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A comparative study of chemical composition, phenolic compound profile and antioxidant activity of wild grown, field and greenhouse cultivated *Physalis* (*P. alkekengi* and *P. peruviana*)

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Abstract

Phytochemical properties and antioxidant activity of medicinal *Physalis alkekengi* fruit (wild and cultivated genotypes) and *P. peruviana* commercial species, were investigated (ten samples). Ripe fruits of *P. alkekengi* in comparison to *P. peruviana*, which is already regarded as an extremely functional fruit, possessed higher values of phytochemicals as well as antioxidant activity. In this present study, greatest phenolics content and antioxidant activity was observed in cultivated *Physalis*. The uppermost antioxidant activity was obtained in the fruit extract of cultivated *P.alkekengi* in C5 (Silvana) with 44.13% by DPPH method and C8 (Urmia) with 0.40 μM Fe^{++} g^{-1} FW by FRAP assay. Extracts obtained C8 and C4 (Khoy) possessed the highest total phenolic (34.12 mg GAE g^{-1} FW) and total flavonoid (7.06 mg QUE g^{-1} FW) contents, respectively. Additionally, the utmost amount of total carotenoid (614.18 mg$100g^{-1}$ FW), β-carotene (0.47 mg/100 g FW), and ascorbic acid (84.61 mg g^{-1} FW) was obtained in C5. Ferulic acid (in C2 sample: Maragheh) and quercetin (in WA sample: Qaradag) were found to be the most abundant phenolic acid and flavonoid by HPLC–MS/MS analysis, respectively. No flavonoid of kaempferol was detected in the studied extracts from different regions. Wild and cultivated species in different regions were classified into four major clusters, according to hierarchical clustering analysis (HCA) method. Considering the cultivation of medicinal plants such as *Physalis*, the growing conditions must be carefully chosen because they affect the phytochemical components and especially the metabolism of polyphenols.

Keywords Ascorbic acid, Carotenoid, Flavonoid, Phenolic acid, Phytochemicals

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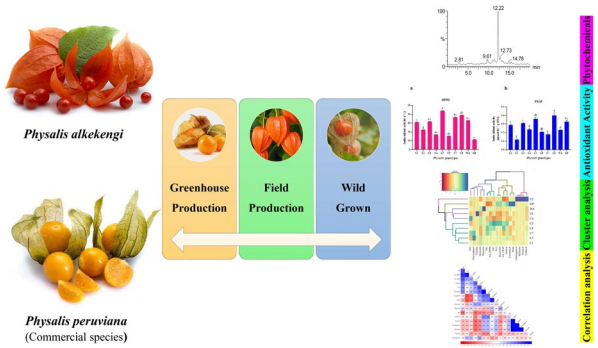
Graphical Abstract



Introduction

The genus *Physalis* as a member of the Solanaceae family, with more than 100 species (Whitson & Manos, 2005; Zhang & Tong, 2016), endemic to Central and South America, Europe and Asia (Yang et al., 2022). *Physalis*, due to containing of above-mentioned bioactive compounds and strong antioxidant properties, exhibit a wide variety of biological properties such as anti-inflammatory (Castro et al., 2008), anti-tumor (Hseu et al., 2011), immune-suppressive, anti-microbial (Yang et al., 2016), anti-leishmanial, anti-diabetic (Hu et al., 2018; Vicas et al., 2020; Zhang et al., 2018), anti-asthmatic, among others (Yang et al., 2022).

P. alkekengi (bladder cherry or Chinese lantern) is an herbaceous, perennial plant, about 30 to 60 cm in height, with a branched rhizome, an erect and hairless stem and alternate leaves, as well as round, cherry-sized berry fruits (Saremi et al., 2021). Mature fruits of *P. alkekengi* have a waxy orange-red peel that is enclosed in an orange-to-red calyx-derived husk (Etzbach et al., 2019). In Iranian indigenous medicine, this plant has been consumed for rheumatism, gout, kidney and urinary tract diseases and accelerating the excretion of uric acid (Bahmani et al., 2016). Traditionally, this plant is used to treat tussis, pharyngitis, jaundice, dysentery, micturition problems, as well as prostatitis. The ripe fruit of *P. alkekengi* as dietary and functional food contains a rich source of ascorbic acid, carotenoids, phenolic compounds (quercetin, rutin, ferulic and caffeic acid), pectin, minerals (P, Ca, and Fe), phytosterols, unsaturated fatty acids and other worthwhile bioactive compounds (Hu et al., 2018; Lu et al., 2011; Sharma et al., 2015). The medicinal properties *P. peruviana* L. are due to the phenolic and carotenoid compounds in this plant, which are considered two important categories of phytochemical compounds. Important phenolic compounds in *P. peruviana* L. include gallic acid, caffeic acid, chlorogenic acid, ferulic acid, coumaric acid, quercetin, rutin, myricetin, kaempferol, catechin, and epicatechin (Olivares Tenorio, 2017). Lycopene and beta-carotene are important carotenoid pigments in *P. peruviana* L., which are obtained through carotenoid biosynthesis. Beta-carotene is a precursor to vitamin A (Ramadan & Moersel, 2009).

Nowadays, the dramatic increase in demand for natural ingredients in the pharmaceutical, cosmetic and food industries has led to increased attention to medicinal plants. Therefore, extensive research is underway to find new drug sources and molecular structures. One of the effective factors in the use of medicinal plants in industries is the object of quality control and their standardization. Examination of medicinal raw materials is an important and fundamental step in controlling the quality of medicinal plants. Because the source and quality of raw materials play a pivotal role in maintaining the quality of pharmaceutical materials. This could be possible through the domestication and breeding of wild plants. It is necessary to cultivate medicinal plants in outside of their native regions in order to domesticate them (Hadi et al., 2017). The identification of different habitats and the assessment of the impact of environmental factors on morphological characteristics and quantitative and qualitative performance of medicinal plant bioactive compounds are considered a crucial step toward domesticating and preserving their genetic diversity (Shaghaghi et al., 2019). In addition to growing demands of pharma industries for uniform and high-quality raw materials of medicinal plants, necessity of preserving and conserving of wild plants has contributed to the cultivation and domestication of medicinal plants. In this regard, domestication is important not only for conservation but also for ensuring the availability and continuous supply of plant material for drug

extraction, in which reduces the pressure on natural populations being collected and exploited (Rao et al. 2003).

In medicinal plants, generally, interactions between environmental (geographical region, temperature, rainfall, among others) and genetic factors influence amount and type (quality and quantity) of constituents of secondary metabolites. Variations in phytochemicals are largely responsible for functional diversity. Previous research on this genus mostly focused on commercial species (Yildiz et al., 2015, Olivares-Tenorio et al., 2016; Wen et al., 2019), or fruits collected from wild-growing regions (Popova et al., 2022). Wild species have a limited growth area due to their physiological adaptation to certain environmental conditions. In order to preserve these species, move towards sustainable agricultural practices (Hazrati et al., 2020), change the amount and content of their phytochemical compounds and benefit from their potential, the cultivation of these medicinal plants is highly recommended.

In accordance with the literature survey, no previous investigations have been executed on the chemical composition and the antioxidant properties of fruit extracts of *P. alkekengi* medicinal species in different regions of Iran and no comparative study has been undertaken on wild-growing, cultivated and commercial species (*P. peruviana*) of this plant. Therefore, in the current study, we investigated the impact of cultivation of wild species of *P. alkekengi* in different regions (eight regions) and evaluated some of the phytochemical parameters in the cultivated with wild-grown species of *P. alkekengi* in natural growth region (also comparing them with a commercial species called *P. peruviana*) with the aim of recognition and exploit of the potential of native populations in breeding programs in the future.

Methodology

Plant material

Fruits of *Physalis* from wild-growing and cultivated of *P. alkekengi* as well as *P. peruviana* plants were collected at mature stage from 10 regions: (1) Wild-growing species were randomly collected from Qaradag (around of Ahar) region (WA) in East Azarbaijan province, (2) Cultivated *P. alkekengi* was collected from eight planting sites (C1 to C8) in East Azerbaijan and West Azerbaijan of Iran; (3) Commercial species of *P. peruviana* (GP) were collected from college Greenhouse of Urmia. Fruits after harvesting in the mature stage were kept at 4˚C until used in the experiments. A list of wild-growing and cultivated *Physalis* genotypes are given in Table 1.

Preparation of fruit extracts

Fresh fruits of distinct regions (1 g) were individually pulverized with liquid nitrogen and then were extracted with methanol/water (80%, v/v) using ultrasound-assisted extraction (at 30°C for 30 min) (Alirezalu et al., 2018). Methanolic extract was used to measure total phenolic and flavonoid contents and antioxidant activity.

Total phenolic content

The Total phenolic content (TPC) was determined by using Folin–Ciocalteu reagent (FCR) according to the procedure described by Ebrahimzadeh et al. (2008) with minor modifications. Methanolic extract of fruit (10 µL) was mixed with 200 µL FCR (10%, v/v). After 5 min, 2 mL sodium carbonate (7.5%, w/v) was added, and the final volume was adjusted to 5 mL with deionized water. Finally, after incubating samples for 30–45 min in the dark at room temperature, their absorbance was read by spectrophotometer (UNICO, China) at 760 nm. Gallic acid was used as the standard for the calibration curve, and the total phenolics were expressed as mg of gallic

Table 1 Geographical characteristics of Wild-growing and cultivated of *Physalis* plants in different regions

Code	Species	Region	Geographical region		
			Longitude (N)	Latitude (E)	Altitude (m)
C1	*P. alkekengi*	Bonab	46° 03′ 34.41″	37° 20′ 14.97″	1292
C2	*P. alkekengi*	Maragheh	46° 14′ 19.89″	37° 20′ 55.23″	1445
C3	*P. alkekengi*	Urmia	45° 10′ 34.01″	37° 31′ 45.98″	1297
C4	*P. alkekengi*	Khoy	44° 45′ 15.07″	38° 28′ 16.19″	1357
C5	*P. alkekengi*	Silvana	44° 15′ 15.82″	37° 25′ 16.25″	1604
C6	*P. alkekengi*	Bukan	46° 13′ 83.00″	37° 39′ 21.60″	1389
C7	*P. alkekengi*	Takab	46° 55′ 42.14″	36° 30′ 00.18″	2067
C8	*P. alkekengi*	Urmia (Greenhouse)	44° 58′ 35.37 ″	37° 39′ 21.60″	1363
WA	*P. alkekengi* (Wild-growing species)	Qaradag	47° 04′ 59.60″	38° 27′ 21.60″	1666
GP	*P. peruviana* (Commercial species)	Urmia (Greenhouse)	44° 58′ 35.37 ″	37° 39′ 21.60″	1363

acid equivalents (GAE) per gram (g) of fresh weight (mg GAE g^{-1} FW). Analyses were carried out in triplicate for each extract.

Total flavonoid content

To measure the total flavonoid content (TFC), 15 µL methanolic extract was combined with 1.5 mL of methanol (80%), 100 µL of aluminum chloride solution (10%), 100 µL of potassium acetate solution (1 M) and 4.78 mL of distilled water. The absorbance of the mixture was read after 40 min at 415 nm in comparison with the control. Quercetin was used as standard. The flavonoid content of all extracts was expressed as mg equivalent of quercetin per gram (g) fresh weight (mg QUE g^{-1} FW) (Chang et al., 2002).

HPLC–MS/MS analysis

The main phenolic acids, flavonoid and anthraquinone profiling were performed with an HPLC system (Alliance waters e2695; Milford, MA, USA), and a Quattro Micro Atmospheric Pressure Ionisation (API) triple quadrupole LC–MS/MS (Waters, Micromass, Manchester, UK). The MS/MS system is equipped with an ESI in positive and negative modes. A Luna Omega PS 1.6 µm C18 column (50 × 2.1 mm) was applied for the chromatographic separation setting the temperature at 25°C.

Elution was conducted using mobile phases B (acetonitrile), and D [water/formic acid/methanol (94.9/0.1/5, v/v)], employing a two-step gradient as follows: 0–17.50 min 90% B; 17.50–20 min 0% B the flow rate was set to 0.6 mL/min and the injection volume was 50 µL. Compounds were analyzed both in the positive and in the negative ion mode. The chromatographic, HPLC-ESI/MS, UV data and external standardization were used for the identification and quantification of the phytochemical compounds. Each standard curve was constructed with 6 different concentrations (5, 10, 20, 50, 100, 200, 500, and 1000 mg/L). The calibration equations and linear ranges were determined by plotting the mean areas ($n = 3$) of the standard solutions against their concentrations.

Antioxidant activity

The antioxidant activities of methanolic extract of cultivated and wild *Physalis* were determined using two complementary tests named, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric (iron (II)) reducing antioxidant power (FRAP) assays.

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant activity of the extract was assessed by bleaching the purple-colored solution of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical according to the method reported by Nakajima et al. (2004). In short, 3 µL of methanolic extract was mixed with 2 mL of DPPH solution. The solution was shaken and stored at room temperature for 30 min in the dark. Afterwards absorbance was determined by using a spectrophotometer (UNICO, China) at 516 nm. Only 50 µL methanol (80%) was used instead of the extract as control. The radical scavenging activity (RSA) which is the criteria for measuring the antioxidant content of the extract was calculated according to the following formula:

$$\mathrm{RSA} = [(\mathrm{Abs\ control} - \mathrm{Abs\ sample})/\mathrm{Abs\ control}] \times 100$$

Where RSA, Abs sample and Abs control were Radical Scavenging Activity, the Absorbance of sample and Absorbance value of the blank, respectively.

Ferric reducing antioxidant power (FRAP) assay

According to Zugic et al. 2014, 250 µL of the extract and 3 µl of fresh FRAP reagent (300 mM sodium acetate buffer (pH 3.6), ferric-tripyridyl-s-triazine and ferric chloride) were mixed together. The resulting mixture was placed in a hot water bath (temperature 37°C) for 30 min and its absorbance was read at 593 nm using a Spectrophotometer (UNICO, China). Iron sulfate was used to draw the standard curve and data results were expressed based on µmol per gram fresh weight (µmol Fe^{++}/g FW).

Total carotenoids and β-carotene content

For the assessment of the total carotenoids (TCC) and β-carotene content, 1 g fresh tissue of fruit was mixed with 5 ml acetone in a cold porcelain mortar and homogenized in an ice bath. Afterwards, 1 g of anhydrous sodium sulfate was added to the homogenate and passed through filter paper. The filtered solution was adjusted to a volume of 10 mL with acetone and centrifuged at 4025 g for 10 min. The supernatant was gathered and absorbance of the solution was measured at 662, 645 and 470 nm compared to the control. Acetone was used as a control. The amount of carotenoids, chlorophyll a and b and β-carotene for each extract was calculated using the following formulae (Lichtenthaler, 1987):

$$\mathrm{Cla} = 11.75A_{662} - 2.350A_{645}$$

$$\mathrm{Clb} = 18.61A_{645} - 3.960A_{662}$$

$$\mathrm{TCC} = 1000A_{470} - 2.270\mathrm{Cla} - 81.4\mathrm{Clb}/227$$

$$\beta - \mathrm{carotene} = 0.854A_{479} - 0.312A_{645} + 0.039A_{663} - 0.005$$

where Cla = Chlorophyll a, Clb = Chlorophyll b, TCC = Total Carotenoid Content.

Ascorbic acid content (Vit C)

According to Bor et al. 2006, the amount of ascorbic acid of fruit extract was measured based on redox titration of vitamin C with 6, 2-dichlorophenol indophenol (DCPIP). Concisely, 1 g of fresh fruit was mixed with 3 mL of metaphosphoric acid (1%, v/v). After half an hour, the mixture was centrifuged at 4025 g, 4˚C for 15 min. 100 µL of the supernatant was taken and 2.5 ml of DCPIP was added to it. The absorbance of the samples was determined at 520 nm in three replicates. The compounds mentioned above except the fruit extract served as blank samples. The standard curve of ascorbic acid was calculated using different concentrations of ascorbic in the presence of DCPIP.

Statistical analysis

The obtained data was based on one-way ANOVA in a completely randomized design (with three replications). Comparisons of means was done using Duncan's new multiple range test (DMRT). Correlation analysis based on Pearson's method and screening of genotypes (HCA analysis) was carried out using the Euclidean distance and the Ward method using RStudio software (version 4.1.0). Kolmogorov–Smirnov normality test was used to evaluate the normality of the distribution of variables in this study. Figures were generated using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA).

Results and discussion

Total phenolic content (TPC)

There were significant cultivation site and genotype-dependent variations ($P<0.01$) (Table 2). The level of phenolic compounds in different areas is presented in Table 3. The uppermost TPC was observed in the methanolic extracts of C8 (*P. alkekengi*) followed by C7 with 34.12 and 32.34 mg GAE g^{-1} FW, respectively, whereas the minimum one found in fruits of cultivated in Greenhouse (GP) *P. peruviana* (8.8 mg GAE g^{-1} FW). The results of mean comparisons showed that there is no significant difference between C2, C3, C6 and WA treatments in terms of TPC (Table 3). The amount of total phenol ranged from 8.8 to 34.12 mg GAE/g FW. Puente et al. 2011 reported that the TPC of *P. peruviana* fruits were 39.15 to 40.45 mg g^{-1}DW. In the study

Table 2 Analysis of variance (ANOVA) of physicochemical characteristics in different samples

SV	DF	Mean Square				
		TPC	TFC	TCC	β-carotene	Vit C
Genotype	9	198.06**	8.235**	100700.36**	0.0621**	384.42**
Error	29	7.0475	0.631	1074.05	0.0008	0.074
CV (%)		12.08	21.02	11.00	12.73	0.44

SV Sources of variation, *DF* Degrees of freedom, *CV* Coefficient of variation, *TPC* Total phenol content, *TFC* Total flavonoid content, *TCC* Total carotenoid content, *Vit C* Vitamin C

* significance at $p<0.01$

Table 3 Phytochemical contents in different *Physalis* samples

Code	TPC (mg GAE g^{-1} FW)	TFC (mg QUE g^{-1} FW)	TCC (mg/100 g FW)	β-carotene (mg/100 g FW)	Vit C (mg/100 g FW)
C1	25.14^{cd} ± 1.97	3.59^{cde} ± 0.16	377.19^{c} ± 9.82	0.29^{c} ± 0.02	57.68^{e} ± 0.97
C2	16.03^{ef} ± 4.09	4.80^{bc} ± 0.66	417.19^{c} ± 7.52	0.33^{c} ± 0.00	53.88^{g} ± 0.09
C3	20.89^{de} ± 0.55	2.51^{de} ± 0.31	257.6^{d} ± 4.30	0.18^{d} ± 0.01	61.14^{d} ± 0.55
C4	14.92^{f} ± 0.36	7.06^{a} ± 0.97	79.20^{f} ± 4.30	0.06^{f} ± 0.00	44.77^{h} ± 0.36
C5	29.18^{bc} ± 0.36	2.09^{e} ± 0.37	614.18^{a} ± 6.91	0.47^{a} ± 0.02	84.61^{a} ± 0.36
C6	19.84^{e} ± 1.06	2.45^{de} ± 0.46	79.39^{f} ± 5.57	0.04^{f} ± 0.01	56.14^{f} ± 0.24
C7	32.34^{ab} ± 2.80	5.90^{ab} ± 1.40	199.39^{de} ± 1.69	0.16^{de} ± 0.005	60.82^{d} ± 0.24
C8	34.12^{a} ± 1.45	3.68^{cd} ± 0.36	238.20^{d} ± 6.30	0.19^{d} ± 0.01	74.93^{b} ± 0.45
WA	18.37^{ef} ± 0.41	3.27^{de} ± 0.62	178.18^{e} ± 2.91	0.12^{e} ± 0.01	68.24^{c} ± 0.24
GP	8.80^{g} ± 0.30	2.41^{de} ± 1.09	535.40^{b} ± 2.6	0.41^{b} ± 0.01	57.76^{e} ± 0.30
Sig	**	**	**	**	**

Mean values with different letters are statistically significant/different at 5% level of probability

TPC Total phenol content, *TFC* Total flavonoid content, *TCC* Total carotenoid content, *Vit C* Vitamin C

** significant at $P<0.01$

of morphological and biochemical properties of *Physalis* fruit, the amount of total phenol varied from 73.6 to 93.5 mg g^{-1} DW (Shokouh et al., 2017). As reported by Yildiz et al. 2015, the amount of total phenol in *P. peruviana* fruits collected from Turkey varied between 136.64 and 154.45 mgg^{-1} DW. In the study of the antioxidant and antibacterial properties of *P. peruviana* fruit, phenol content was determined as 1.50 mgg^{-1} DW (Erturk et al., 2017). Medina-Medrano et al. (2015) pointed out that the total phenolic contents in *P. hederifolia* var. *hederifolia* fruits were 86.51 mg g^{-1} DW. It's well known that several factors, including genetics, climate, planting location, soil characteristics and other environmental conditions affect phenolic compounds in fruits (Scalzo et al., 2005). It was reported that the contents of phenol in the aqueous and ethanol extracts of *P. alkekengi* fruits were 51.80 and 53.09 µg GAE mg^{-1} (Diaz et al., 2012), respectively, being higher than those of our findings.

A comparison of cultivated and wild-growing *P. alkekengi* (WA) showed that the cultivated species contained higher total phenol content in all the cultivation sites except in Maragheh (C2) and Khoy (C4) regions. In addition, the extracts from wild-growing *P. alkekengi* (WA) contained higher phenol content (18.37 mg GAE g^{-1} FW) than those from commercial species *P. peruviana* (8.8 mg GAE g^{-1} FW). Indeed, the cultivation of this species significantly ($P < 0.01$) enhanced the total phenolic content of *P. alkelengi* in comparison with wild-growing genotypes. In the present study, the quantity of TPC was significantly affected by climate, which is consistent with the results reported by Wen et al. 2019. Also, species had a significant impact on the polyphenol content, which agreed with Wen et al. 2019 results. Bautista et al. (2016) detailed height as the main single parameter associated with significantly increased phenolic concentrations and flavonoids. Perhaps the reason for the high content of phenolic compounds in sample C7 is the high altitude of the cultivation site. It should be understood that various environmental factors depend on altitude, such as mean and extreme temperatures, precipitation, soil type, snow cover periods, seasonal changes and exposure to the sun. An increase in altitude means a significantly higher ratio of UV to total solar radiation. Thus, the altitude-dependent increase in the content of phenolics can be partially attributed to higher UV radiation (Bautista et al., 2016). Indeed, solar and UV radiation have been recognized as important external elements affecting the generation of secondary metabolite with radical activity (Vázquez-León et al., 2017).

As a matter of fact, abiotic stress such as climatic conditions can induce oxidative stress and production of active oxygen species (ROS) and subsequently would bring on the generation of higher phenolic compounds in plants (Lamien-Meda et al., 2010) or influence phenylpropanoid metabolism with inducing of the biosynthesis pathways of phenolic compounds via increasing the activity of the phenylalanine ammonia-lyase (PAL) enzyme (Goldson Barnaby et al., 2017). Developmental stages of plant (Li et al., 2020; Verma & Shukla, 2015) and the maturity stage of fruit at harvest time are also important and influential factors for phenolic content (Alirezalu et al., 2015; Licodiedoff et al., 2013; Wu et al., 2013). The amount of phenolic compound in the species of *Physalis* demonstrated that the total phenol content in these species is diverse, which is consistent with findings of Wen et al. 2019.

Total flavonoid contents (TFC)

The TFC was significantly variable amongst the different genotypes of *Physalis* ($p < 0.01$) (Table 2). The results of the analysis revealed that different planting sites and genotype affected the flavonoid content of all *Physalis* fruits (Table 3). The average of TFC *Physalis* fruits in cultivation sites ranged between 2.09 to 7.06 mg QUE g^{-1} FW which was higher than reported amount of flavonoid in *P. alkekengi* in Bulgaria (15.84–18.03 mg QE/100 g DW) (Popova et al., 2022). The results of mean comparisons showed that C4 genotype contained the highest amount of flavonoids and the lowest amount was found in C3, followed by C6, C5, and GP (*P. peruviana*), respectively (Table 3). Hence, According to Medina-Medrano et al. (2015), the total flavonoids in *P. solanacea* and *P. subulata* fruits were 2.49 and 5.46 mg QUE g^{-1} DW, respectively. In another study, the average flavonoid content of *Physalis* fruits was 156 mg QUE g^{-1} DW (Shokouh et al., 2017).

Flavonoids as naturally-occurring phenolic compounds in various parts of plants have a variety of biological activities. Owing to this, the identification, quantification and assessment of the antioxidant properties of phenolic compounds is therefore of great importance (Shetty, 2004). The biosynthesis, regulation and accumulation pathways of phenolic compounds are hypersensitive to environmental changes, because the expression and activity of genes and enzymes involved in these pathways change by biotic and abiotic stresses (Borges et al., 2017) and genotypes as well (Fernandez-Orozco et al., 2010; Shameh et al., 2019). Additionally, morphological changes and physiological functions due to changes in climatic conditions may impact the accumulation of flavonoids (Shameh et al., 2019).

The type and amount of flavonoids are the quality parameters of the plant. Numerous biological effects have been assigned to the presence of

flavonoids in plants. These compounds play a crucial role in defense against plant pathogens (Yang et al., 2007). The antioxidant properties of flavonoids make them especially important for preventing various diseases including cardiovascular diseases and various types of cancers and many others. Besides, it has been confirmed an antioxidant role of flavonoids in inhibiting of generation of free radicals. In addition, the photoprotective ability of flavonoids (e.g., quercetin) under abiotic stress (e.g., drought, salinity, UV radiation, etc.), which is associated with over-reduction in electron transport during photosynthetic process and increase of generation of ROS have been reported (Agati et al., 2020). In particular, chalcone synthase (CHS), the primary enzyme in the flavonoid biosynthetic pathway, is transcriptionally stimulated by ultraviolet light. In conclusion, UV radiation is an important parameter observed with UV-B modulating the interaction between phenolics and essential oils in medicinal plants through the expression of genes involved in UV-B absorbing flavonoids and terpenoid biogenesis (Dolzhenko et al., 2010).

Variations in TFC have been reported in other studies. These variations have been attributed to a variety of factors including, genotype, climatic condition, geographic area, as well as fruit maturity state in harvest time (different stages of fruit development) (Shameh et al., 2019, Alirezalu et al., 2018, Bravo et al., 2015; Medina-Medrano et al., 2015, Li et al. 2013, Hamouz et al., 2010). Solvent utilized for extraction can also have a significant impact on fruit phenolic constituents (Diaz et al., 2012). For the fruit of the same species, such factors could clarify the discrepancies encountered in the phenol and flavonoid contents reported by different researchers (Puente et al., 2020).

Fruit phenolic compounds

The main phenolic acids such as coumaric, caffeic, ferulic, gallic and chlorogenic acids, as well as flavonoid compounds, namely, catechin, kaempferol, quercetin, rutin and myricetin were also detected in the studied genotypes. In addition, anthraquinone compounds such as chrysophanol and physcion were identified by HPLC–MS/MS. Depending on the cultivation regions and species, three categories of phenolic compounds were detected in the extracts, namely, phenolic acids, flavonoids and anthraquinones. Fruits from different regions contained different amounts of phenolic and flavonoid compounds. Table 4 shows the calibration curve, the correlation coefficient and the limits of detection (LOD) and quantification (LOQ) of phenolic compounds studied. The contents of individual phenolic compounds of *Physalis* species in the studied regions are illustrated in Table 5. The main phenolic acids such as coumaric, caffeic, ferulic, gallic and chlorogenic acids, as well as flavonoid compounds, namely, catechin, quercetin, rutin and myricetin were also detected in the studied genotypes. In addition, anthraquinone compounds such as chrysophanol and physcion were identified in some samples (Table 5). Results showed that ferulic acid and quercetin were the predominant phenolic acid and flavonol in extracts. The flavonoids quercetin, along with myricetin and rutin inhibit superoxide radical production (Letan et al., 1966). The highly active effect of anthraquinones such as physcion and chrysophanol on plant powdery mildew is reported by Yang et al. 2007. The results highlighted that planting sites had an impact on both the quality and quantity of chemical composition from wild and cultivated *Physalis*. The phenolic compounds of cultivated *P. alkekengi* were partially resembling profiles of wild one. The phenolic components of *P. peruviana* extracts also

Table 4 Phenolic compounds quantified by HPLC–MS/MS with the calibration curve, correlation coefficient and limits of detection (LOD) and quantification (LOQ)

Phenolic compounds	Calibration curve	R^2	LOD (μg g^{-1})	LOQ (μg g^{-1})
Coumaric acid	y = 74.205x—34.942	0.9998	3.15	9.55
Caffeic acid	y = 65.587x + 88.052	0.9957	15.67	47.50
Ferulic acid	y = 4.8818x + 49.691	0.9871	37.27	112.94
Gallic acid	y = 65.599x + 87.825	1	5.01	15.18
Chlorogenic acid	y = 63.101x + 170.92	0.9998	9.53	31.78
Catechin	y = 2.2327x + 10.584	0.9997	34.08	103.29
Quercetin	y = 56.795x—4833.2	0.9913	223.37	676.90
Rutin	y = 30.178x—516.58	0.9973	69.09	209.38
Kaempferol	y = 4.0763x—54.709	0.9984	74.22	224.91
Chrysophanol	y = 0.7499x—5.7121	0.9991	39.79	120.60
Physcion	y = 3.4561x—54.723	0.9995	40.48	122.68
Myricetin	y = 2.7016x—27.874	0.9989	52.39	158.78

Table 5 Content of phenolic compounds in different *Physalis* samples by HPLC–MS/MS

Identification and characterization of Phenolic compounds (μg g^{-1} FW)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Rt (min)	9.05	7.98	9.41	6.60	7.37	2.71	11.25	8.47	11.09	3.89	1.74	2.10
MF	$C_9H_8O_3$	$C_9H_8O_4$	$C_{10}H_{10}O_4$	$C_7H_6O_5$	$C_{16}H_{18}O_9$	$C_{15}H_{14}O_6$	$C_{15}H_{10}O_7$	$C_{27}H_{30}O_{16}$	$C_{15}H_{10}O_6$	$C_{15}H_{10}O_4$	$C_{16}H_{12}O_5$	$C_{15}H_{10}O_8$
MW (g/mol)	164.16	180.16	194.18	170.12	354.31	290.27	302.23	610.5	286.24	254.24	284.26	318.23
m/z	162.9 > 118.9	178.9 > 134.9	193 > 133.9	169 > 125	355 > 163	288.9 > 108.7	300.9 > 150.9	609 > 300.1	285 > 185	253 > 225	283 > 240	317 > 151
Code												
C1	1.93	30.23	nd	13.31	15.39	nd	85.81	6.62	nd	nd	nd	nd
C2	2.48	135.01	2465.41	nd	344.97	nd	nd	13.69	nd	nd	nd	45.97
C3	1.42	36.43	79.7	21.12	9.79	nd	85.53	3.47	nd	59.18	nd	nd
C4	0.77	7.67	nd	4.31	1.78	nd	85.96	6.14	nd	nd	nd	nd
C5	1.9	45.09	nd	27.77	8.84	nd	85.48	3.18	nd	nd	nd	nd
C6	1.91	5.22	nd	4.35	nd	6.11	nd	4.56	nd	nd	nd	nd
C7	0.77	43.35	3.51	20.5	3.76	0.85	nd	8.66	nd	47.88	nd	nd
C8	0.67	13.77	152.02	8.22	8.34	nd	nd	5.35	nd	nd	nd	nd
WA	2.9	3.86	6.67	8.63	10.29	nd	85.99	9.52	nd	nd	41.44	nd
GP	5.7	25.39	nd	27.81	2.63	nd	43.92	28.64	nd	nd	nd	nd

1: Coumaric acid, 2: Caffeic acid, 3: Ferulic acid, 4: Gallic acid, 5: Chlorogenic acid, 6: Catechin, 7: Quercetin, 8: Rutin, 9: Kaempferol, 10: Chrysophanol, 11: Physcion, 12: Myricetin

nd not detected, *Rt* Retention time, *MF* Molecular Formula, *MW* Molecular Weight

were similar to the cultivated *P. alkekengi* in C5, C1 and C4. The highest concentration of *p*-coumaric acid, caffeic acid, ferulic acid, gallic acid, chlorogenic acid, quercetin, catechin, rutin, chrysophanol and physcion was measured as 5.70, 135.01, 2465.41, 344.97, 27.81, 92.43, 6.11, 28.64, 59.18 and 41.44 μg g-^{1} FW, respectively (Table 5). Concentration of kaemferol also was evaluated, whilst no flavonoid of kaempferol was detected in any of regions studied.

HPLC–MS/MS analysis detected the presence of phenolic compounds chlorogenic acid (10.29 μg g^{-1} FW) and quercetin (85.99 μg g^{-1} FW) being the prominent compounds in the wild-growing *P. alkekengi*, whereas gallic acid (27.81 μg g^{-1} FW) and quercetin (92.43 μg g^{-1} FW) being predominant for commercial one (*P. peruviana*). As stated by Sheikha et al. (2010), the methanolic extract of *P. pubescens* contained protocatechuic acid 0.93 μg g^{-1} FW, coumaric acid 0.17 μg g^{-1} FW, vanillic acid 2.72 μg g^{-1} FW, paracoumaric acid 5.36 μg g^{-1} FW, ferulic acid 10.03 μg g^{-1} FW, rutin 0.44 μg g^{-1} FW, catechin 4.96 μg g^{-1} FW and myricitin 0.10 μg g^{-1} FW, while in the present study, the constituents of this species extracts included rutin (28.64 μg g^{-1} FW), quercetin (92.43 μg g^{-1} FW), coumaric acid (5.7 μg g^{-1} FW), caffeic acid (25.39 μg g^{-1} FW), gallic acid (27.81 μg g^{-1} FW), and chlorogenic acid (2.63 μg g^{-1} FW). Indeed, the individual phenolic acid level of fruits were higher than the amount reported by Sheikha et al. (2010) for the fruits of *P. pubescens*. The highest amount of *p*-coumaric acid, gallic acid, quercetin and rutin was obtained in the greenhouse (*P. peruviana*), which can be attributed to the environmental conditions, including temperature and carbon dioxide level. In strawberries, the increase of temperature and carbon dioxide level increased the amount of phenolic compounds (Wang et al., 2003). Temperature variation is another abiotic habitat parameters that has a remarkable effect on the synthesis of phytochemicals such as individual phenolic components and their biological activity. It is reported that low temperatures promote the formation of phenolic compounds (Sharma et al., 2019). Conversely, a certain degree of elevated temperature favors the biosynthesis of phenolics. Some phenolics, such as caffeic acid and coumaric acids, can prevent heat oxidative damage through accumulation (Commisso et al., 2016). Therefore, depending on the temperature, plants biosynthesize more or less phenolic compounds, which ultimately help protect plant cells (Sharma et al., 2019).

In this study, the level of various constituents revealed high variations, which was in accordance with Medina-Medrano et al. 2015, who reported that constituents of different parts of wild studied species of *Physalis* were species-specific and diverse. Quercetin and ferulic acid were identified as the main flavonoids and phenolic acids, respectively. Ferulic acid has also been reported as the dominant cinnamic acid in *P. angulata* (de Oliveira et al., 2020), as well as citrus (Zhang et al., 2014). In this study, it was found that among the studied compounds, the highest concentrations were also related to phenolic acids. The flavonoids quercetin, along with myricetin and rutin inhibit superoxide radical production (Letan et al., 1966). Studies have indicated that ferulic acid displays scavenging properties for superoxide, nitrogen dioxide, hydroxyl radical and hydrogen peroxide free radicals and possesses strong antioxidant activity (Li et al., 2022). Furthermore, nowadays, the beneficial effects of ferulic acid against a variety of viruses and strengthening the body's immunity against viral infections, preventing platelet aggregation, preventing thrombosis and atherosclerosis, as well as anticancer, antimicrobial, anti-inflammatory properties and in particular, its potential in preventing and treatment of diabetes mellitus and its-related complications have been reported (Neto-Neves et al., 2021, Wang et al. et al. 2017, Narasimhan et al., 2015, Mancuso et al., 2014, Alam et al. 2019, Nankar et al., 2017, Saija et al., 2000), which there were/being abundant in the extracts of *P. alkekengi* grown in C2 (Maragheh region).

In this study, the main phenolic acids detected in *P. peruviana* were gallic acid, caffeic acid, *p*-coumaric acid and chlorogenic acids. Ferulic acid was not found in this species. On the other hand, ferulic acid identified in phenolic compounds of *P. peruviana* studied by Olivares-Tenorio et al., 2016, Namiesnik, et al., 2014; Vega-Gálvez et al., 2014. In terms of both amount and type, there is a wide variation of phenolic compounds in the *Physalis* species. The highest concentration of *p*-coumaric acid (5.70 μg g^{-1} FW), gallic acid (27.81 μg g^{-1} FW), rutin (28.64 μgg-1 FW) and quercetin (92.43 μg g^{-1} FW) were found in fruit extracts from greenhouse (*P. peruviana*). In addition, extracts derived from cultivated *P.alkekengi* in C2 possessed the uppermost concentration of caffeic acid (135.01 μg g^{-1} FW), ferulic acid (2465.41 μg g^{-1} FW), and chlorogenic acid (344.97 μg g^{-1} FW). Chromatograms of individual phenolic compound in C2 samples shown in Fig. 1. Catechin was found in genotypes of cultivated in C6 (6.11 μg g^{-1} FW). Chrysophanol was also found in C3 and C7, 59.18 and 47.88 μg g^{-1} FW, respectively (Table 5). Physcion was only detected in the wild-grown P. alkekengi and myricetin in C2. Moreover, fruit extracts of C2 had the highest amount of phenolic compounds (ferulic acid) among all the studied areas. The highly active effect of anthraquinones such as physcion and chrysophanol on plant powdery mildew is reported by Yang et al. 2007. Therefore, it can be concluded that *P. alkekengi* serves as a rich source of phenolic compounds,

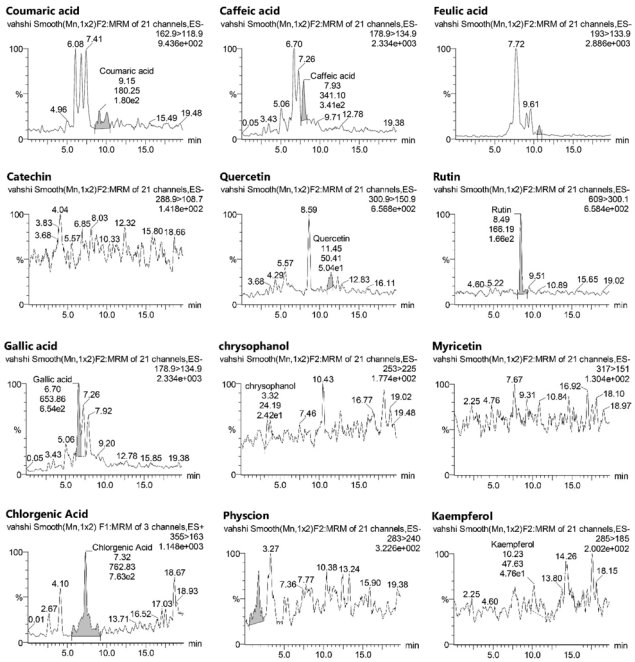


Fig. 1 Chromatograms of individual phenolic compound in WA samples

which might be responsible for antioxidant properties of this species. Of these, ferulic acid and quercetin were the predominant phenolic acid and flavonol in extracts. The flavonoids quercetin, along with myricetin and rutin inhibit superoxide radical production (Letan et al., 1966) which was found in extracts of C2.

The flavonoid of kaempferol was evaluated but was not detected in any of the areas in the species studied. In our findings, quercetin (92.43 μg g^{-1}) was found as a predominant compound in *P. peruviana* and myricetin was not identified in this species; on the contrary, in the report by Licodiedoff et al. 2013, quercetin was not detected and amount of rutin and myricetin were 6.76 and 1.17 μg g^{-1}, respectively. The amount of rutin in the current survey was higher than that published by Licodiedoff et al. 2013 in *P. peruviana*. According to the results of the present research and previous literature, it can be concluded that the place of planting and species significantly influenced this plant's phytochemical concentrations and compositions.

Antioxidant activity

In the present study, the antioxidant activity of *Physalis* fruits in different areas was evaluated by both DPPH and FRAP assays. The results of analysis revealed that the antioxidant activity of this plant, like other phytochemical compounds affected by location of planting and genotype ($p < 0.01$) (Table 6). In the FRAP assay, the amount of antioxidant activity in different planting sites ranged from 0.12 to 0.40 μmol Fe^{++} g^{-1} FW (Fig. 2b). The highest antioxidant activity in *P. alkekengi* fruits was observed in C8, whereas the C2 had the lowest level. The results of mean comparisons showed that there is no significant difference between C1, C3 and GP treatments in terms of antioxidant activity based on FRAP assay. In the DPPH assay, the amount of antioxidant activity of the extracts varied from 11.36 to 44.13% (Fig. 2a). The highest antioxidant activity was obtained in the fruit extract from C5 and C8 with 44.13% and 39.07%, respectively; whereas the lowest level was found in the GP (*P. peruviana*) with 11.36%. Hence, *P. alkekengi* had higher antioxidant activity than *P. peruviana* based on the mechanisms DPPH analyzed. The results of mean comparisons showed that

Table 6 Analysis of variance (ANOVA) of antioxidant characteristics in different samples

SV	DF	Mean Square	
		Antioxidant activity (DPPH assay)	Antioxidant activity (FRAP assay)
Genotype	9	364.67**	0.0229**
Error	29	16.31	0.0008
CV (%)		14.33	10.80

SV Sources of variation, *DF* Degrees of freedom, *CV* Coefficient of variation

** significance at $p < 0.01$

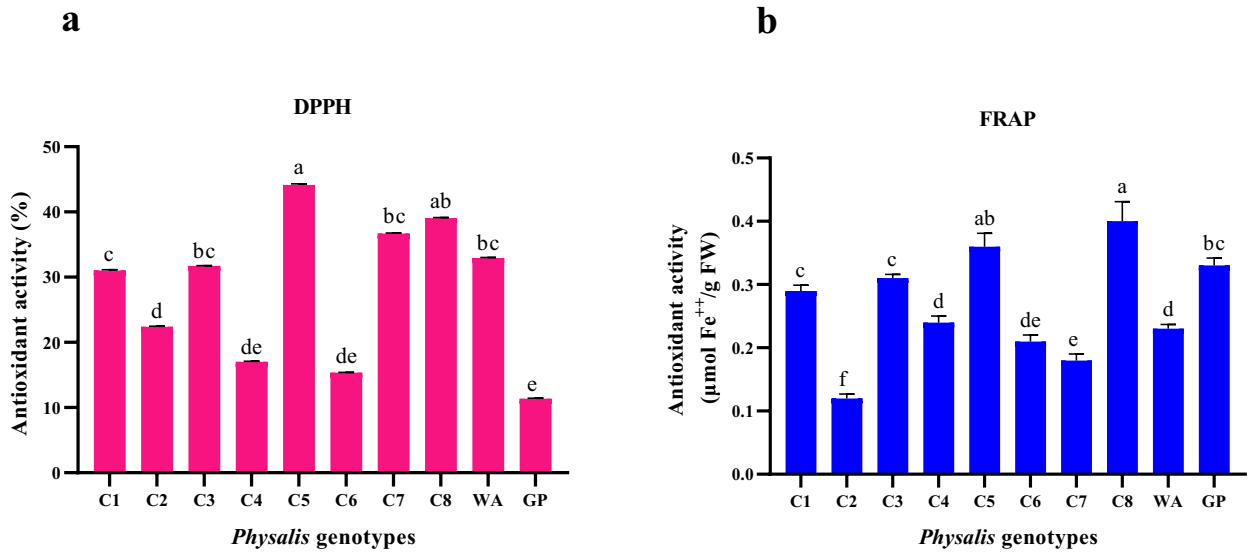


Fig. 2 The antioxidant activity in different *Physalis* genotypes by DPPH (**a**) and FRAP (**b**) assays

there is no significant difference between C3, C7, C8 and WA treatments in terms of antioxidant activity based on DPPH assay (Fig. 2b).

As reported by Diaz et al. 2012, the ethanol extracts of *P. alkekengi* fruits had greater flavonoid and phenol contents and exhibited higher antioxidant activities (DPPH) (53.09%) than the aqueous extract (51.08%) of fruits. In other study conducted in China, the antioxidant activity of *P. alkekengi* fruits was assessed by DPPH assay. Moreover, Mier-Giraldo, et al. 2017 stated that the level of antioxidant activity by the FRAP assay in fruits of *P. peruviana* collected from two different locations of Colombia varied from 35.02 to 47.87 mol g^{-1} DW. These variations in values of antioxidant capacity can be attributed to the differences in origin, geographical coordinates, climate conditions, performance methods and harvest time of fruit, and extraction methods (Wu et al. 2013). The ethanol extract of *P. alkekengi* fruits had greater flavonoid and phenol contents and exhibited higher antioxidant activities than the aqueous extract (Diaz et al., 2012). Methanolic extracts of cultivated *Physalis* fruits contained more phenolics (34.12 mg GAE g^{-1} FW) and flavonoids (3.68 mg QUE g^{-1} FW) than wild *P. alkekengi* fruits. In the current study, the high antioxidant capacity of fruits of *Physalis* in C5 and C8 could have been highly correlated to their ascorbic acid and total phenolic content. On the other hand, the antioxidant properties of *P. alkekengi* can be attributed to its high total phenol content and flavonoids such as catechin, rutin, quercetin and chrysophanol; in addition, phenolic acids such as ferulic acid, caffeic acid and coumaric acid, gallic acid (Antonopoulou et al., 2022) which can be played a major role in the antioxidant activity of this plant. Significant correlation among TPC with high antioxidant activity by DPPH method also has been reported by Shameh et al. 2019; Katalinic et al. 2006.

Vitamin C and TPC (phenolic compounds) are the main contributors to antioxidant capacity in *Physalis* fruit (Fig. 4). The FRAP and DPPH assays gave comparable results for the antioxidant capacity determined in Physalis fruit extracts. The DPPH assay revealed high reproducibility, rapidly performed and revealed the highest correlation with both Vitamin C and TPC. Therefore, it would be a suitable assay for measuring antioxidant activity in Physalis fruit extracts.

Total carotenoid (TCC) and β-carotene contents

There were significant cultivation site and genotype-dependent variations ($p < 0.01$) (Table 2). The level of TCC and β-carotene contents in different areas is presented in Table 3. The comparison of the data mean showed that the amount of carotenoid and β-carotene in different samples of *Physalis* had a significant difference with each other ($p < 0.01$). As shown in Table 3, the TCC of fruits in different planting areas was in the range between 79.20 and 614.67 mg 100 g^{-1} FW with large variations among *Physalis* genotypes. C5, followed by GP (*P. peruviana*) contained the highest amount of carotenoids and the lowest amount was related to C6. The results of mean comparisons showed that there is no significant difference between C3, C7 and C8 treatments in terms of total carotenoid and β-carotene contents (Table 3). As reported by Wen et al. 2019, *P. alkekengi* fruits contained strikingly high quantities of

carotenoids (19.8–21.6 mg/100 g fresh weight). In the present research, the TCC variation was higher than those reported in previous study (Wen et al., 2019). The presence of high levels of carotenes like β-carotene and lutein esters impart the intense orange color of *P. peruviana* fruit (Wen et al., 2019), whereas intense red color in red-colored *P. alkekengi* could be due to zeaxanthin esters (Etzbach et al., 2019; Wen et al., 2019).

Olivares-Tenorio et al. 2016 reported the amount of β-carotene in *Physalis* species varied from 0.2 to 1074.7 mg 100 g^{-1} FW. However, in the present study, the β-carotene content *Physalis* fruits in different climates ranged from 0.04 to 0.47 mg 100 g^{-1} FW. Here again, the uppermost quantity of β-carotene (0.47 and 0.41 mg 100 g^{-1} FW) was related to C5 followed by GP (*P. peruviana*), whereas the minimum one (0.04 mg 100 g^{-1} FW) obtained in C6 (Table 3). According to the previous literature, temperature and radiation can play a decisive role in the biosynthesis and accumulation of carotenoids, so the harvest time is an influential factor in the color of the fruit (Shameh et al., 2019). In addition, climate change can alter the phytochemical composition of a fruit. Nawirska-Olszańska et al. (2017) pointed out that the amount of carotenoids in *Physalis* fruits was dependent on climatic conditions of cultivation sites, so that this amount was higher in cold regions. In addition, amount of β-carotene in *Rosa canina* was different among ten different habitats, and cold habitants contained high levels (Saeedi et al., 2014), that results of the present study were consistent with those. Studies have shown that the variability in TCC of tropical fruits is very high, with the greatest variability due to variety type. The growing season, cultivation site and harvest time also affect the nutritional composition of the fruit. Other reports have shown that exposure to UV radiation improved TCC in some species and decreased it in others. Several studies on the effect of UV radiation on TCC in plant species have shown that the effect can be species-dependent, because each plant species behaves differently to adapt to its environment (Dolzhenko et al., 2010).

Ascorbic acid (Vitamin C) contents

The location of planting had a significant effect ($p < 0.01$) on the amount of ascorbic acid in *Physalis* fruits (Table 2). The levels of ascorbic acid in fruits harvested from different planting areas are displayed in Table 3. The results of mean comparisons showed that the highest amount of ascorbic acid (84.61 mg/100 g FW) was observed in the fruits of C5 sample and the lowest one (44.77 mg/100 g FW) was found in C4 sample. In the studied cultivation areas, the vitamin C contents ranged from 44.77 to 84.61 mg ascorbic acid/100 g, in which C5 (*P. alkekengi*) contained the highest content. In the current study, *P. peruviana* had about 57 mg 100 g^{-1}, being higher than the values reported for *Physalis* fruits 43 mg and 27 mg 100 g^{-1} by Ramadan et al. (2011) and Guiné et al. 2020, but inferior to those reported for ripe *Physalis* fruits (151–163 mg 100 g^{-1}) by Licodiedoff et al. (2013). Accordingly, in comparison with other commonly consumed fruit sources, cultivated *P.alkekengi* in C5 (84.61 mg ascorbic acid.100 g^{-1} FW) followed by C8 (74.93 mg 100 g^{-1} FW) and then wild-growing *P. alkekengi* (68.24 mg 100 g^{-1} FW) are good sources of vitamin C, much higher than mango (15–36 mg 100 g^{-1}FW) and orange (50 mg 100 g^{-1}FW), but less than guava (120–228 mg 100 g^{-1} FW) or marula (120 mg 100 g^{-1} FW) (Sogi et al., 2012). Here again, a variety of factors may contribute to vitamin C levels, including cultivars, varieties, or ecotypes of studied plants, the site of the cultivation, the ripeness stage of the fruit, or analytical methods used to measure vitamin C levels. Cultivated *P.alkekengi* in Silvana (C5) and Greenhouse (C8) contained the vitamin C contents higher than wild one in Qaradag. Indeed, cultivation of this species in greenhouse (controlled ecosystem) increased the amount of Vitamin C. The high vitamin C content of the C8 specimen grown in the greenhouse is probably due to the high amount of carbon dioxide in the environment. Doddrell et al. 2023 reported that growth in a carbon-enriched atmosphere such as greenhouse thus significantly improves flavor and increases ascorbic acid (Vit C) (an essential nutrient compound) among other antioxidant compounds by up to 13.3%.

Hierarchical cluster analysis (HCA)

In the current study, HCA was performed to assort groups of cultivation areas of *Physalis* with similar characteristics based on 18 phytochemical parameters including total phenol and flavonoid, total carotenoid, β-carotene, ascorbic acid (Vitamin C) contents, antioxidant activity by DPPH and FRAP assay, as well as quercetin, rutin, catechin, myricetin, ferulic acid, coumaric acid, caffeic acid, chlorogenic acid, gallic acid, chrysophanol and physcion. As can be shown in Fig. 3, different genotypes were categorized into four distinct groups. The first group was comprised of C1, C3, C7, C8, and C5. The main characteristics of these genotypes that differentiated them from other places were the presence of high levels of phytochemical compounds (total phenols and Vitamin C) along with antioxidant activity by DPPH assay, as well as phenolic compounds (gallic acid, chrysophanol and quercetin). In this group, C5 sample had higher amounts

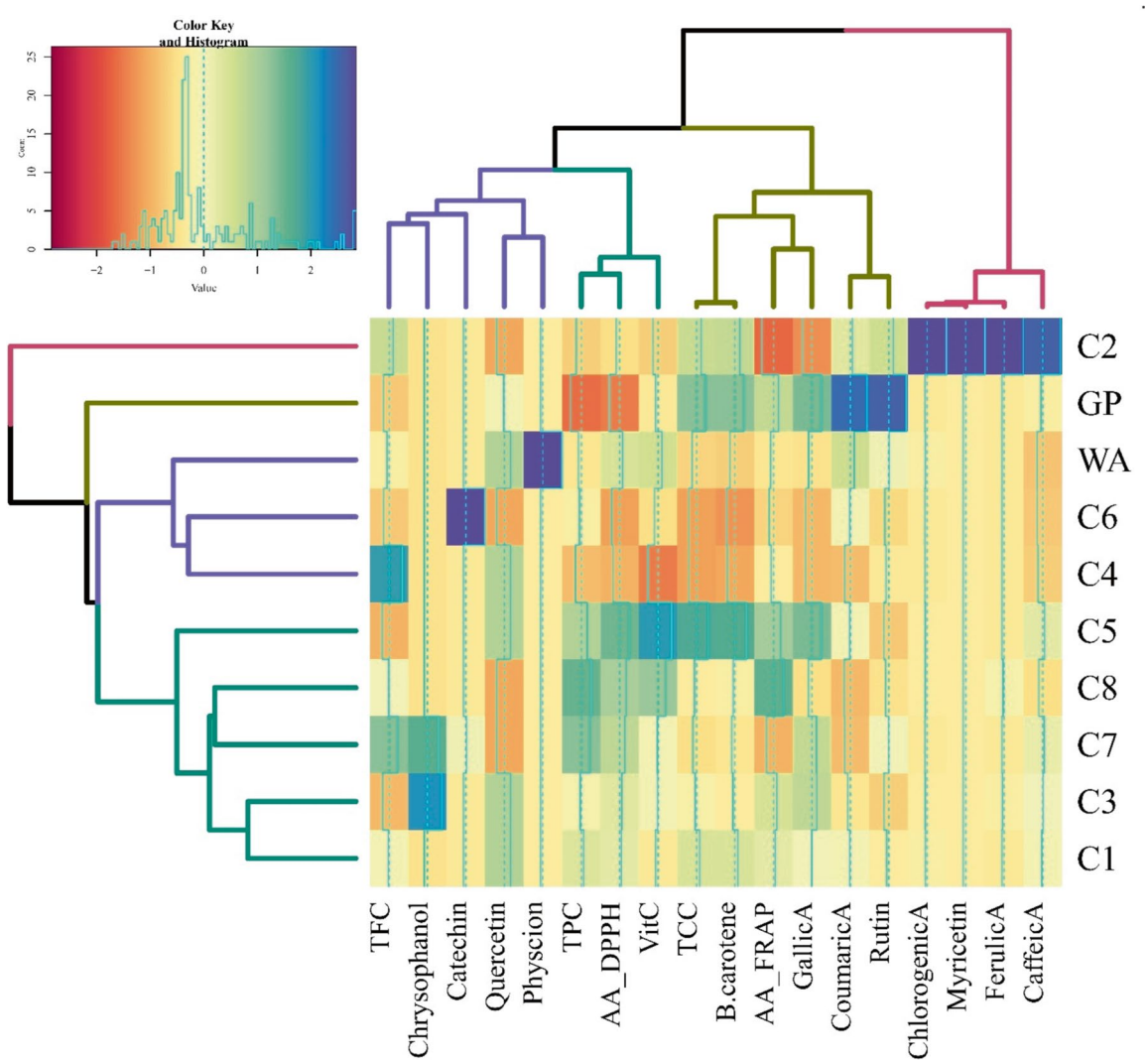


Fig. 3 Hierarchical cluster analysis (HCA) among different *Physalis* genotypes based on 18 phytochemical parameters. VitC: Vitamin C, TPC: Total phenolic content, AA-DDPH: Antioxidant activity by DPPH assay, AA-FRAP: Antioxidant activity by FRAP assay, GallicA: Gallic acid, B.caroten: β-caroten, CoumaricA: Coumaric acid, TFC: Total flavonoid content, CaffeicA: Caffeic acid, FerulicA: Ferulic acid, ChlorogenicA: Chlorogenic acid

of phytochemical compounds than the other genotypes, which can be considered. The second group consisted of the genotypes of C4, C6 and WA with low levels of antioxidant activity based on both methods, and some phytochemical compounds among other genotypes, as well as high levels of TFC (C4), catechin (C6) and physcion (WA). In contrast, the third group consisted of the sample of GP with the high amount of antioxidant activity by FRAP assay, some phytochemical compounds (total carotenoid and β-carotene contents), as well as phenolic compounds (gallic acid, coumaric acid and rutin). Probably, the reason why sample GP is placed in a separate group is due to the correlation of carotenoids with antioxidant activity based on FRAP assay. Müller et al. 2011 reported that carotenoids are the one of the most effective compounds in reducing ferric ions (FRAP assay) due to steric hindrance and the low chemical reactivity of cyclic carotenes and their carbonyl substituted derivatives. The fourth group exclusively comprised samples of C2, which exhibited low antioxidant activity according to both methods. Among other genotypes, this group also stood out due to its phytochemical compounds, particularly its high levels of phenolic compounds, notably ferulic acid, chlorogenic acid, caffeic acid, and myricetin.The high level of ferulic acid was the most noticeable feature of the region in this group.

Having higher individual phenolic compounds does not necessarily mean a high antioxidant capacity

because the antioxidant capacity depends on structure and chemical function of phenolics (presence of a carbohydrate moiety, number and location of hydroxyl group, etc.). There is not one universal assay by which the antioxidant capacity can be quantitatively determined in a very precise way. Considering that medicinal plants have many compounds with different structures, different antioxidant evaluation methods (each of which has different principles) should be used to evaluate antioxidant activity (such as DPPH, β-carotene bleaching, FRAP, ABTS/TEAC, CUPRAC, etc.) (Christodoulou et al., 2022).

Correlation analysis

The simple correlation coefficients of the measured traits are shown in Fig. 4. Simple correlation coefficients

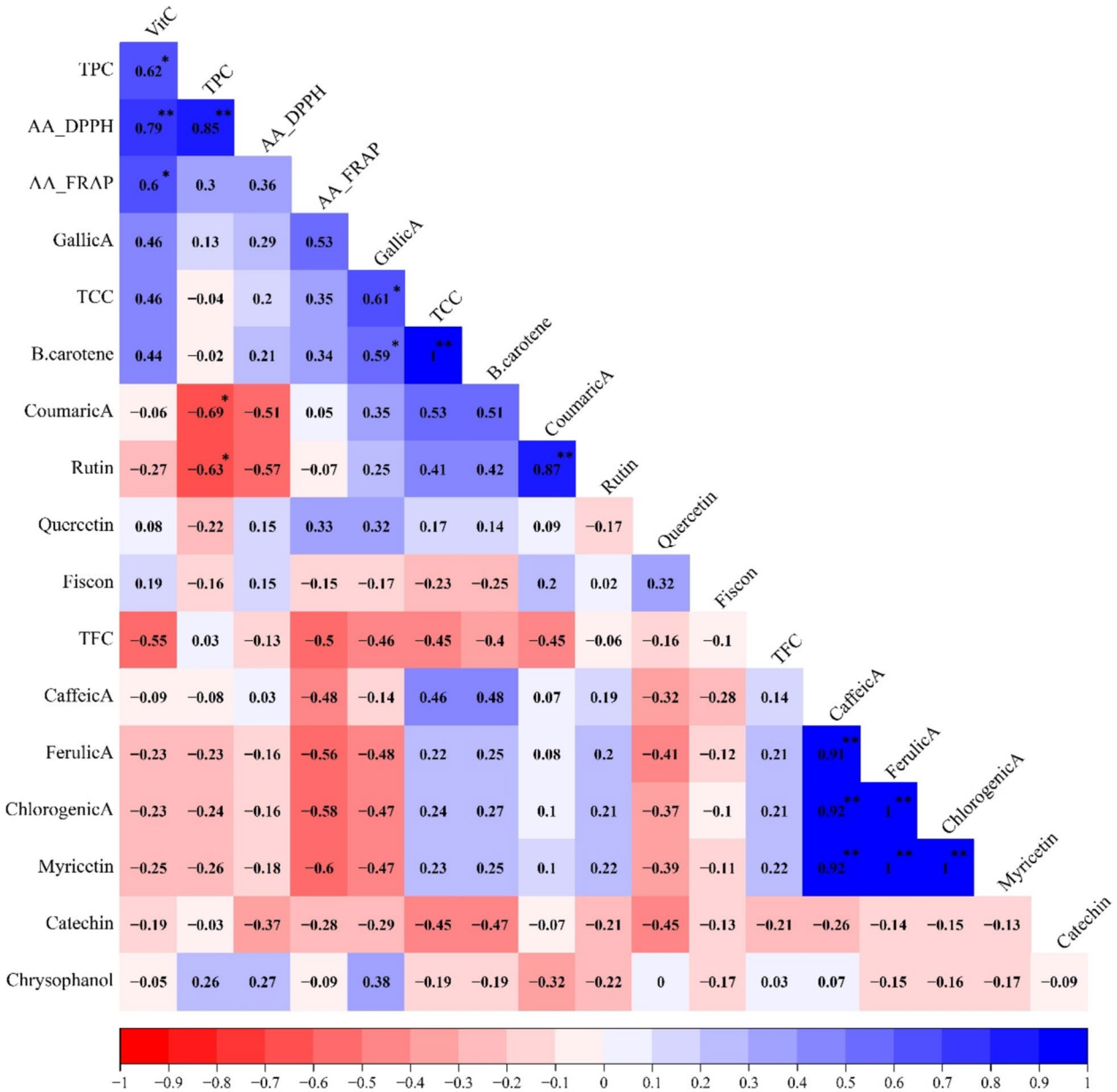


Fig. 4 Correlation coefficients between phytochemicals and phenolic compounds on *Physalis* genotypes. VitC: Vitamin C, TPC: Total phenolic content, AA-DDPH: Antioxidant activity by DPPH assay, AA-FRAP: Antioxidant activity by FRAP assay, GallicA: Gallic acid, B.caroten: *β*-caroten, CoumaricA: Coumaric acid, TFC: Total flavonoid content, CaffeicA: Caffeic acid, FerulicA: Ferulic acid, ChlorogenicA: Chlorogenic acid. ** and *: significant at $p < 0.01$ and $p < 0.05$, respectively

between phytochemical compounds showed that some of them had a significant correlation ($P \leq 0.01$, $P \leq 0.05$), and in some cases no significant correlation was seen, as shown in the Fig. 4. In the present study, it was found a positive and remarkable correlation between antioxidant activity based on the DPPH method with vitamin C (r = 0.79 at $P \leq 0.01$) and with total phenol contents (r = 0.85 at $P \leq 0.01$). As regards the antioxidant properties are attributed to the presence of various phytochemical compounds such as phenolic compounds, carotenoids, and ascorbic acid in foods (Schmitz-Eiberger et al., 2003; Shameh et al., 2019); hence, the high antioxidant capacity of fruits of C5 and C8 genotypes may be strongly correlated to their high ascorbic acid and total phenolic contents (Fig. 4). Traits that had a positive and significant correlation include, the amount of *p*-coumaric acid with rutin (r = 0.87 at $P \leq 0.01$), the amount of caffeic acid with ferulic acid (r = 0.91 at $P \leq 0.01$) and chlorogenic acid (r = 0.92 at $P \leq 0.01$), myricetin with ferulic acid (r = 1 at $P \leq 0.01$) and chlorogenic acid (r = 1 at $P \leq 0.01$), chlorogenic acid with myricetin (r = 1 at $P \leq 0.01$), and total carotenoid with β-carotene ((r = 1 at $P \leq 0.01$). Also, the amount of total phenol had a negative and significant correlation with the amount of coumaric acid (r = -0.69, $P \leq 0.05$) and rutin (r = -0.63 at $P \leq 0.05$).

Conclusion

Considering the cultivation of medicinal plants such as *Physalis*, the growing conditions must be carefully chosen because they affect the phytochemical components and especially the metabolism of polyphenols. The research aimed to inquire the similarities and differences in antioxidant activity and chemical constituents among wild-growing, cultivated *Physalis alkekengi* and commercial species namely *P. peruviana*. Methanolic extracts of cultivated *P. alkekengi* fruits contained more phenolics and flavonoids than wild *P. alkekengi* fruits and commercial species (*P. peruviana*). *P. alkekengi* fruits are of great importance in some phenolic compounds, including total flavonoids (C4 and C7), total phenols (C8 and C7), carotenoids (C5), vitamins C and also antioxidant properties (C5 and C8). Ferulic acid (in C2 sample) and quercetin (in WA sample) were found to be the most abundant phenolic acid and flavonoid by HPLC–MS/MS analysis, respectively. In conclusion, the results obtained from this research revealed that the different regions of cultivation, particularly the fruits obtained from the C5 and C8 genotypes were rich in some valuable medicinal compounds (such as TPC, TCC, Vit C, antioxidant activity). The changes observed in the quantity of phytochemical compounds of *Physalis* fruit were caused by the difference in the various climatic conditions of the ten samples studied. The results of this study and identifying the potentials of each climate can be used to plan the domestication of this plant and to develop new cultivars with greater phenolic accumulation. Also, the results obtained in this work made it possible to find out the best region growth conditions for the higher content of phytochemicals of the studied samples and their possible use in different fields such as pharmaceutical and food industries. However, more studies are needed to clarify the causes of site effects, continue research after several years, and determine the heritability and antioxidant properties of phytochemicals, and to develop a production system that ensures usable biomass.

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Authors' contributions
Parisa Yari: Investigation, Resources, Methodology, Validation. Abolfazl Alirezalu: Supervision, Writing–review & editing, Investigation, Resources, Methodology, Validation, Software, Formal analysis. Soghra Khalili: Writing – review & editing, Validation.

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