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Regional environmental impacts on growth traits and phytochemical profiles of *Glycyrrhiza glabra* L. for enhanced medicinal and industrial use

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Abstract

Identifying the optimal cultivation regions and evaluating the impact of environmental factors are crucial for selecting the best conditions for the commercial production of important medicinal and industrial plants. This study examined the effects of different cultivation areas-Rayen, Eghlid, Kalat, and Zanjan-on the agro-morphological and phytochemical traits of *Glycyrrhiza glabra*. The findings revealed that the location where the plants were grown significantly influenced their physical and chemical characteristics. The Kalat region produced the tallest plants, measuring 96.86 cm, along with the highest shoot dry weight at 205.17 g, root dry weight of 318.00 g, root yield of 1590.12 g/m^2, and glabridin content of 2.92 mg/g dry weight (DW). Conversely, samples from the Rayen region had the highest glycyrhizic acid content at 17.92 mg/g DW and liquritigenin content at 1.22 mg/g DW. The Eghlid region showcased the highest total phenol content and antioxidant activity. Additionally, the study found a negative and significant correlation between altitude and glabridin content, indicating that glabridin levels decrease with increasing altitude. Based on the needs of the food and pharmaceutical industries, the study recommends the Rayen region for the production of glycyrhizic acid, the Kalat region for glabridin, and the Eghlid region for phenolic compounds.

Keywords Functional traits, Glycyrhizic acid, Glabridin, Altitude, Licorice

Introduction

Glycyrrhiza glabra L., commonly known as licorice, is a herbaceous perennial plant that belongs to the Fabaceae family. It is widely distributed throughout Asia and some parts of Europe [1]. This plant is considered one of the most valuable commercial plants in the world, used extensively in the pharmaceutical, cosmetic, tobacco, and food industries [2]. *Glycyrrhiza glabra* is cultivated in various countries, including Italy, China, Spain, France, Greece, Germany, India, Russia, England, Uzbekistan, and the United States [3]. G. glabra L. is rich in biologically active substances, which include proteins, amino acids, polysaccharides, pectin, starch, mineral salts,

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gums, sterols, and resins [1]. It also contains important flavonoids, such as glabridin, liquiritigenin, and liquiritin, as well as triterpenoid saponins like glycyrrhizin and glycyrrhizic acid [3, 4]. These compounds exhibit multiple biological activities, including anticoagulant and anti-inflammatory properties, liver protection, anti-tumor effects, anti-arteriosclerosis, antiviral and antimicrobial actions, antioxidant activity, and cytotoxic effects [5–8].

G. glabra is a valuable and strategic plant in Iran, but it is at risk of extinction due to excessive harvesting. Despite government efforts to enforce strict regulations to prevent indiscriminate harvesting and protect this plant, serious threats remain, including habitat destruction and ongoing unsustainable harvest practices. Endangered plants like *G. glabra* have a slower rate of habitat recovery compared to the rate at which they are removed from their natural environment. Their slow reproduction further underscores the importance of protecting them [6]. Moreover, *G. glabra* plays a significant role in Iran's economy, with a substantial portion of the country's medicinal plant exports attributed to this species. Iran's large area (1,648,195 km^2) and the presence of 11 out of the 13 known climates worldwide create highly favorable conditions for plant growth and development [9].

There is a growing global trend towards the consumption of natural products. These products are increasingly being utilized in pharmaceuticals, food, cosmetics, health industries, and as dietary supplements. The biological effects of these products on the human body have received widespread approval [10]. However, the rapid growth of the world's population, our dependence on nature, and the overexploitation of medicinal plants have led to significant erosion of ecosystems and the loss of natural habitats for many medicinal species [5].

Cultivating and producing medicinal plants can be an effective solution to protect ecosystems and meet the demands of various industries [11]. By focusing on the cultivation of medicinal plants, issues related to wild collection-such as high species diversity, incorrect identification of plants, instability of active compounds, contamination with pollutants, and the presence of toxic substances-can be minimized. This approach allows for the production of high-quality products with consistent efficacy [12].

Geographical and climatic factors play a significant role in the production of secondary metabolites and the morphological traits of medicinal plants. While the synthesis of effective substances is driven by genetic processes, environmental factors such as temperature, light, and altitude greatly influence their production [13, 14]. Various environmental and ecological conditions have been shown to affect specific species, including Cistanche [15], *Orthosiphon aristatus* [16], *Astragalus membranaceus* [17], and *Trachyspermum ammi* [18]. Research indicates that these environmental factors can impact the performance, quantity, and quality of effective substances produced by these plants.

Genotype-environment interactions can limit the genetic potential of cultivars and restrict their ability to perform well in specific environments. Therefore, it is important to evaluate various factors such as agro-climatic regions, yield stability, and cultivar adaptability to make more accurate predictions about successful cultivation [19]. To gain a clearer understanding of genotypic stability patterns, researchers use both univariate and multivariate statistical analyses. Multivariate methods, such as cluster analysis, pattern analysis, and principal component analysis (biplots), are effective tools for uncovering patterns of genotype-environment interactions. Pattern analysis, which combines classification and ordination techniques, is particularly useful for elucidating the structure of genotype-environment interactions in the data being examined [20].

The objective of this research is to identify suitable areas for cultivating *G. glabra* and to evaluate its agro-morphological and phytochemical traits across different regions. Additionally, the study seeks to understand the relationships between these traits and environmental factors. To accomplish this, the plant was cultivated in four distinct regions-Rayen, Eghlid, Kalat, and Zanjan-and its morphological, functional, and phytochemical characteristics were assessed in the third year of cultivation.

Materials and methods

Cultivation regions of *G. Glabra*

The cultivation regions of *G. glabra* include Rayen (Kerman Province), Eghlid (Fars Province), Kalat (Khorasan Razavi Province), and Zanjan (Zanjan Province). The climate and soil characteristics of these regions are presented in Table 1; Fig. 1. Climatic data were obtained from the nearest meteorological station. To assess the physical and chemical properties of the soil in different cultivated areas, soil samples were collected from a depth of 30 cm. Soil texture was determined using the Bouyoucos hydrometer method (Gee and Bauder, 1979). pH and electrical conductivity (EC) were measured using the CPD-65 N multi-meter (ISTEK, South Korea). Organic carbon content was measured using the modified Walkley and Black method [21], while calcium carbonate was determined by the Bernard calcimeter method [22]. Olsen's method was employed to assess the levels of phosphorus, potassium, and calcium [21]. Finally, the nitrogen content was measured using the Kjeldahl method [23].

Plant cultivation

To prepare the land for cultivating samples, initial measures included plowing, removing stones, and clearing

Table 1 Geographical, climate and soil characteristics of *Glycyrrhiza glabra* cultivation regions

Region	Rayen	Eghlid	Kalat	Zanjan
Environmental characteristics				
Longitude (°E)	57°23′ 18″	52°29′ 38″	59°45′ 41″	48°23′ 11″
Latitude (°N)	29°36′ 19″	30°50′ 34″	36° 59′ 19″	36° 40′ 09″
Altitude (m)	2318	2328	950	1583
Average temperature (°C)	15.4	22.1	28	14.2
Average Annual rainfall (mm)	93.5	291	240.5	320
Organic matter (%)	1.81	0.82	1.14	1.18
Clay (%)	15	20	37	33
Silt (%)	12	52	26	27
Sand (%)	73	28	37	40
Texture	Sandy Loam	Silt Loam	Clay loam	Clay loam
pH	8.04	7.92	7.74	8.28
EC (ds/m)	0.59	1.8	2.55	0.72
N (%)	0.08	0.08	0.11	0.09
P (mg/kg)	6.17	7.3	16.4	9.7
K (mg/kg)	251	136	315	276

out plant remains and weeds. After this preparation, the land was plotted into sections measuring 4 m in length and 3 m in width (12 m^2 per plot). For planting, rhizomes with a diameter of 2 cm and a length of 15 cm were used. These were spaced 50 cm apart in rows and 40 cm apart within the row. The rhizomes were collected during the dormant season from their natural habitat in Kerman province. The experimental design was structured as a randomized complete block design with 3 replications conducted between 2020 and 2023. Each plot consisted of four rows, each 4 m long. During the initial stages of cultivation, irrigation was conducted weekly to promote better establishment of the plants. Throughout the growing season, irrigation was adjusted based on the climatic conditions of the region and the water needs of the plants. Manual weed control was implemented during the growth period to maintain the health of the crops.

Evaluation of morphological and functional traits

At the end of the third year of cultivation, measurements were taken in October for various traits, including plant height, leaf length, leaf width, the number of branches, and stem diameter. After harvesting both the aerial and underground parts of the plants, the samples were dried in the shade and at room temperature in the laboratory. Functional traits were then assessed, which included the fresh and dry weights of the aerial parts, the fresh and dry weights of the roots, and root yield per square meter. Quantitative traits related to the length and width of the organs were measured using a digital ruler and caliper, while a digital scale was employed to measure the functional traits (Table 2).

Preparation of *G. Glabra* root extract

To extract the desired compounds, 200 mg of the powdered root was combined with 20 ml of 80% methanol. This mixture was subjected to ultrasonic treatment using the SingenHtw Elmasonic-D device for 30 min. Afterward, the mixture was centrifuged and filtered using 0.2-µm filters [5].

Determination of total phenol and flavonoid content and antioxidant activity

The total phenol content (TPC) was determined by mixing 0.5 ml of methanolic extract with 5 ml of Folin-Ciocalteu solution (diluted 1:10 with distilled water), followed by the addition of 4 ml of 1 M sodium carbonate. For the control, methanol was used instead of the extract. The absorbance of the solutions was measured at a wavelength of 765 nm after a 30-minute incubation in the dark in a 40 °C steam bath [24, 25]. A calibration curve was constructed using various concentrations of a gallic acid standard, resulting in an R^2 value of 0.998, with the equation $y = 0.01x + 0.0075$.

To measure the total flavonoid content (TFC), 0.5 ml of the methanol extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride in ethanol (prepared by dissolving 10 g of aluminum chloride in 100 ml of ethanol), 2.8 ml of distilled water, and 0.1 ml of 1 M potassium acetate. The control was prepared using pure methanol instead of the methanolic extract. The absorbance of the solution was measured after a 30-minute incubation in the dark at a wavelength of 415 nm [26].

The amount of total flavonoid was determined using the quercetin standard curve, with the equation from the standard line yielding an R^2 value of 0.985, expressed as $y = 0.0112x + 0.0004$. To measure antioxidant activity, the percent inhibition method of DPPH (2,2-diphenyl-1-picrylhydrazyl) was employed [27]. A mixture of 2 ml of DPPH (0.1 mmol) and 2 ml of the prepared methanolic extract was allowed to stand in the dark for 30 min, after which the absorbance of the samples was measured with a spectrophotometer at a wavelength of 517 nm. The control consisted of 2 ml of DPPH mixed with 2 ml of methanol, with methanol serving as the blank. The calculation for DPPH radical scavenging activity was determined using the following equation:

$$\mathrm{DPPH} = \mathrm{Ac} - \mathrm{As}/\mathrm{Ac} \times 100$$

In this equation, AC is the DPPH radical absorption of the control sample and AS is the DPPH absorption of the sample.

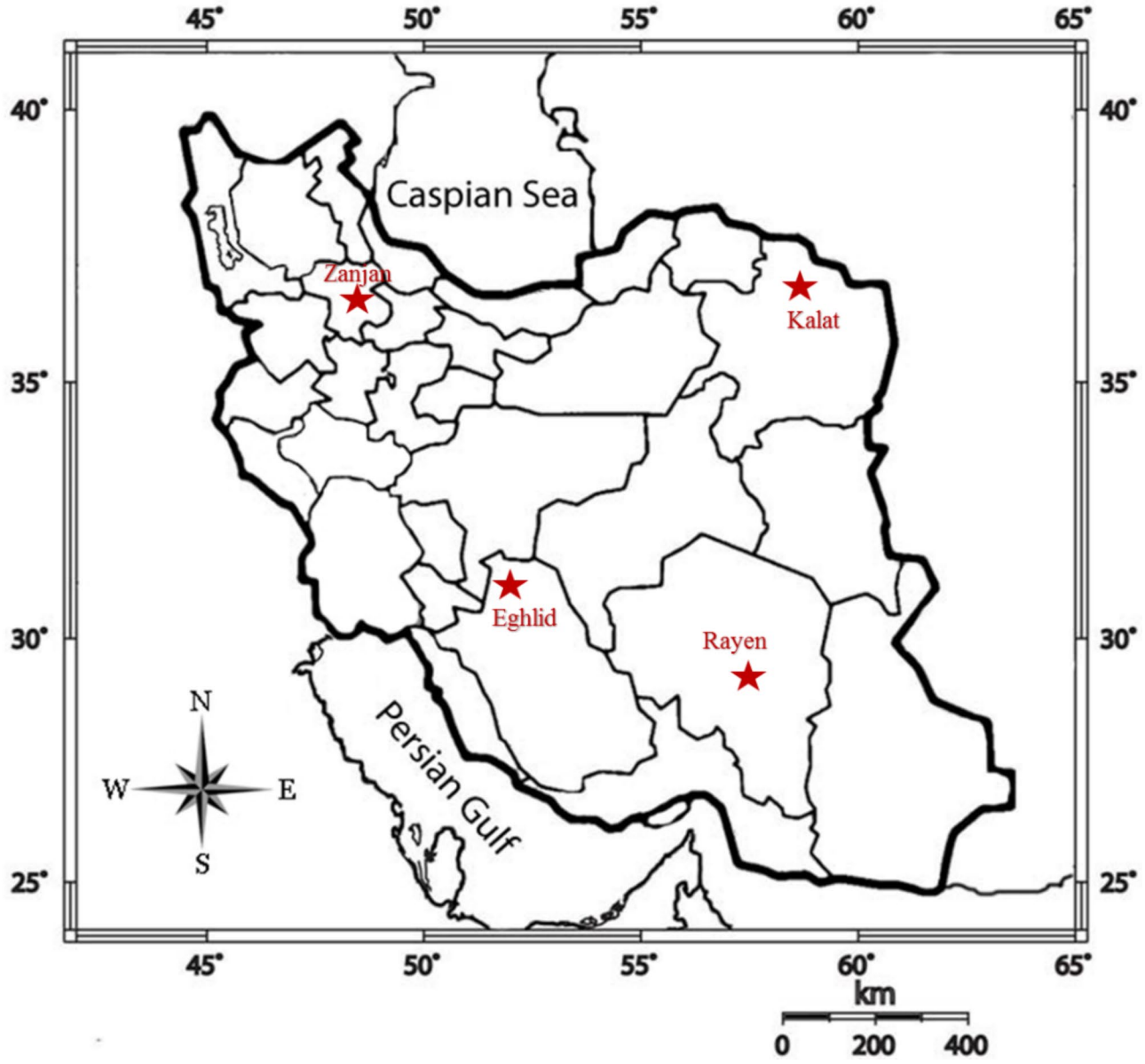

Fig. 1 The investigated cultivation regions of *G. glabra* (Zanjan, Kalat, Eghlid and Rayen)

Table 2 Agro-morphological traits of the *G. Glabra* in different cultivation regions

Traits	Region			
	Rayen	Eghlid	Kalat	Zanjan
Plant height (cm)	73.26 ± 0.75^{b}	47.73 ± 1.23^{d}	96.86 ± 0.75^{a}	58.03 ± 0.75^{c}
Number of branches	7.00 ± 0.57^{b}	3.34 ± 0.35^{c}	10.33 ± 0.33^{a}	5.67 ± 0.57^{b}
Stem diameter (mm)	7.33 ± 0.08^{b}	3.41 ± 0.20^{d}	8.73 ± 0.20^{a}	4.56 ± 0.17^{c}
Leaf length (mm)	15.86 ± 0.26^{d}	22.93 ± 0.31^{b}	19.50 ± 0.23^{c}	28.20 ± 0.21^{a}
Leaf width (mm)	7.10 ± 0.21^{d}	10.23 ± 0.12^{b}	8.26 ± 0.12^{c}	13.16 ± 0.34^{a}
Shoot fresh weight (g)	294.67 ± 1.69^{b}	155.26 ± 2.39^{d}	366.07 ± 2.67^{a}	188.43 ± 1.73^{c}
Shoot dry weight (g)	149.17 ± 1.34^{b}	74.61 ± 1.71^{d}	205.17 ± 2.02^{a}	90.76 ± 1.78^{c}
Root fresh weight (g)	394.83 ± 2.07^{b}	191.97 ± 3.48^{d}	485.93 ± 2.81^{a}	251.70 ± 3.17^{c}
Root dry weight (g)	245.01 ± 2.31^{b}	124.34 ± 0.88^{d}	318.00 ± 1.73^{a}	163.00 ± 1.15^{c}
Root yield (g/m2)	1225.13 ± 11.54^{b}	621.67 ± 4.40^{c}	1590.12 ± 8.66^{a}	815.00 ± 5.77^{c}

Data are mean ± standard error ($n = 3$). Values followed by the same letter within each row are significantly different ($p < 0.05$)

Quantitative analysis by HPLC

A high-performance liquid chromatography (HPLC) device manufactured by Knauer in Germany was utilized for separation and measurement. This device features two Wellchron-K1001 model pumps and a PDA detector (K2800 model). The chromatographic column, produced by Eurospher, is of the RP-C18 type, with an inner diameter of 4.6 mm and a length of 250 mm. The mobile phases used were acetonitrile and HPLC-grade water, with a flow rate set at 1 mL/min. The procedure included washing the column with 95% water for 5 min, followed by a gradient change in the solvent composition from 95 to 5% over a period of 30 min. The samples were assessed at specific absorption and emission wavelengths: 276 nm for liquiritin and liquiritigenin, 230 nm for glabridin, and 250 nm for glycyrrhizic acid. Standard samples of glycyrrhizic acid, liquiritin, liquiritigenin, and glabridin were obtained from Phytolab, Germany. To create standard curves, concentrations of 10, 20, 50, 100, and 250 ppm of these standards were prepared and injected into the HPLC device. EZChrom software, installed on a Windows operating system, was utilized to integrate the results and calculate the area under the curve. The peak areas corresponding to the standard concentrations were computed, and standard curves were generated using Excel software, leading to the formulation of the line equation ($y = bx + a$) [6].

Data analysis

A data variance analysis was conducted using a factorial design within a randomized complete block design, with three replications. Average data comparisons were performed using the Duncan test at a significance level of $P < 0.05$, utilizing SAS software (Version 9.4). Graphs were created using Excel 2016. Additionally, heat maps, correlation plots, principal component analysis (PCA), and hierarchical cluster analysis (HCA) were generated using Origin software (Version 2024).

Results

Morphological and functional traits

The results of this study indicate that the cultivation region significantly affects both morphological and functional traits ($P < 0.01$). Plant height ranged from 47.73 ± 1.23 cm to 96.86 ± 0.75 cm across different cultivation regions, with the lowest height in Eghlid and the highest in Kalat. The Kalat region showed the highest number of branches (10.33 ± 0.33), while the Eghlid region had the lowest (3.34 ± 0.35). The diameter of the main stem increased in the following order: Eghlid < Zanjan < Rayen < Kalat. Regarding leaf characteristics, the Zanjan region exhibited the highest leaf length (28.20 ± 0.21 mm) and width (13.16 ± 0.34 mm), while the Rayen region had the lowest measurements. There were also significant differences in the fresh weight (ranging from 155.26 ± 2.39 g to 366.07 ± 2.67 g) and dry weight (ranging from 74.61 ± 1.71 g to 205.17 ± 2.02 g) of the shoots among different regions, with Kalat having the highest weights and Eghlid the lowest. The root is the most important organ utilized by *G. glabra* and plays a critical role in its medicinal properties. The fresh and dry weights of the root, as well as its overall yield, also varied significantly across cultivation regions. The Kalat region produced the highest fresh (485.93 ± 2.81 g) and dry (318.00 ± 1.73 g) root weights, while the Eghlid region had the lowest fresh (191.97 ± 3.48 g) and dry (124.34 ± 0.88 g) root weights. The root yields for the Rayen, Eghlid, Kalat, and Zanjan regions were 1225.13 ± 11.54 g/m², 621.67 ± 4.40 g/m², 1590.12 ± 8.66 g/m², and 815.00 ± 5.77 g/m², respectively.

Table 3 Glycyrrhizic acid, glabridin, liquiritin and liquiritigenin content of the *G. Glabra* in different cultivation regions

Region	Glycyrhizic acid (mg/g DW)	Glabridin (mg/g DW)	Liquritin (mg/g DW)	Liquritigenin (mg/g DW)
Rayen	17.92 ± 1.43	0.34 ± 0.02	Trace	1.22 ± 0.14
Eghlid	12.25 ± 1.08	0.15 ± 0.01	0.18 ± 0.01	0.98 ± 0.07
Kalat	9.61 ± 0.38	2.92 ± 0.28	Trace	0.87 ± 0.04
Zanjan	11.32 ± 0.53	0.98 ± 0.11	0.13 ± 0.01	1.08 ± 0.10

Data are mean ± standard error ($n = 3$)

Glycyrrhizic acid, glabridin, liquritin, and liquritigenin content

The cultivation area had a significant effect on the content of glycyrrhizic acid, glabridin, liquiritin, and liquiritigenin in *G. glabra* ($P < 0.01$). The amount of glycyrrhizic acid ranged from 9.64 to 17.92 mg/g dry weight (DW) across different cultivation regions, with the lowest content found in the Kalat region and the highest in the Rayen region. Regarding glabridin, the highest concentration was noted in the Kalat region at 2.92 mg/g DW, while the lowest, at 0.15 mg/g DW, was recorded in the Eghlid region. For the Rayen and Zanjan regions, the glabridin content was 0.34 mg/g DW and 0.98 mg/g DW, respectively. In terms of liquiritin, *G. glabra* from the Eghlid and Zanjan regions contained 0.17 mg/g DW and 0.13 mg/g DW, respectively, while the amounts in Rayen and Kalat areas were negligible. Lastly, the content of liquiritigenin was measured at 1.22 mg/g in the Rayen region, 0.98 mg/g in Eghlid, 0.87 mg/g in Kalat, and 1.08 mg/g in Zanjan (Table 3; Fig. 2).

TPC, TFC, and antioxidant activity

The results indicate that the TPC, TFC, and antioxidant activity of *G. glabra* were significantly influenced by the cultivation region ($P < 0.01$). A comparison of the averages revealed that the TPC in the Eghlid region was

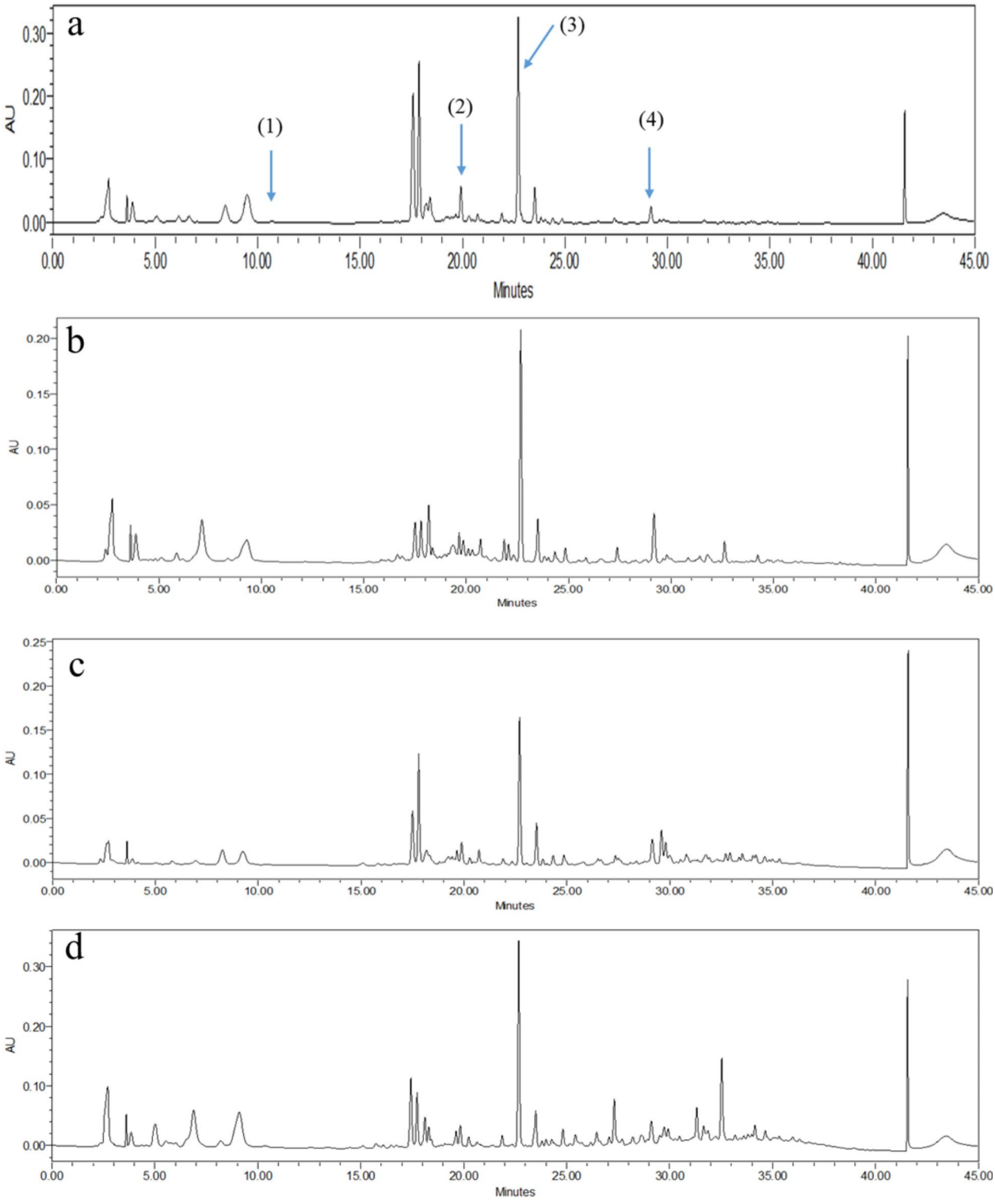

Fig. 2 The HPLC chromatogram of Rayen (**a**), Eghlid (**b**), Kalat (**c**), and Zanjan (**d**) Regions (liquiritigenin (**1**), liquiritin (**2**), glycyrrhizic acid (**3**) and glabridin (**4**))

higher at 14.83 mg GAE/g DW, and the TFC was also higher at 17.46 mg RE/g DW compared to other cultivation regions. In contrast, the Zanjan area exhibited the lowest levels, with a TPC of 6.72 mg GAE/g DW and a TFC of 9.79 mg RE/g DW (Fig. 3). The antioxidant capacity of licorice grown in different regions was assessed using the DPPH method. The comparison of samples from various regions showed that the IC_{50} values ranged from 35.25 to 59.39 µg/ml (Fig. 4).

Correlation between environmental and geographical factors with agro-morphological and phytochemical traits

The Pearson correlation coefficients are displayed in Fig. 5. Plant height showed a positive and significant correlation with several traits, including the number of branches, diameter of the main stem, and both fresh and dry weights of the shoot and roots, as well as root yield. Additionally, root yield was positively and significantly correlated with plant height, number of branches, diameter of the main stem, and both fresh and dry weights of the shoot and roots. On the other hand, the IC_{50} value was negatively and significantly correlated with TPC and TFC. This indicates that antioxidant activity had a positive and significant correlation with both TPC and TFC. Furthermore, liquiritin content demonstrated a negative and significant correlation with stem diameter, as well as fresh weights of the shoot and roots, while glabridin showed a negative and significant correlation with plant height.

PCA and HCA of agro-morphological and phytochemical characteristics

The PCA analysis results indicated that the first two components accounted for 82.67% of the total variance. The factor analysis successfully reduced the 17 traits under investigation to two main factors, which explained 61.00% and 21.67% of the total variance, respectively (Fig. 6a). Figure 6b illustrates the agro-morphological and phytochemical traits, highlighted with color scores; higher values of better quality traits are represented in pink, while lower values are shown in purple. Additionally, the dendrogram reveals the relationships between groups displayed in the columns and rows. The cultivation areas of Zanjan and Eghlid were clustered together, while Kalat and Reyen formed a separate cluster. Overall, the plants cultivated in Kalat and Reyen exhibited the highest values for fresh and dry root weight, fresh and dry shoot weight, diameter of the main stem, number of branches, root yield, glycyrrhizic acid, and glabridin. Conversely, the plants from the Zanjan and Eghlid regions demonstrated the highest levels of leaf length, leaf width, liquiritin, total phenol content, total flavonoid content, and antioxidant activity.

Discussion

The findings of this research indicated that the cultivation region and environmental conditions significantly influence the growth and performance of *G. glabra*. The highest fresh weight, dry weight, and root yield were recorded in the Rayen region. Several factors, including light, temperature, humidity, nutrition, water, altitude, soil composition, and geographical location, can impact the growth and functional characteristics of medicinal and aromatic plants in different ecological settings [28]. Each plant species has specific environmental requirements for optimal growth and development, which highlights the relationship and interaction between plants and their environments [29, 30]. While genetic factors influence the quality and quantity of bioactive compounds in medicinal plants, environmental factors play a crucial role as well [31]. Numerous reports indicate that the metabolic activities of medicinal plants vary under different environmental conditions-such as diverse cultivation areas or habitats-leading to variations in the rates and qualities of the chemical compounds produced by the cells [32, 33]. Studies have shown that environmental factors like temperature, light (both intensity and quality), humidity, and soil characteristics play significant roles in regulating gene expression in various horticultural crops [29, 34–36]. Moghtader et al. [32] reported that different populations of *Hyssopus officinalis*, influenced by varying ecological conditions, exhibited distinct amounts of secondary metabolites. Additionally, Ghorbani et al. [29] evaluated the phytochemical compounds of Iranian populations of *Ruscus hyrcanus* across different distribution areas. Their findings revealed that a range of intrinsic and extrinsic factors affects both the type and quantity of bioactive compounds in this medicinal plant. Specifically, extrinsic factors such as differing climatic and soil conditions influence metabolic pathways, gene expression, and enzymatic activities involved in plant growth and the biosynthesis of active substances. This leads to varying growth habits and the synthesis of diverse secondary metabolite profiles under different environmental conditions. A comparison of *G. glabra* samples gathered from various regions of Uzbekistan has revealed that the glycyrrhizic acid content ranges from 3.3 to 6.1%, while the glabridin content varies from 0.08 to 0.35% of dry weight [37]. Additionally, an examination of *G. glabra* root metabolites from different sources showed varying amounts of glycyrrhizic acid in samples from Italy, China, Turkey, and Iran, with concentrations of 51 mg/g DW, 53 mg/g DW, 33 mg/g DW, and 32 mg/g DW, respectively [38]. A study on *G. glabra* populations in different ecosystems of the central Zagros region in Iran also highlighted significant diversity in glycyrrhizin content, ranging from 0.03 to 0.23% [39].

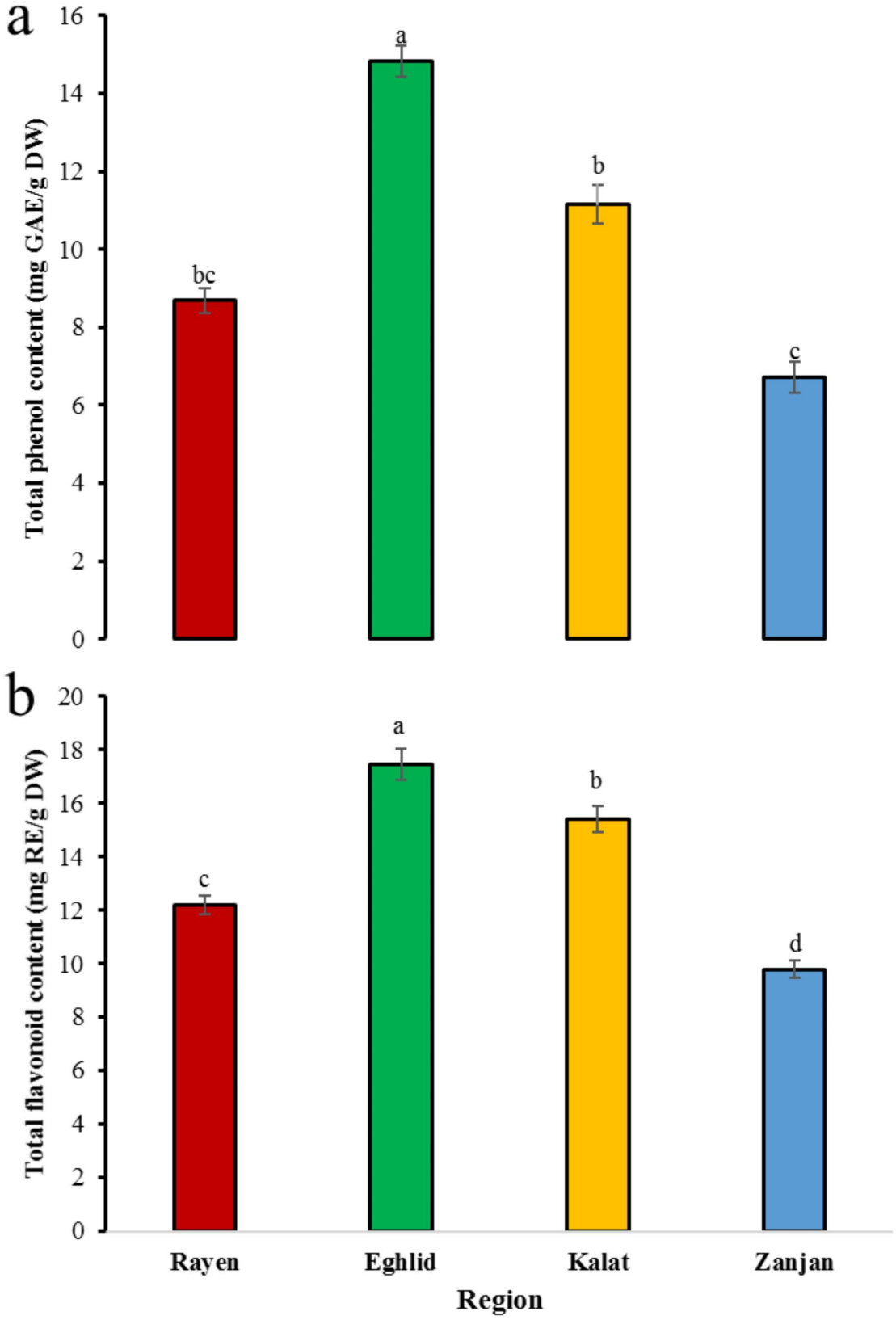

Fig. 3 Effect of cultivation regions on total phenol and flavonoid content (**b**) of *G. glabra*. Columns with at least one common letter are not significantly different at 5% probability level based on Duncan's test. The bars on each column indicate the standard error

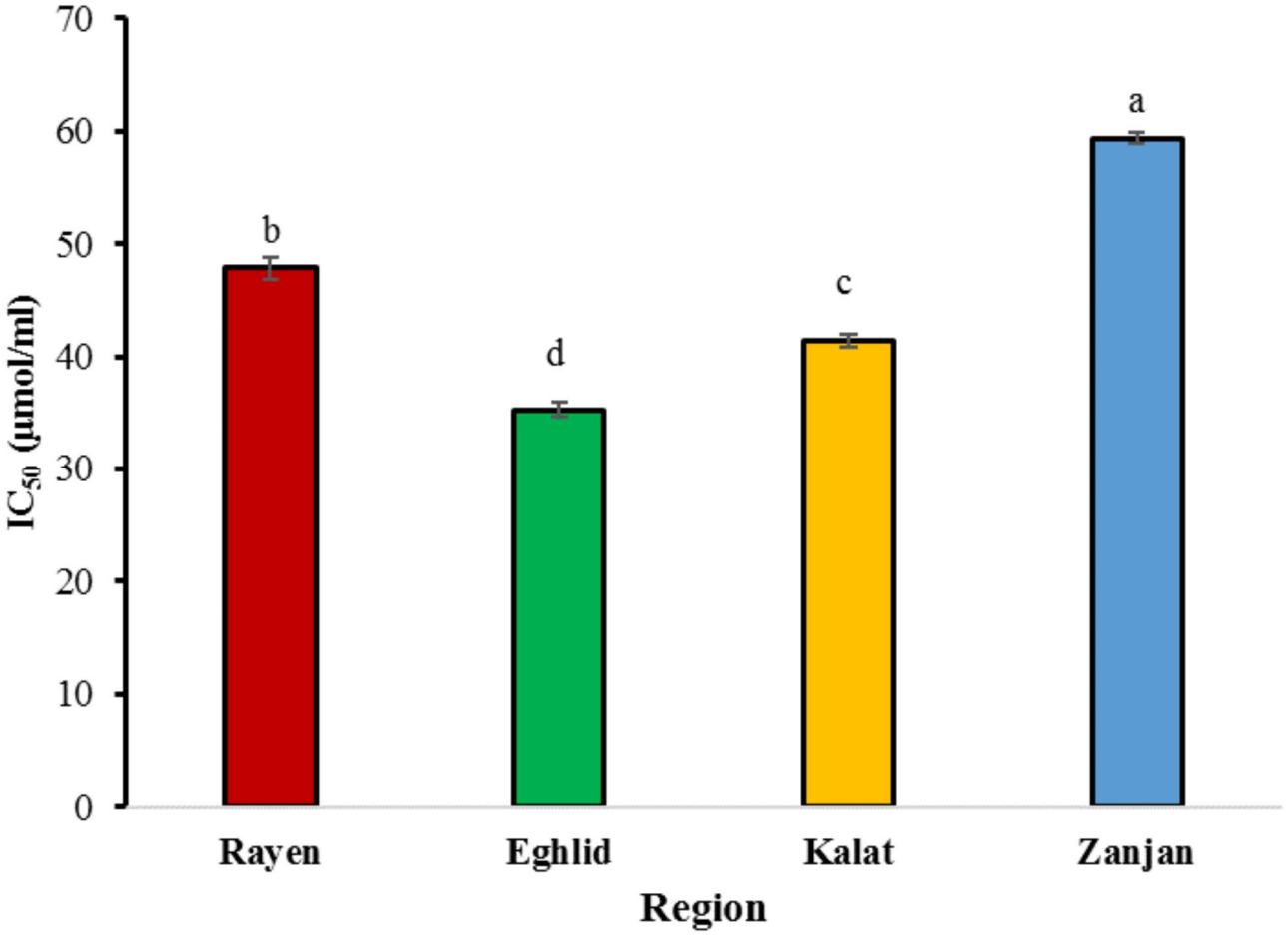

Fig. 4 Effect of cultivation regions on antioxidant activity of *G. glabra*. Columns with at least one common letter are not significantly different at 5% probability level based on Duncan's test. The bars on each column indicate the standard error

The variation in TPC and TFC among different samples of *G. glabra* can be attributed to factors such as climate, geographical conditions, and genetic differences [40]. Additionally, the type of solvent used, along with various environmental and climatic factors, significantly influences the quantity of phenolic and flavonoid compounds extracted from botanical sources [41]. Phenolic compounds are well-documented for their antimicrobial and antioxidant properties, playing a crucial role in plant defense mechanisms by protecting against infections caused by pathogens and harmful microorganisms. Furthermore, these compounds within plant tissues act as a barrier against the damaging effects of reactive oxygen species [42]. The differences in antioxidant capacity among samples grown in various regions can be linked to variations in their phenolic and flavonoid compound content [43].

The height of the plant is positively and significantly correlated with several traits, including the number of branches, the diameter of the main stem, and both the fresh and dry weights of the shoot and root, as well as root yield. Since the aerial parts of the plant are where photosynthesis occurs and where carbohydrates necessary for growth and development are produced, an increase in height and the number of lateral branches leads to enhanced photosynthesis and the generation of essential primary metabolites for both vegetative and reproductive growth. The results indicate that greater plant height and a higher number of lateral branches correlate with increased photosynthetic rates, larger stem diameters, and an increased number of branching stems from the base. These factors contribute to a greater capacity for photosynthesis, resulting in higher carbohydrate production. Carbohydrates are essential for the synthesis of secondary metabolites and phytoalexins in plants, which help reduce damage from pests and diseases, ultimately improving the yield [5]. Furthermore, root yield shows a positive and significant correlation with traits such as plant height, the number of branches, stem diameter, and both fresh and dry weights of the shoot and root. Since the desired metabolites in plants are primarily produced in the underground organs and roots,

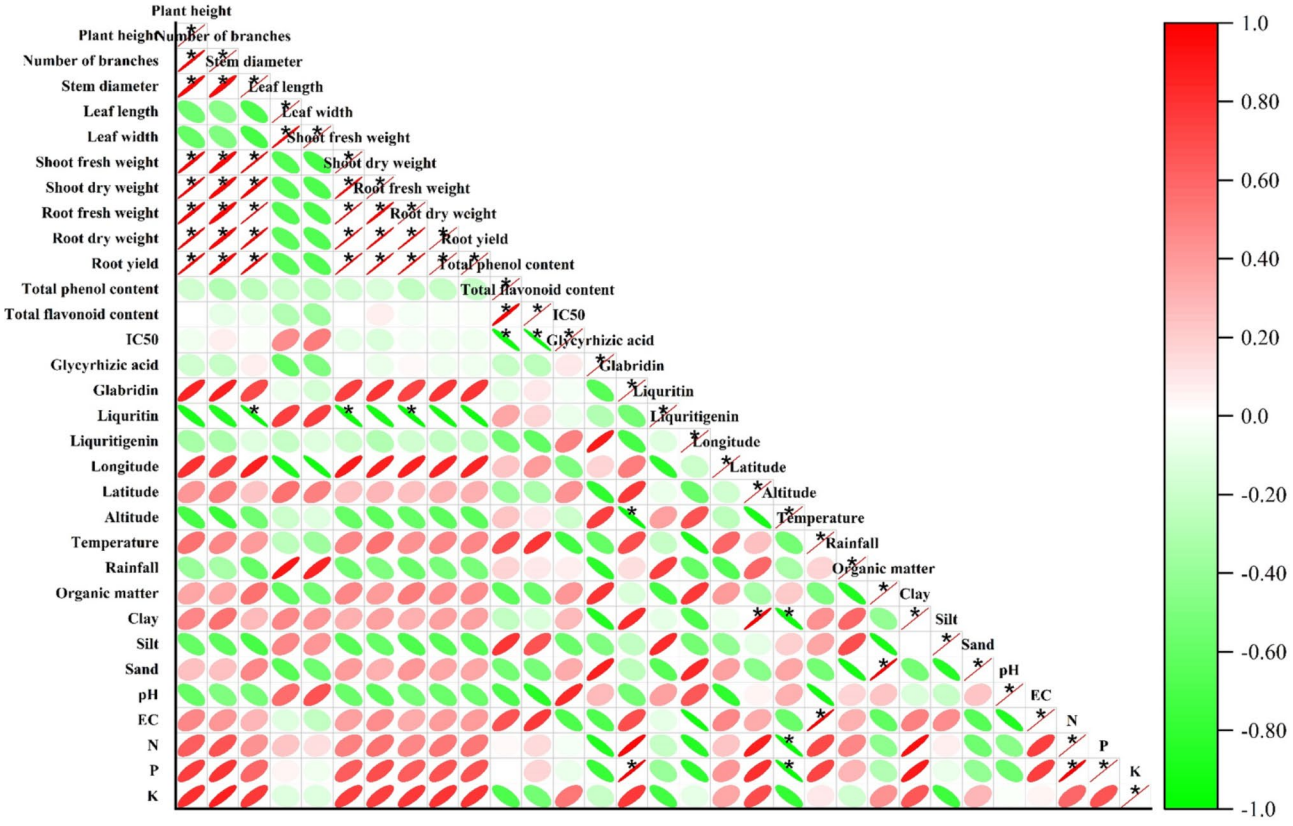

Fig. 5 Linear correlation between the climatic, soil, agro-morphological and phytochemical traits. Significant difference in 5% level

factors that enhance root performance are crucial for maximizing metabolite production. Therefore, traits that effectively increase root development can be considered as modifiers in this context. The IC_{50} values exhibited a negative and significant correlation with TPC and TFC. This indicates that antioxidant activity correlates positively and significantly with both TPC and TFC. Numerous studies have demonstrated that phenolic compounds possess exceptional antioxidant capacities [5, 6, 44]. Additionally, the content of liquiritin showed a negative and significant correlation with traits such as stem diameter, as well as shoot and root fresh weight. Conversely, glabridin was negatively correlated with plant height, consistent with the findings of Esmaeili et al. [6]. As the altitude of the cultivation area increased, the amount of glabridin decreased. Higher altitudes are associated with variations in temperature, solar radiation, humidity, water availability, and wind, all of which can influence the production and accumulation of secondary metabolites [28]. However, different species may respond uniquely to these environmental conditions due to variations in genetic composition and specific ecological factors [45]. To identify the optimal locations for plant production and understand the relationship between ecological factors and phytochemicals, it is essential to take environmental influences into account. In the current study, we observed a strong and significant positive correlation between environmental factors and the phytochemicals of *G. glabra*. Consistent with our findings, Xu et al. [46]. reported correlations between the amounts of metabolites and the immune activity of *Echinacea purpurea* with geographical factors such as latitude, longitude, climate, and soil. Similarly, Liu et al. [47] found a negative correlation between tannin and rutin levels and TPC in *Potentilla fruticosa* related to annual rainfall. Furthermore, Najjar et al. [48] documented an increase in the synthesis of total phenol, flavonoids, chlorogenic acid, caffeic acid, and rutin in nettle plants subjected to higher geographical conditions. Zhang et al. [49] noted that lower precipitation, humidity, and temperature, combined with longer sunlight exposure, resulted in elevated levels of tanshinone in *Salvia miltiorrhiza*.

Principal Component Analysis (PCA) is a multivariate statistical method used to identify important traits. The relative variance of each factor indicates its importance concerning the total variance of the traits being investigated, and this importance is expressed as a percentage. Another multivariate analysis, Hierarchical Clustering Analysis (HCA), was employed to visually assess the variation in agro-morphological traits and phytochemicals of the *G. glabra* plant. This was done using the Euclidean distance and the average linkage method.

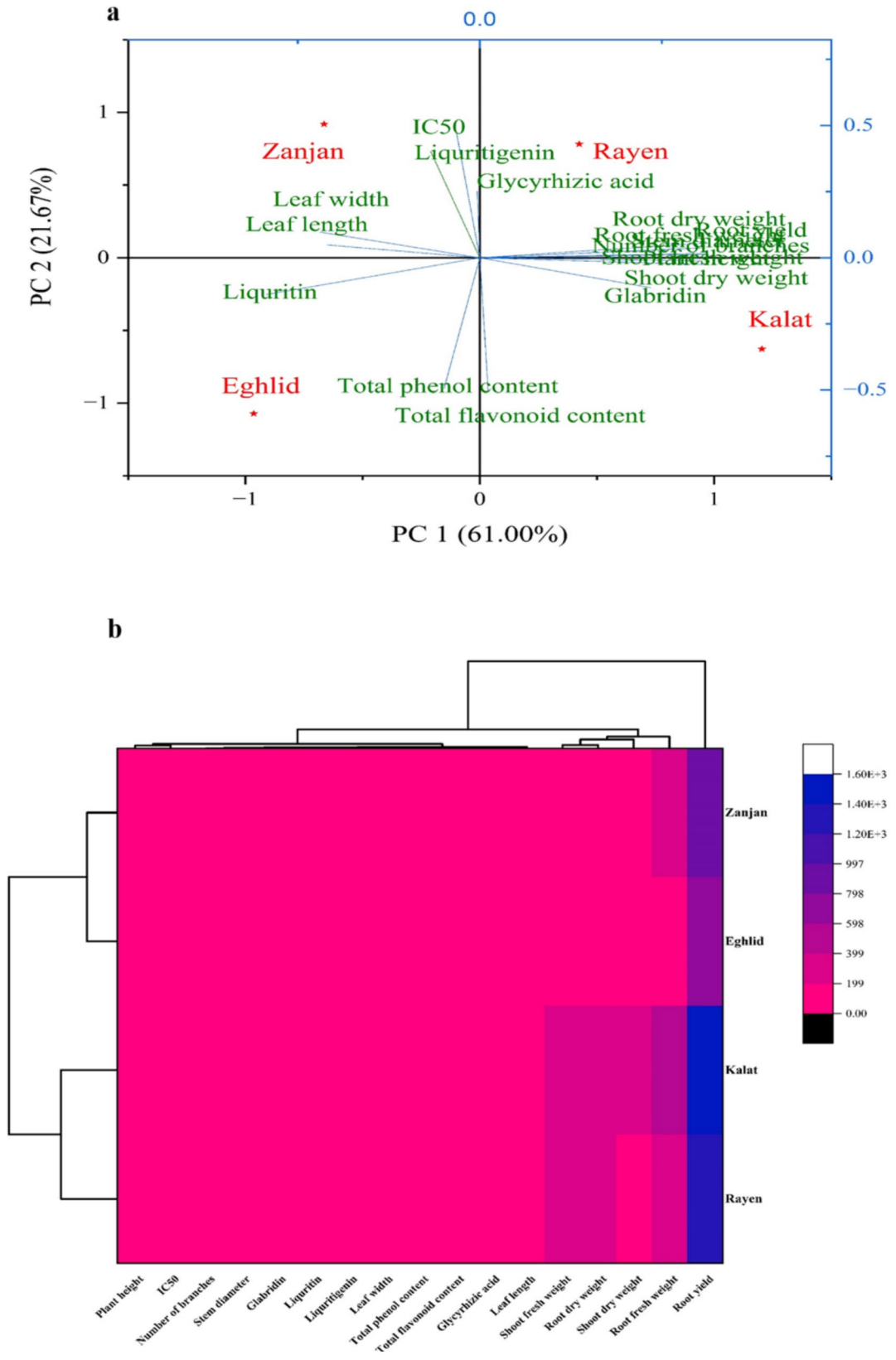

Fig. 6 Multivariate analysis of phytochemical compounds of *G. glabra* plants in four cultivation regions. Biplot of PCA (**a**), clustered heatmap (**b**)

Multivariate analyses, including PCA and heatmap clustering, can help identify the best areas for cultivating *G. glabra* plants and producing phytochemicals. Ben Arfa et al. [50] demonstrated that in rosemary, two chemotypes were identified based on the concentration of the main components in the essential oil, using multivariate analyses such as PCA and heatmaps. This information is valuable for selecting the appropriate season and harvest region for extracting high-potential essential oils from rosemary. Additionally, research on *Prunus scoparia* revealed two distinct clusters through heatmap analysis. Population 2 (Markazi) formed one cluster, while Population 1 (Lorestan) and Population 3 (Tehran) formed another. Notably, Population 2 exhibited superior traits, while Population 1 had a higher content and yield of essential oil [51].

Conclusions

In the current study, *Glycyrrhiza glabra* plants grown in the Kalat region exhibited the highest measurements in several factors: plant height, number of lateral branches, diameter of the main stem, fresh and dry weights of shoots, fresh and dry weights of roots, root yield, and glabridin content. The root yield and glabridin content in the Kalat region were 2.5 and 19.46 times higher, respectively, compared to those in the Eghlid region. Additionally, the highest levels of glycyrrhizic acid and liquiritin were found in the Rayen region, while the highest concentration of liquirtin was observed in the Zanjan region. The Eghlid region showed the highest TPC, TFC, and antioxidant activity. Among various environmental and geographical factors, altitude was found to have a negative and significant correlation with glabridin content, indicating that glabridin levels decreased as altitude increased. In summary, for the production of glycyrrhizic acid, glabridin, and phenolic compounds in the food and pharmaceutical industries, the Rayen, Kalat, and Eghlid regions are recommended, respectively.

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Author contributions
GE: methodology, farm work, conceptualization, supervision, data curation, data analysis and writing-original draft, YMT: lab work, analysis data; FA: reviewing, and editing; HA: methodology and editing; MH: methodology, conceptualization, data curation, reviewing, and editing.

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Data availability
The datasets used during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All methods performed in this study including the collection of plant materials were in compliance with the relevant institutional, national, and international guidelines and legislation.

Consent for publication
Not applicable.

Competing interests
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