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Phenolic compounds and antioxidant activity in seven populations of *Lepidium sativum* L. Leaves

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ABSTRACT

Background & Aim: Garden cress (*Lepidium sativum* L.) is anannual herbaceous species native to Egypt and south west Asia. The leaves of *L. sativum* possesse various medicinal properties. This study was conducted to evaluate the diversity of phytochemical constituents of seven populations of *L. Sativum* from Iran.

Experimental: The crude extracts of *L. sativum* populations leaves were obtained with methanol and were evaluated for the total phenol content, total flavonoid content, antioxidant activity using DPPH radical scavenging assay, and phenolic compounds by HPLC analysis.

Results: The total phenolic content ranged from 1.25 to 2.36 mg GAE/g extract and the total flavonoid content was 0.74- 1.61 mg QE/g extract. Chlorogenic acid was the most abundant phenolic acid, followed by ferulic and caffeic acids. The content of kaempferol flavonoid was also 5.2-fold of quercetin. Leaf extracts of Tabriz and Kerman populations exhibited higher yields of phenolic constituents and antioxidant activity. The total phenolic content was positively correlated to total flavonoid content and phenolic acids (except caffeic acid) and negatively correlated to DPPH free radical scavenging activity (IC_{50}). A negative correlation was found among total flavonoid content, caffeic acid, p-coumaric acid and ferulic acid with precipitation and relative humidity.

Recommended applications/industries: Our research is the first report to study the phytochemical profiles and antioxidant activity in different Iranian populations of *L. sativum* leaves for their health benefit.

1. Introduction

Garden cress (*Lepidium sativum* L.), belongs to the family Brassicaceae, is a fast-growing erect annual herb, 10-60 cm in height, leaves entire or pinnately dissected, 4-15 cm long, andvariously lobed in 0.3-1.2 cm size (Farag and Shaaban, 2021; Raval and Pandya, 2011; Roughani *et al.*, 2018a). It is native to Egypt and south west Asia and widely distributed throughout the world, including Iran (Farag and Shaaban, 2021; Jelvehgar *et al.*, 2022; Roughani *et al.*, 2021). The leaves are consumed as salad, vegetableor garnish (Mali *et al.*, 2007). The leaves of *L. sativum* contain minerals, protein, vitamin E, phenolic compounds, alkaloids, terpenoids, and other metabolites with beneficial biological activities (Sangekar *et al.*, 2018; Vazifeh *et al.*, 2022). In traditional medicine, *L. sativum* is used to treat bronchial asthma, diabetes, hypertension, hepatitis, local and rheumatic pain,menstrual problems, sexual debility, diarrhea, constipation and migraine (Hekmatshoar *et al.*, 2022; Malar *et al.*, 2014; Roughani and Miri, 2018). In addition, *L. sativum* leaves have antioxidant and anti-inflammatory effects due to the presence of sulforaphane, flavonoids, flavonols, alkaloids, tannins,

glucosinolates, sterols, and triterpenes compounds (Farag and Shaaban, 2021; Vazifeh *et al.*, 2022).

Plant phytochemical such as phenolic and flavonoid compounds are exhibiting antioxidant and antibacterial effects, and have a high potential in human health (Birhanie *et al.*, 2021; Keshavarzi *et al.*, 2016). In addition to agro-morphological traits, the quantity and quality of plant bioactive constituents are greatly influenced by environmental and genetic factors (Batubara *et al.*, 2020; Roughani *et al.*, 2018c). Therefore, it is necessary to select genotypes with more phytochemical and pharmacological activities in order to develop cultivars in breeding programs (Alizadeh *et al.*, 2017). In previous works, the essential oil compounds of Iranian *L. sativum* genotypes identified using GC-MS analysis (Mirza and Najafpour Navaei, 2006; Rahimi, 2019). However, to the best of our knowledge, no information has been published on the variation of total phenolic and flavonoid content, antioxidant capacity and phenolic compoundsin the leaves of Iranian *L. sativum* genotypes.

This research was conducted to identify the total phenolic and flavonoid contents and HPLC analysis of phenolic compounds of leaves' extracts of seven *L. Sativum* populations from different geographic locations and evaluate their antioxidant activity.

2. Materials and Methods

2.1. Plant materials

The seeds of *L. sativum* populations were collected from seven Iranian cities with different climatic conditions (Table 1), and planted in early spring in a field located in Shahriar, Iran. The leaves were harvested at full growth stage (6-7 weeks after seed planting), and stored in a freezer at -30 °C until analysis to avoid the oxidation of phytochemical compounds.

Table 1. Geographical coordinates and 10-year average of climatic parameters of the collection sites of *L. sativum* seeds.

-	Latitude	Longitude	Altitude (m)	Temperature (°C)	Precipitation (mm)	Relative humidity (%)
Hamedan, Hamedan	34°48′10″N	48°28′54″E	1860	12.7	241	48
Alborz, Karaj	35°49′57″N	50°59′29″E	1341	16.1	256	47
Kerman, Kerman	30°16′59″N	57°04′43″E	1760	16.9	115	29
Qazvin, Qazvin	36°16′40″N	50°00′26″E	1310	15.2	294	53
Tehran, Shahriar	35°38′59″N	51°02′60″E	1140	17.5	220	41
Fars, Shiraz	29°36′37″N	52°31′52″E	1545	18.6	261	36
East Azerbaijan, Tabriz	38°04′18″N	46°17′32″E	1392	14.0	222	51

2.2. Extrac preparation

The frozen leaves were crushed with liquid nitrogen in a mortar. 10 g of the sample was homogenised with 10 mL of 80% methanol for two minutes, and placed on a shaker for 24 hours. The extracts were placed in an ultrasonic bath for 15 min at 34 °C and centrifuged at 4000 rpm for 10 min. The supernatant phase was removed for phytochemical analysis.

2.3. Total phenolic content

Total phenolic contents were determined using Folin–Ciocalteu method, slightly modified as described by Singleton *et al*. (1999). 0.5 mL of diluted extract (1 : 2) was mixed with 5 mL of 1 N Folin–Ciocalteu reagent. Following incubation for 5 min in room temperature, 4 mL of 7.5% Na_2CO_3 was added. Absorbance at 765 nm was measured in an UV-Vis spectrophotometer (Jenway 6705, UK) after 15 min and the concentration of phenolic compounds was calculated using the calibration curve (y = 0.0023x + 0.018, R^2 = 0.9978) of gallic acid (50–200 µg/mL). Total phenolic content was expressed as mg gallic acid equivalent per gram of the extract (mg GAE/g extract).

2.4. Total flavonoid

Total flavonoids were determined according to a slightly modified colorimetric method as described by Chang *et al*. (2002). 0.5 mL of diluted extract (1 : 5) was added to 1.5 mL methanol, 0.1 mL 10% $AlCl_3$ and 1 M acetate potassium followed by addition of 2.8 mL of distilled water and incubated for 5 min. Absorbance was measured at 415 nm and total flavonoids were calculated using quercetin as standard. The results were expressed as mg quercetin equivalent per gram of the extract (mg QE/g extract).

2.5. Antioxidant activity

The antioxidant activity of the extracts was evaluated by monitoring their ability in 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay according to Takao *et al.* (1994), with a few modifications. Two ethanol dilutions of each sample at 100 and 180 µL/90 mL 80% methanol were tested. A solution of 4 g DPPH in 100 mL 80% methanol was used as a negative control. The absorption was measured at 517 nm after 30 min. The DPPH scavenging ability was expressed as IC_{50}.

2.6. Determination of phenolic compounds by HPLC

Determination of seven phenolic compounds, including chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, gallic acid, quercetin and kaempferol was performed using high performance liquid chromatography (HPLC) apparatus (Agilent Series 1200, USA) equipped with G1367C autosampling injector, and reciprocating pumps connected to a G1322A degasser. UV-DAD B1315G detector and Chemstation software were used. Reverse phase chromatography analyses were carried out on a C-18 column (4.6 mm × 250 mm), and volume injection was 20 µL. Column temperature was maintained at 30 °C. Mobile phase consists of water containing 0.1% phosphoric acid, pH = 2.3 (solvent A) and acetonitrile (solvent B). The UV absorption spectra of the standards as well as the samples were recorded in the range of 280–360 nm. Stock solutions of standards were prepared as 5 mg/50 mL solutions in methanol. Samples and standards solutions as well as the mobile phase were degassed and filtered through 0.2 µm membrane filter. Identification of the compounds was done by comparison of their retention's time and UV absorption spectrum with those of the standards. Quantification of phenolic compounds was carried out based on peak area computation.

2.7. Statistical analysis

Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at $P< 0.01$ using SPSS ver. 23 software. Correlation coefficient was calculated using Pearson method. Cluster analysis was performed to classify the *L. sativum* populations based on the phytochemical compounds.

3. Results and discussion

3.1. Total phenolics and flavonoids content

Total phenolics and flavonoids content of *L. sativum* leaf extracts were found to vary significantly ($P< 0.01$) among populations (Table 2). The highest total phenolic content (TPC) was observed in Tabriz and Shiraz populations, followed by Kerman (2.36, 2.33 and 1.99 mg GAE/g extract), respectively. The lowest TPC was obtained in Qazvin (1.46 mg GAE/g extract) and Karaj (1.25 mg GAE/g extract), respectively. The total flavonoid content (TFC) ranged from 1.61 mg QE/g extract (Kerman) to 0.74 mg QE/g extract (Karaj) (Table 2).

Table 2. Total phenolic contents, total flavonoid content and DPPH radical scavenging assay in leaves' extracts of *L. sativum* populations.

Population	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)	DPPH (IC_{50}, mg/mL)
Hamedan	1.65± 0.28bc	1.27± 0.005 b	1.04± 0.09cd
Karaj	1.25± 0.31 c	0.74± 0.006 g	1.35± 0.01 b
Kerman	1.99± 0.34 ab	1.61± 0.007 a	0.70± 0.04 e
Qazvin	1.46± 0.36 c	0.88± 0.006 f	1.10± 0.02 c
Shahriar	1.48± 0.27bc	0.96± 0.006 e	1.55± 0.01 a
Shiraz	2.33± 0.16 a	1.19± 0.007 c	1.01± 0.01 b
Tabriz	2.36± 0.10 a	1.04± 0.005 d	0.99± 0.02 d
Sig.	**	**	**

Means with different letters are significantly different according to DMRT.**: $P< 0.01$

The phytochemical profiles of medicinal and aromatic plants can be affected by environmental conditions and genotypes (Birhanie *et al.*, 2021; Keshavarzi *et al.*, 2016). Mohanad Sultan and Katib (2021) reported that TPC and TFC of *L. sativum* methanolic leaves extract were 9.93 mg GAE/g and 1.53 mg QE/g, respectively. Kaur *et al*. (2013) revealed total phenols and flavonoid content of *L. latifolium* leaves collected from Ladakh region of Jammu and Kashmir State, India, are different. These results showed that genotype plays an important role in TPC and TFC of *L. Sativum* leaves, enable us to ease selection of populations that might be more useful for medicinal applications. These variations in TPC and TFC are likely based on differences in genotypic background of *L. sativum*, which exhibits high genetic diversity due to annual nature and partly of its sporophytic self-incompatibility system (Jelvehgar *et al.*, 2021a, 2011b; Roughani *et al.*, 2018b).

3.2. DPPHfree radical scavenging activity

DPPH free radical scavenging activity (IC_{50}) ranged from 1.55 mg/mL (Shahriar) to 0.70 mg/mL (Kerman). Efficient scavenging of free radicals makes Kerman population as an effective antioxidant. Antioxidant capacity of *L. sativum* populations in the present study was higher than that noted for the DPPH assay of *L. sativum* seed oil (IC_{50} = 15.7 mg/ml) by Getahun *et al*. (2020), but was similar to Chatoui *et al*. (2016) findings on *L. sativum* seeds from Morocco (IC_{50} = 0.92 mg/ml).

3.3. Phenolic compounds

The results for phenolic acid and flavonoids contents revealed that chlorogenic acid was the most abundant phenolic acid, followed by ferulic and caffeic acids (Table 3).

Table 3. Phenolic acids and flavonoids contents (µg/g DW) in leaves' extracts of *L. sativum* populations.

Population	Chlorogenic acid	Caffeic acid	p-coumaric acid	Ferulic acid	Gallic acid	Quercetin	Kaempferol	Mean
Hamedan	279.8	74.8	23.7	214.6	4.1	9.3	33.4	639.7
Karaj	338.8	62.2	17.3	103.1	1.7	8.8	61.7	593.6
Kerman	678.9	121.0	54.3	401.5	2.0	13.9	98.1	1369.7
Qazvin	412.0	69.7	16.4	19.0	2.2	14.1	65.6	599
Shahriar	532.9	68.8	19.1	189.4	2.4	16.1	62.6	891.3
Shiraz	448.2	93.2	32.3	215.3	2.3	11.9	31.5	834.7
Tabriz	642.7	65.7	33.9	222.8	6.0	15.7	119.0	1105.8
Mean	476.2	79.4	28.1	195.1	2.9	12.8	67.4	

Similarly, Orlovskaya and Chelombit´ko (2007) reported chlorogenic acid as the predominant phenolic compound in *L. sativum* seeds. Chlorogenic acid is an important and biologically active phenolic acid, that has therapeutic properties including antioxidant, antibacterial, hepatoprotective, cardioprotective, anti-inflammatory, antipyretic, neuroprotective, anti-obesity, antiviral, anti-microbial, and anti-hypertension activities (Naveed *et al.*, 2018). The content of kaempferol flavonoid was also 5.2-fold of quercetin. Bensaid Sara *et al*. (2018) identified 13 phenolic acids and 6 flavonoids in aerial parts of *L. Draba* using HPLC-TOF/MS technique, whichseven reported phenolic compounds were also identified in our study. Necip and Durgun (2022) detected 20 phenolic compounds, including chlorogenic, caffeic and p-coumaric acids, in the aerial parts of *L. draba* using LC-MS/MS technique, that quinic and rosmarinic acids were the predominant compounds.

The populations of Kerman and Tabriz were richer than others in terms of phenolic compounds, so that the highest contents of chlorogenic, caffeic, p-coumaric and ferulic acids belonged to Kerman, while the most gallic acid and kaempferol were observed in Tabriz population. The highest quercetin was related to Shahriar population (Table 3). Due to the role of phenolic compounds in human health, the two populations of Kerman and Tabriz have higher medicinal and nutritional value.

3.4. Cluster analysis

According to the phytochemical profiles, *L. sativum* populations were divided into two main clusters (Fig. 1).

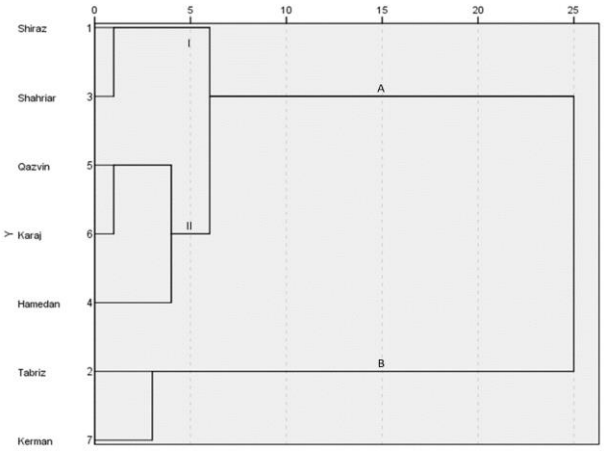

Figure 1. Cluster analysis of *L. sativum* populations based on phytochemical compounds using Ward method.

Cluster I subdivided into two groups: Cluster I-A consists of two populations (Shiraz and Shahriar) characterized by lower antioxidant activity and kaempferol content, and cluster I-B contains three populations (Qazvin, Karaj and Hamedan), which are identified by lower amounts of TPC, TFC, phenolic acids and quercetin. Cluster II contains two populations including Tabriz and Kerman, characterized by higher quantities of TPC, TFC, phenolic acids, flavonoids and antioxidant activity. Cluster analysis based on secondary metabolites was proven to be a helpful tool to classify chemotypes. Cluster analysis on *Zataria multiflora* populations was able to identified three different chemotypes (Karimi *et al.*, 2020).

3.5. Correlation among phytochemical compounds

The correlation coefficients among TPC, TFC, DPPH and phenolic compounds are presented in Table 4.

Table 4. Pearson correlations phytochemical constituents of leaves' extracts in *L. sativum* populations.

	TPC	TFC	DPPH	ChA	CA	pCA	FA	GA	Q
TFC	0.46*								
DPPH	-0.56**	-0.77**							
ChA	0.49*	0.44*	-0.38						
CA	0.38	0.89**	-0.72**	0.48*					
pCA	0.58**	0.87**	-0.80**	0.72**	0.87**				
FA	0.49*	0.89**	-0.58**	0.62**	0.79**	0.91**			
GA	0.49*	0.07	-0.21	0.23	-0.31	0.10	0.14		
Q	0.29	0.09	-0.00	0.79**	0.09	0.24	0.17	0.25	
K	0.27	0.13	-0.31	0.80**	0.10	0.47*	0.32	0.43	0.61**

TPC: total phenolic content, TFC: total flavonoid content, DPPH: DPPH free radical scavenging activity (IC_{50}), ChA: chlorogenic acid, CA: caffeic acid, pCA: p-coumaric acid, FA: ferulic acid, GA: gallic acid, Q: quercetin, K: kaempferol
*: $P< 0.05$, **: $P< 0.05$

TPC was positively correlated to TFC and phenolic acids (except caffeic acid) and negatively correlated to DPPH. Chlorogenic acid had a significant positive correlation with TFC and phenolic compounds (except gallic acid). DPPH free radical scavenging activity (IC_{50}) showed highly significant negative correlation to total phenolic and flavonoid contents, and caffeic, p-coumaric and ferulic acids. It indicates that TPC, TFC and some of phenolic compounds contribute to the antioxidant activity of *L. sativum* plants. Phenolic compounds due to their hydroxyl group (OH) are usually considered as the source of scavenging activities of the plant extracts (Birhanie *et al.*, 2021; Uroko *et al.*, 2022). These findings were consistent with the previous reports, which confirmed that the *L. sativum* extracts with higher phenolic compounds had higher antioxidant activity (Malar *et al.*, 2014; Mohanad Sultan and Katib, 2021). Similar results have been obtained for other *Lepidium* species, which could be used to evaluate the antioxidant potential of plants. Asnaashari *et al.* (2018) reported that *L. vesicarium* leaf essential oil has a weak radical scavenging potential, which may be due to the absence of phenolic compounds in the essential oil. Lee and Chang (2019) also found that the antioxidant activities of *L. meyenii* leaves extract were positively correlated with total phenol and flavonoid content. No significant correlations were found among the quercetin with TPC, TFC, DPPH and phenolic compounds (except chlorogenic acid).

3.6. Correlation among phytochemical compounds and climatic conditions

The caffeic acid of extract had negative and positive significant correlations with latitude and longitude of the seed collection site of *L. sativum* populations, respectively (Table 5).

Table 5. Pearson correlations among phytochemical constituents in leaves' extracts of *L. sativum* populations and climatic conditions.

Compound	Latitude	Longitude	Altitude	Temperature	Precipitation	Relative humidity
TPC	-0.31	0.00	0.33	0.10	-0.29	-0.33
TFC	-0.65	0.57	0.78*	0.04	-0.77*	-0.72*
DPPH	0.44	-0.33	-0.75*	0.16	0.48	0.37
ChA	-0.09	0.33	-0.10	0.29	-0.70	-0.45
CA	-0.84*	0.85*	0.60	0.40	-0.73*	-0.88*
pCA	-0.56	0.59	0.57	0.19	-0.85*	-0.73*
FA	-0.55	0.53	0.59	0.16	-0.91**	-0.78*
GA	0.54	-0.72	0.14	-0.67	-0.01	0.44
Q	0.21	0.03	-0.46	0.21	-0.27	-0.07
K	0.38	-0.02	-0.17	-0.16	-0.51	0.02

*: $P< 0.05$, **: $P< 0.05$; Symbols as in Table 4.

The amount of TFC and DPPH (IC_{50}) had positive and negative significant correlations with altitude, respectively. However, temperature was not correlated with phytochemical compounds and antioxidant capacity of *L. sativum* extract. A negative correlation was found between TFC, caffeic acid, p-coumaric acid and ferulic acid with precipitation and relative humidity. Secondary metabolites play important roles in adaptation of plants to the environmental conditions. Phenolic compounds are synthesized as an antioxidant defense mechanism against abiotic and biotic stress conditions (Albergaria *et al.*, 2020). Phytochemical evaluation in *Ageratina petiolaris* showed that the phenolic compounds, flavonoids and antioxidant activity in the samples collected from the natural environment with limiting edaphic-environmental factors, were increased compared to cultivated plants (Pérez-Ochoa *et al.*, 2022). Negative correlation between altitude with essential oil content and positive correlation between temperature with essential oil yield were found in *Zataria multiflora* (Karimi *et al.*, 2020)

4. Conclusion

This research allowed to obtain new information about the phenolic composition and antioxidant capacity of leaves' extracts in some Iranian *L. sativum* populations. The phytochemical constituents differed among the populations, which was affected by the genotype and partly the climatic conditions of the collection site. Overall, Tabriz and Kerman populations possessed the highest yields of phenolic constituents and antioxidant activity among the populations analyzed in this study. A negative correlation was found among total flavonoid content, caffeic acid, p-coumaric acid and ferulic acid with precipitation and relative humidity. The obtained results confirm that *L. Sativum* could be potential source of phenolic compounds with antioxidant capacity that could be used as medicinal herbs in addition to vegetables.

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