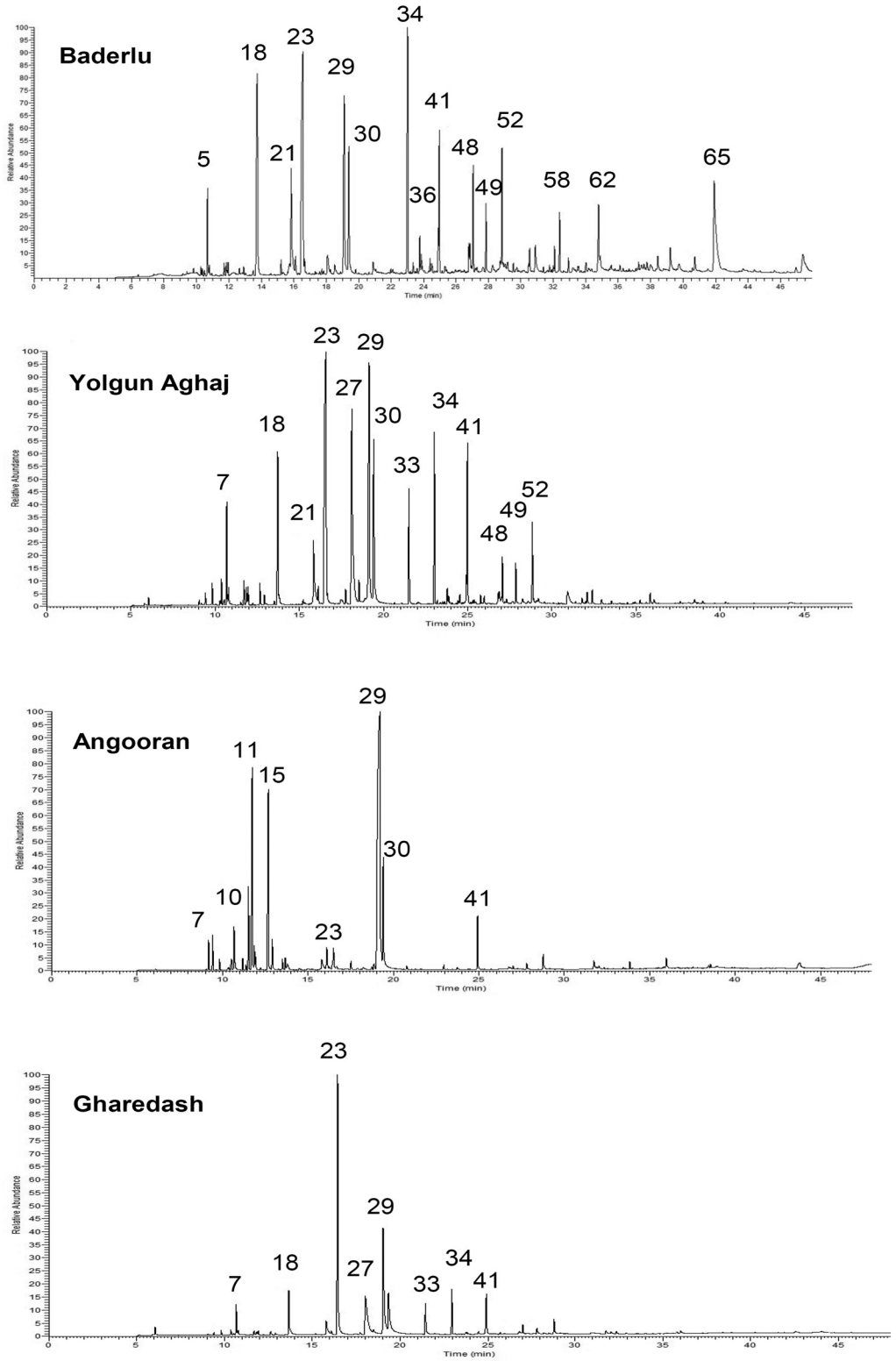


Fig. 3 Gas chromatography–mass spectrometry (GC–MS) chromatograms of the essential oil from Baderlu, Yolgun Aghaj, Angooran, and Gharadash populations of *Thymus persicus*



Fig. 4 Heatmap of the essential oil profile of the studied *Thymus persicus* populations. Mean values refer to colors from minimum displayed in bright yellow to maximum represented with dark green

and bract width had the most variation among the studied traits. Morphological changes can be related to the genetic and environmental diversity of the species [39].

Variation in the essential oil content among the plant species collected from different geographical locations has been widely reported. For example, the essential oil content of ten species of *Thymus* from different geographical regions in Iran was recorded in the range of 0.29% to 3.87% [73]. The essential oil yield of 0.35% has also been reported in Turkish endemic thyme (*T. spathulifolius* Haussken. & Velen.) [24]. The chemical polymorphism can be due to environmental factors and plant species [52].

Thymol as the major constituent in the EOs of the studied samples including *TPP3* (43.9%), *TPP4* (17.7%), and *TPP2* (13.4%) has also been reported at high content in the other *Thymus* species [67]. The essential oil of *TPP3* contained a high percentage of thymol can be considered as a good source of this valuable compound for further applications. Al-Maqtari et al. [6] have reported the essential oil fraction of *T. vulgaris* L. rich in oxygenated monoterpenes (56.5% of total oil). This high diversity in

Table 6 Variability in the content of phytochemical compounds in *Thymus persicus* populations (*TPPs*)

Compounds	Baderlu (*TPP1*)	Yolgun Aghaj (*TPP2*)	Angooran (*TPP3*)	Gharadash (*TPP4*)
Betulinic acid (mg 100 g^{-1} DW)	856.89 ± 6.76^{a}	530.55 ± 13.04^{c}	790.53 ± 14.49^{b}	709.22 ± 9.80^{b}
Oleanolic acid (mg 100 g^{-1} DW)	480.64 ± 11.12^{b}	419.35 ± 11.44^{c}	471.94 ± 10.08^{bc}	584.43 ± 12.67^{a}
Ursolic acid (mg 100 g^{-1} DW)	941.66 ± 11.49^{b}	1057.34 ± 17.00^{a}	1070.82 ± 10.14^{a}	977.98 ± 9.78^{b}
Total tannins (mg 100 g^{-1} DW)	690.13 ± 9.38^{a}	637.98 ± 12.10^{ab}	246.32 ± 6.87^{c}	502.54 ± 11.28^{b}
Total saponins (mg DE g^{-1} DW)	18.46 ± 1.22^{c}	22.57 ± 1.45^{b}	31.23 ± 1.16^{a}	36.78 ± 1.85^{a}
Total phenols (mg GAE g^{-1} DW)	87.26 ± 4.35^{a}	71.44 ± 2.97^{ab}	24.31 ± 1.26^{c}	42.39 ± 1.54^{b}
Total flavonoids (mg RE g^{-1} DW)	72.34 ± 2.63^{a}	69.14 ± 1.25^{a}	21.12 ± 1.08^{c}	39.83 ± 2.40^{b}
1,1-diphenyl-2-picrylhydrazyl assay (IC_{50} µg ml^{-1})	64.28 ± 4.57^{a}	87.43 ± 3.36^{ab}	209.73 ± 4.32^{c}	186.62 ± 2.79^{c}
Ferric reducing antioxidant power assay (µmol Fe^{+2} g^{-1} DW)	61.68 ± 1.10^{a}	54.86 ± 1.50^{ab}	34.11 ± 1.75^{b}	48.11 ± 2.32^{ab}

SE standard error

The data represent mean ± SE of replicates ($n = 3$). Different letters mean significant difference at 95% (Tukey test—$p < 0.05$)

Tukey's pairwise comparison test; $p < 0.05$)

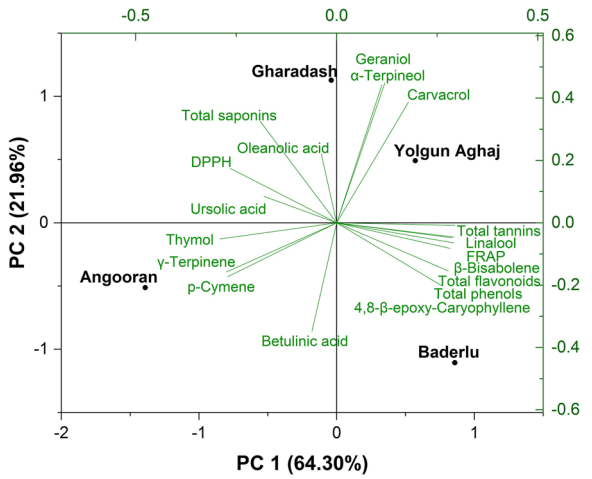


Fig. 5 Biplot of PCA analysis based on the essential oils composition, HPLC–PDA analysis of triterpenic acids, and other phytochemical compounds

the oils has also been reported in the other species [28, 33].

In another study on *Thymus* species from Ukraine, α-terpineol and carvacrol chemotypes were reported [45]. In the present study, α-terpineol (34.2%) was the dominant compound in the essential oil of *TPP4*, while carvacrol was ranged from 5.2% to 7.2% among *TPPs*. Mancini et al. [49] also reported that the variation among the major compounds of *Thymus* EOs can be due to the biosynthetic relationships between thymol and carvacrol. All these data help us to have a better understanding for future works on this valuable medicinal plant.

Triterpenic acids have been determined in many plant species so far [1, 51, 66]. In the present study, analysis of the same *T. persicus* extract by HPLC showed the presence of the three major TAs. These compounds had higher contents compared to *Origanum vulgare* L., *Origanum majorana* L., *Salvia officinalis* L., and *Melissa*

Table 7 Antibacterial minimal inhibitory concentration (MIC) (mg ml^{-1}) and minimal bactericidal concentration (MBC) (mg ml^{-1}) of *Thymus persicus* populations (*TPPs*)

Populations	Gram-positive cocci				Gram-negative bacilli			
	Staphylococcus aureus ATCC 25923		*Enterococcus faecalis* ATCC 29212		*Escherichia coli* ATCC 25922		*Pseudomonas aeruginosa* ATCC 27853	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Baderlu (*TPP1*)	1.190	2.416	0.780	0.156	0.780	1.560	1.190	2.416
Yolgun Aghaj (*TPP2*)	1.165	2.330	0.298	0.616	0.270	0.540	0.780	1.560
Angooran (*TPP3*)	0.080	0.160	0.005	0.010	0.019	0.038	0.023	0.061
Gharadash (*TPP4*)	0.626	1.125	0.053	0.126	0.270	0.540	0.056	0.112
Streptomycin	0.0005	0.001	0.0005	0.001	0.0005	0.001	0.0005	0.001

Table 8 Antifungal minimal inhibitory concentration (MIC) (mg ml^{-1}) and minimal fungicidal concentration (MFC) (mg ml^{-1}) of *Thymus persicus* populations (*TPPs*)

Populations	*Candida albicans* ATCC 90028		*Candida glabrata* ATCC 90030		*Candida krusei* ATCC 6258		*Candida parapsilosis* ATCC 22019	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Baderlu (*TPP1*)	0.312	0.586	0.312	0.686	0.250	0.500	0.500	1.000
Yolgun Aghaj (*TPP2*)	0.260	0.524	0.210	0.524	0.205	0.412	0.416	0.833
Angooran (*TPP3*)	0.077	0.154	0.100	0.201	0.083	0.167	0.080	0.165
Gharadash (*TPP4*)	0.260	0.524	0.210	0.412	0.156	0.312	0.250	0.500
Fluconazole	0.001	0.002	0.001	0.002	0.001	0.002	0.001	0.002

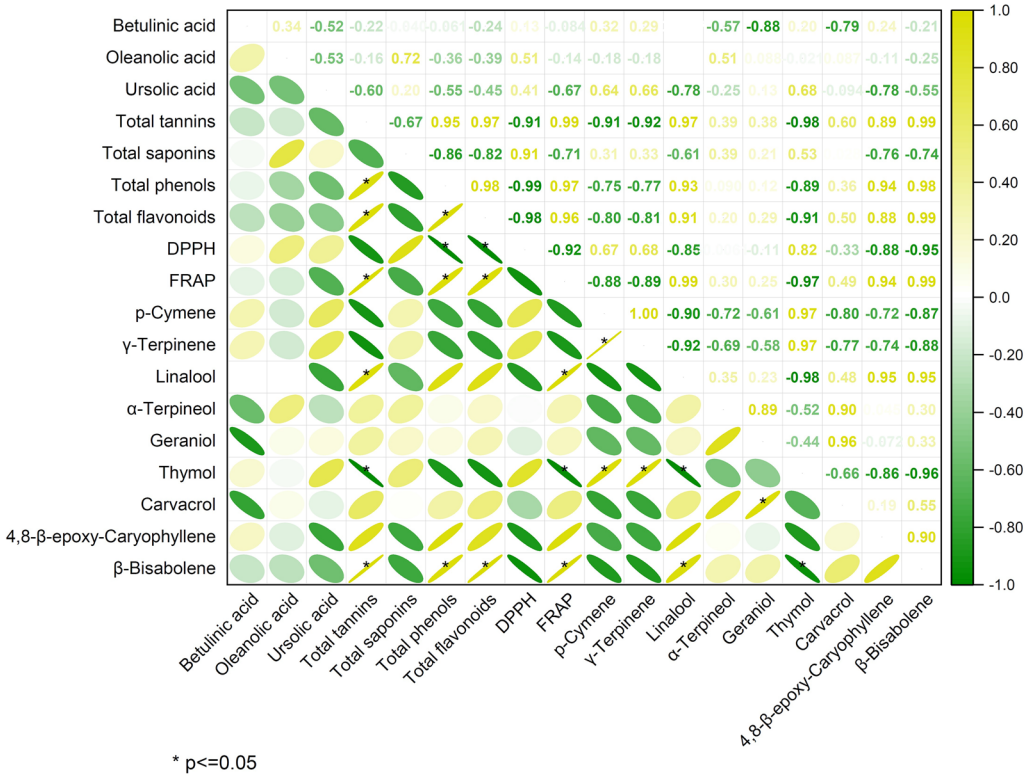


Fig. 6 Correlation coefficients between phytochemical components on studied *Thymus* populations

officinalis L. [7, 48] although these contents were less than *Rosmarinus officinalis* L. [8, 19].

Kindil et al. [44] reported the TTC in the aerial parts of six *Thymus* species from different locations in Croatia in the range of 0.77 ± 0.07% to 1.59 ± 0.04%. In a study on four species of *Thymus* from Romania, total tannin content was also found in the range of 0.27% to 1.53%, which was higher than the values obtained in *TPPs* in the present study. However, the TTC in *T. vulgaris* (0.27–0.94%) was near to *TPPs* [23]. In another study, tannins content (mg catechin g^{-1}) in leaves and stems of four local Moroccan species *Thymus* was found in the range of 1.7 ± 0.049 to 22.6 ± 0.512. The phytochemical screening of their plant materials revealed an abundance of tannins and flavonoids and the absence of saponins in the stems and leaves of some species [68]. It proposed that the difference in the SMs of different MAPs can be related to genetic, ontogenic, morphogenetic, and environmental factors [75].

All studied samples exhibit high TPC. Variations in TPC and antioxidant activity (0.8–48,680 μg ml^{-1}) have been reported for *Thymus* species [72]. In a study on

Table 9 Morphological traits associated with phytochemical compositions in *Thymus persicus* populations (*TPPs*) as illustrated using multiple regression analysis and coefficients

Morphological marker	Phytochemical composition	*r*	R_2	Standardized beta coefficients	*t* value	*P* value
PH	Betulinic acid	0.965[a]	0.931	0.965	5.208	0.035
CanD	Total tannins content	0.983[a]	0.967	0.983	7.688	0.017
DMW	Total saponins content	0.965[a]	0.930	0.965	5.171	0.024
	p-Cymene	0.888[b]	0.789	0.888	6.113	0.000
	Ursolic acid	0.936[c]	0.876	0.721	5.387	0.000
	Betulinic acid	0.988[d]	0.976	0.333	5.695	0.000
RL	4,8-β-*epoxy*-Caryophyllene	0.978[a]	0.957	0.978	6.656	0.022
FSL	Total saponin content	0.966[a]	0.932	− 0.966	− 5.247	0.034
LLWR	Caryophyllene oxide	0.983[a]	0.966	0.983	7.590	0.017
LCo	α-Terpineol	0.952[a]	0.906	0.952	4.387	0.048
BolW	DPPH	0.983[a]	0.965	0.983	7.479	0.019
CaL	Total flavonoids content	0.968[a]	0.937	− 0.968	− 5.451	0.032
FSL	Carvacrol	0.729[a]	0.531	0.729	3.366	0.007
IntL	α-Terpineol	0.931[a]	0.866	0.558	4.262	0.002
BL	Oleanolic acid	0.984[a]	0.969	− 1.031	− 12.937	0.000
LL	Oleanolic acid	0.620[a]	0.384	0.620	2.496	0.028
NFI	4,8-β-*epoxy*-Caryophyllene	0.980[a]	0.960	0.279	5.407	0.000
	Betulinic acid	0.994[b]	0.988	− 0.110	− 2.708	0.000
	α-Terpineol	0.996[c]	0.994	− 0.416	− 12.868	0.000
	γ-Terpinene	0.997[d]	0.994	0.195	7.197	0.000
NIP	4,8-β-*epoxy*-Caryophyllene	0.531[a]	0.282	0.531	2.657	0.016
	Ursolic acid	0.691[b]	0.478	− 0.691	− 3.024	0.013
	Betulinic acid	0.944[c]	0.891	0.944	9.051	0.000
	Linalool	0.960[d]	0.921	0.818	7.653	0.000
	γ-Terpinene	0.979[e]	0.959	− 0.507	− 6.549	0.000
	p-Cymene	0.982[f]	0.964	− 0.477	− 6.594	0.000
	Thymol	0.997[g]	0.993	− 0.659	− 21.081	0.000

three *Thymus* species, high TPC and TFC were detected in *T. kotschyanus* Boiss. & Hohen. (337.0 ± 8.31 mg rutin mg^{-1}) and *T. pubescens* Boiss. & Kotschy ex Celak. (50.39 ± 0.75 mg rutin mg^{-1}), respectively. The highest antioxidant activity was also reported for *T. pubescens* (IC_{50} = 31.47 µg ml^{-1}) [58]. *Thymus* species are the best sources of chemical components and antioxidant agents for the cure of many diseases. The extracts from *TPPs* showed high value of TPC, TFC, and antioxidant activity, so these extracts can be used as antibiotics or preservatives in the pharmaceutical and food industries.

Essential oils are known to have inhibitory activity against a variety of microbes [41]. The EOs of the Lamiaceae members have been shown strong antimicrobial activity [60, 76]. *Origanum vulgare* and *T. vulgaris* are the most studied EOs exhibiting antimicrobial activity against a wide range of bacterial and fungi strains [21]. The antimicrobial activity of the EOs of *Thymus* species is well documented in the literature for *T. vulgaris* [5], *T. daenensis* L. [53], *T. zygis* L. and *T. mastichina* [15], *T. maroccanus* Ball and *T. broussonetii* Boiss. [30], and *T. caramanicus* Jalas [57] so far. It has been reported that EOs of the plant with MIC of 2 mg ml^{-1} or lower show significant antimicrobial activity [34, 74]. Therefore, the EOs of the *TPPs* could be considered as a potent and valuable antimicrobial agent for further exploitation in food and pharmaceutical products.

The results are similar to those of Khadivi-Khub et al. [43] on *Satureja mutica* Fisch. & C.A. Mey. Also, carvacrol showed associations with flower stem length and dry matter weight. Based on the results of multiple regression analysis, leaf and flower variables were associated with phytochemical compounds, which showed the main role of these morphological traits in the production of these compounds. This finding was in agreement with the obtained results by Berardi et al. [18]. Studies on correlations between morphological and phytochemical traits can help plant breeder's select suitable populations.

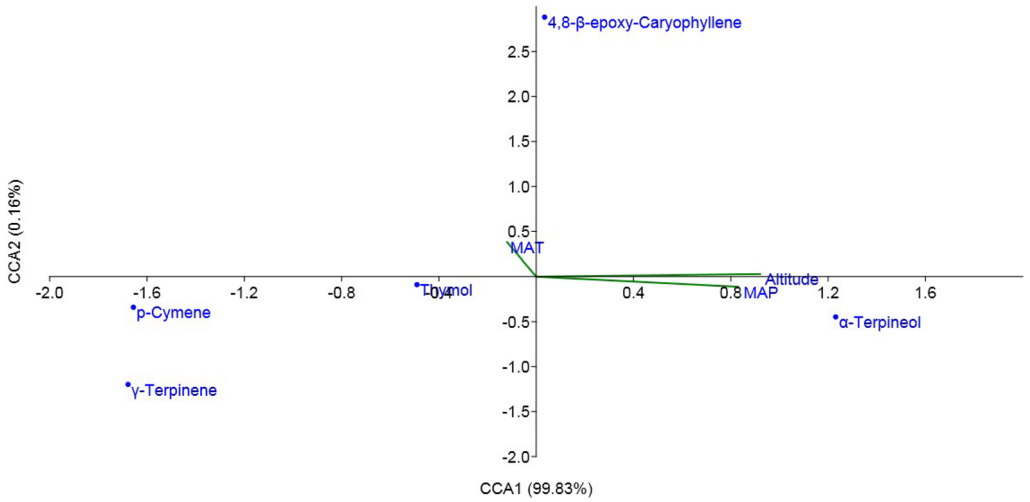


Fig. 7 Canonical correspondence analysis biplot of *Thymus persicus* populations, linking percentages of the major and important constituents, collected from different environmental conditions

The studied *TPPs* are distributed within the latitude of 36° 26′ N to 36° 45′ N and longitude of 47° 13′ E to 47° 26′ E encompassing different geographical regions. All populations were located in the northwest of Iran, and their mean rainfall is between 340 and 390 mm/year. To evaluate the correlation between environmental factors and the essential oil components, canonical correspondence analysis (CCA) was performed based on the three environmental factors and five important main components of the plants EOs, including thymol, α-terpineol, 4,8-β-*epoxy*-caryophyllene, *p*-cymene, and γ-terpinene (Fig. 7). Involved environmental factors were mean annual precipitation (MAP), altitude, and mean annual temperature (MAT). The first CCA variable (CC1) concerning environmental parameters showed that MAP and altitude had a positive share, while MAT had a negative share on this CCA construction. Also, this analysis highlights the role of each environmental factor in the grouping of *TPPs*. By considering these data, the first canonical variable in connection to the phytochemical characteristics showed that the thymol, *p*-cymene, and γ-terpinene had a negative share in the formation of CCA1 variables.

4,8-β-*epoxy*-Caryophyllene and α-terpineol had a positive share with altitude and MAP. Also, thymol correlated with *p*-cymene and γ-terpinene, and is distinct from α-terpineol and *E*-caryophyllene. The most important factor of the second CCA (CCA2) was MAT. The three groups were identified based on the PCA and the cluster analyses. According to the analysis of UPGMA (heatmap), the *TPP3* collected from the northwest region of Iran is characterized by a high content of thymol. This population was collected from a location with high temperature, low rainfall, and relatively low elevation. In general, it may be assumed that the content of essential oil and thymol is high in arid and semi-arid conditions [47], as illustrated in this CCA analysis. The correlation of thymol with *p*-cymene and γ-terpinene was not only obvious but also distinct from them. The distance might be due to the biosynthesize pathway. The main precursors for the biosynthesis of thymol are γ-terpinene and *p*-cymene [10]. The higher content of γ-terpinene and *p*-cymene to produce thymol as a finished product can lead to a decrease in the content of precursors. Furthermore, the heatmap cluster placed γ-terpinene and *p*-cymene together, which showed their correlation. The present study investigated the role of some environmental factors. However, phytochemicals can also be attributed to genetics [36].

Conclusions

In this research, morphological and phytochemical characteristics and biological activities of *TPPs* were evaluated for the first time. Morphological analysis of *TPPs* showed a high diversity between qualitative and quantitative traits that help the breeder to select the desired genotype. Analysis of the EOs exhibited high diversity among major compounds. Thymol was the most abundant one that present in *TPP3*. Other major constituents were α-terpineol, *p*-cymene, geraniol, γ-terpinene, and (*E*)-caryophyllene. Assessment of the extracts represented considerable contents of anticancer compounds (BA, OA, and UA), TTC, TSC, TPC, TFC, and antioxidant and antimicrobial activity, which can be utilized in scaling up through biotechnological methods. Association and the relationship between various characters are good tools to select the best plant for future breeding programs. It

also helps to distinguish of correlation between chemical, morphological, and environmental characteristics. The results showed that extracts and EOs of *TPPs* can be exploited in food and pharmaceutical industries.

Acknowledgements
The authors express their appreciation to the Research Council of Shahid Beheshti University, Tehran, Iran for their support. We also wish to thank Prof. Ali Sonboli and Mr. Hamid Ahadi for their kind help in plant materials collection and HPLC analysis, respectively.

Author contributions
ZB: Conceptualization, Investigation, Data interpretation, Formal analysis, Writing-Original draft. MHM: Supervision, Methodology, Funding acquisition, Data curation, Validation, Writing, Review & editing. MS: Review & editing, Data interpretation, Formal analysis, Visualization. AY: Resources, Review & editing. MG: Data curation, Formal analysis, Validation, Review & editing.

Funding
Not applicable.

Data availability
All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate
This manuscript is original research and has not been published or submitted in other journals.

Consent for publication
All authors listed have read the complete manuscript and have approved submission of the paper.

Competing interests
The authors declare no conflict of interest.

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Received: 31 August 2023 Accepted: 12 December 2023
Published online: 18 December 2023

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