**Journal of Medicinal Plants and By-products (2026) 02: 281– 289**

# Phytochemical Compounds, Antioxidant Activity and HPLC Polyphenols Profiling of *Echinops ritro* L. ecotypes

Bashir Mohammadi[1], Shahnaz Fathi[1]*, Shahla Shameh[1] and Abolfazl Alirezalu[2]

[1] *Department of Medicinal plant department, Shahid Bakeri Higher Education Center of Miandoab, Urmia University, Urmia, Iran*
[2] *Department of Horticultural Sciences, Faculty of Agriculture, Urmia University, Urmia, Iran*

**Article Info**

**Article Type**
Original Article

**Article History**
Received: 04 October 2025
Accepted: 22 October 2025


***Corresponding author**
sh.fathi@urmia.ac.ir

**ABSTRACT**

In this research, phytochemical compounds, antioxidant activity and polyphenolic compounds of 27 ecotypes of *Echinops ritro* were investigated. Total carotenoid and beta-carotene were evaluated as plant pigments. Total phenol, total flavonoid, antioxidant activity and total carbohydrate content were measured using Folin-Ciocalteu, aluminum chloride, anthrone and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods, respectively. Hierarchical cluster analysis (HCA) and Principal component analysis (PCA) were done among the traits using Minitab software. R software based on Pearson's method was used for correlation between data. The highest levels of total carotenoid content (16.17 µg/g FW) and beta-carotene (0.024 mg/g FW) were obtained in G19. The value of total phenol, total flavonoid, antioxidant activity and total carbohydrate content were ranged between 5.42-22.60 mg GAE/g FW, 1.56-4.21 mg QUE/g FW, 2.30-16.52 mg AA/g FW and 1.76-6.66 mg/g FW, respectively. *p*-coumaric acid (538.29 µg/g FW) were revealed as major phenolic compounds of *Echinops* ecotypes, being an order of magnitude higher than the others. Based on HCA and PCA, twenty-seven of *Echinops* ecotypes were divided into four main groups. Quantitative phenolic compounds were detected by high-performance liquid chromatography. The findings showed that G19, G25, G12, G4, G15 and G4 of *Echinops* ecotypes can be considered as an excellent source of total carotenoid content and beta-carotene, total phenol content, total flavonoid content, antioxidant activity, total carbohydrate content and *p*-coumaric acid, respectively. Therefore, the information from this research can be useful for introducing valuable *Echinops* ecotypes for use in the food and pharmaceutical industries as well as breeding projects.

**Keywords**: *Echinops ritro*; phytochemical, Antioxidant activity, High-performance liquid chromatography

**Abbreviations:** $\beta$-carotene, beta-carotene; TCC, total carotenoid content; Chla, Chlorophyll a; Chlb, Chlorophyll b; TPC, total phenolic content; A, absorbance; A1, the absorbance of sample; AA, ascorbic acid; TCH, total carbohydrate content; HPLC, high-performance liquid chromatography; A0, the absorbance of control; FW, fresh weight; DW, dry weight

**How to cite this paper**
Mohammadi, B., Fathi, Sh., Shameh, SH., Alirezalu, A. Phytochemical Compounds, Antioxidant Activity and HPLC Polyphenols Profiling of *Echinops ritro* L. ecotypes. Journal of Medicinal Plants and By-products, 2026; 15(2): 281-289. doi: 10.22034/jmpb.2025.370803.2058

## INTRODUCTION

The *Echinops* genus commonly known as globe thistles belongs to Asteraceae family, which has approximately 120 species [1]. The geographical distribution of this genus has been reported in the tropical regions of Africa, Asia and the Mediterranean [2]. According to Flora Iranica, 54 species of *Echinops* have been found in Iran, of which 35 species are endemic to Iran [3], Therefore, Iran is considered as one of the most important centers of *Echinops* diversity [4]. The general reasons for this diversity include the existence of 11 out of 13 climates in the world and the diversity of plant species in Iran [5]. Thiophenes, terpenoids, phenolic compounds, flavonoids, alkaloids as well as essential oils and lipids are important bioactive compounds detected in *Echinops* plants [6-13]. According to bioactive compounds, *Echinops* species have been traditionally used in the treatment of various diseases, including the treatment of skin diseases, cold, liver diseases, hydrocele, kidney stones, eye diseases, cough, food poisoning, antipyretic and analgesic [6,7]. Many studies have been conducted on the identification of phytochemical compounds of *Echinops* spp., for example, in a research, echinopsine in *E. echinatus*, *E. nanus*, *E. albicaulis*, *E. orientalis*, echinozolinone in *E. echinatus*, echinopsidine in *E. echinatus*, 7-hydroxyechinozolinone in *E. echinatus* and 1-methyl-4- quinolone in *E. heterophyllus* were detected as alkaloid compounds in different organs of *Echinops* spp. [6]. In another study, apigenin and rutin were reported as major flavonoid compounds in *E. albicaulis* and *E. heterophyllus*, respectively [10,14]. Dong et al. found that chlorogenic acid was the essential compound in *E. grijisii* [15]. However, similar comprehensive phytochemical profiling for *E. ritro* remains limited. Therefore, the aim of the present study was to investigate phytochemical traits (including measurement of plant pigments such as total carotenoids and beta-carotene, total phenols, total flavonoids, and soluble carbohydrate content), evaluate the antioxidant activity of plant extracts using by DPPH method, and identify polyphenolic compounds of *E. ritro* ecotypes in northwest Iran using HPLC compared to other regions for the first time.

## MATERIALS AND METHODS

### Plant Material

The aerial parts of twenty-seven *E. ritro* ecotypes were collected from two provinces of Iran (West Azerbaijan and Kurdistan) in August 2022 (Table 1). Every sample was collected during the full flowering stage for 30 days. After cleaning, the plant samples were kept frozen until the factors were measured. Figure 1 shows the 27 ecotypes of *E. ritro* at the collection location. Adel Jalili and Ziba Jamzad's "Red Data Book of Iran," a preliminary assessment of endemic, uncommon, and endangered plant species in Iran, states that *E. ritro* is not a threatened species and is classified as a stable species by the IUCN. Nevertheless, the Iranian Natural Resources Organization granted the required licenses and permits for the collection of this species with least concern for harvesting. According to the authors, the collection of *E. ritro* ecotypes was conducted in accordance with institutional, national, and international regulations and recommendations.

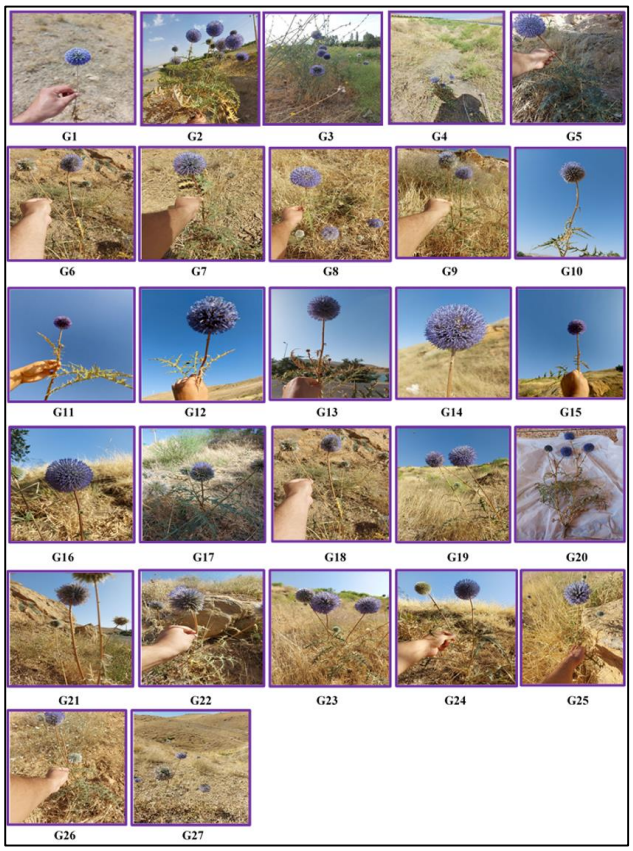


**Fig. 1** Picture of different *E. ritro* ecotypes.

### Measurement of Total Carotenoid Content and beta-carotene

In the first step, 1 g of each plant sample was powdered with a Chinese mortar and then a homogeneous mixture was prepared from it with 10 ml of acetone. In the second step, the obtained mixture was centrifuged (ROTINA 380/380R, Hettich, Germany) at 10,000 rpm for 5 minutes. In the next step, the separated upper phase of each sample was poured into the cuvette. Finally, the absorbance of the samples was read by a spectrophotometer (Dynamica Halo XB-10, England) at 470, 479, 645 and 663 nm. Acetone was used as control. The amount of carotenoid [16] and beta-carotene [33] was calculated using the following formulas:

Chla = $(11.75A_{662} - 2.350A_{645})$

Chlb = $(18.61A_{645} - 3.960A_{662})$

TCC = $(1000A_{470} - 2.270\text{Chla} - 81.4\text{Chlb}/227)$

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

### Preparation of Methanolic Extract

Methanolic extraction was performed in an ultrasonic (Elmasonic, Singen, 120 Hz power, Germany) in methanolic solvent. For this purpose, 1 g of each plant sample was mixed with 10 ml of methanol (80% v/v) and transferred into the falcons. The duration of ultrasonic extraction was 30 minutes at 30 °C [33].

### Measurement of Total Phenolic Content

The phenol content (TPC) was measured using Folin–Ciocalteu reagent [17]. 180 µl of distilled water and 1200 µl of Folin (10%) were added to 50 µl of the methanolic extract (80% v/v) of each sample. 960 µL of sodium carbonate (7%) was added to them after 5 minutes. Then the samples were transferred for 30 minutes in the dark at 25 °C. The absorbance of the samples was measured by a spectrophotometer (Dynamica Halo XB-10, England) at 760 nanometers. Gallic acid was used as a standard to draw the calibration curve (Fig. 2). The TPC was presented based on the milligrams of gallic acid (mg GAE) per gram of fresh weight (g FW).

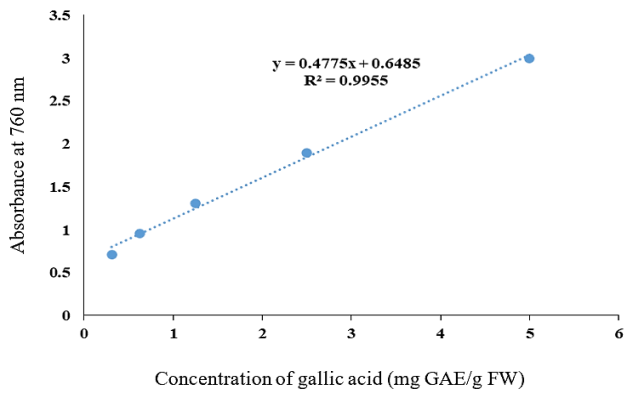


**Fig. 2** Standard curve for determination of TPC

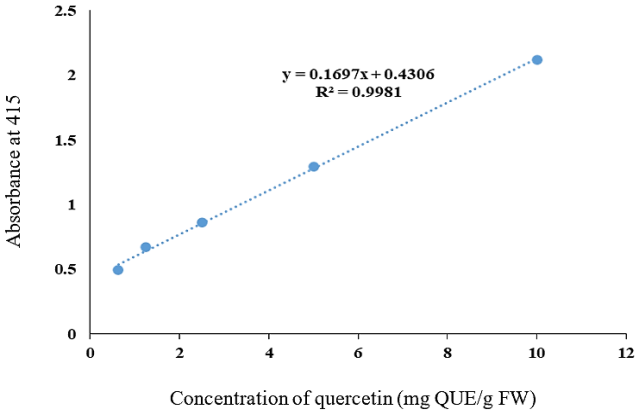


**Fig. 3** Standard curve for determination of TFC

### Measurement of Total Flavonoid Content

Total flavonoid content (TFC) was measured using aluminum chloride reagent [18]. The reaction components for each sample in the tube included 100 µl of methanolic extract (80% v/v), 500 µl of aluminum chloride (10%), 500 µl of potassium acetate (1 M), and 1400 µl of distilled water. After storing the samples for 30 minutes in the dark at 25 °C, the absorbance of the samples was read by a spectrophotometer at 415 nm. Quercetin was used as a standard to draw the calibration curve (Fig. 3). The TFC was recorded based on milligrams of quercetin (mg QUE) per gram of fresh weight (g FW).

**Table 1** Characteristics and data of the study areas of *E. ritro* ecotypes

| Code | Voucher of samples | Province | Locality | Longitude | Latitude | Height (m) |
|---|---|---|---|---|---|---|
| G1 | 1510 | West Azerbaijan | Mahabad | 45° 44´ 30.41" | 36° 48´ 46.70" | 1360 |
| G2 | 1511 | West Azerbaijan | Mahabad | 45° 41' 38.49" | 36° 45´ 21.23" | 1416 |
| G3 | 1512 | West Azerbaijan | Mahabad | 45° 40' 10.46" | 36° 41' 0.16" | 1483 |
| G4 | 1513 | West Azerbaijan | Oshnavieh | 45° 7' 52.98" | 37° 2' 12.27" | 1436 |
| G5 | 1514 | West Azerbaijan | Oshnavieh | 45° 7' 22.39" | 37° 3' 45.68" | 1440 |
| G6 | 1515 | West Azerbaijan | Oshnavieh | 45° 3' 52.96" | 37° 0' 4.18" | 1513 |
| G7 | 1516 | West Azerbaijan | Naqadeh | 45° 21' 27.84" | 36° 59' 36.29" | 1316 |
| G8 | 1517 | West Azerbaijan | Naqadeh | 45° 22' 5.23" | 36° 56' 49.39" | 1326 |
| G9 | 1518 | West Azerbaijan | Naqadeh | 45° 24' 29.05" | 36° 56' 22.33" | 1305 |
| G10 | 1519 | West Azerbaijan | Piranshahr | 45° 9' 20.31" | 36° 38' 9.56" | 1430 |
| G11 | 1520 | West Azerbaijan | Piranshahr | 45° 8' 10.26" | 36° 43' 30.09" | 1491 |
| G12 | 1521 | West Azerbaijan | Piranshahr | 45° 9' 35.05" | 36° 41' 16.31" | 1420 |
| G13 | 1522 | West Azerbaijan | Sardasht | 45° 24' 29.35" | 36° 9' 46.48" | 1574 |
| G14 | 1523 | West Azerbaijan | Sardasht | 45° 33' 0.81" | 36° 12' 17.11" | 1134 |
| G15 | 1524 | West Azerbaijan | Sardasht | 45° 28' 39.16" | 36° 9' 9.52" | 1529 |
| G16 | 1525 | Kurdistan | Baneh | 45° 48' 25.33" | 36° 1' 13.81" | 1429 |
| G17 | 1526 | Kurdistan | Baneh | 45° 55' 12.12" | 35° 59' 30.58" | 1647 |
| G18 | 1527 | Kurdistan | Baneh | 45° 52' 5.24" | 35° 59' 21. 84" | 1511 |
| G19 | 1528 | Kurdistan | Mariwan | 46° 14' 7.17" | 35° 32' 22.35" | 1376 |
| G20 | 1529 | Kurdistan | Mariwan | 45° 6' 7.24" | 35° 34' 33.64" | 1313 |
| G21 | 1530 | Kurdistan | Mariwan | 46° 9' 28.39" | 35° 31' 35.66" | 1304 |
| G22 | 1531 | Kurdistan | Divandarreh | 47° 3' 28.15" | 35° 53' 28.10" | 1792 |
| G23 | 1532 | Kurdistan | Divandarreh | 47° 1' 17.70" | 35° 45' 37.42" | 1827 |
| G24 | 1533 | Kurdistan | Divandarreh | 47° 1' 21.92" | 35° 65' 14.38" | 1941 |
| G25 | 1534 | Kurdistan | Saqez | 46° 15' 21.51" | 36° 14' 0.51" | 1483 |
| G26 | 1535 | Kurdistan | Saqez | 46° 13' 18.18" | 36° 12' 15.96" | 1547 |
| G27 | 1536 | Kurdistan | Saqez | 46° 12' 53.09" | 36° 22' 34.21" | 1544 |

### Antioxidant Activity

To evaluate the antioxidant activity, 2 ml of freshly prepared 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution with 0.004 % of inhibitory concentration was homogenized with 50 µl of methanol extract of each sample. After 15 minutes, the absorbance of the samples was read by a spectrophotometer at 517 nm. Ascorbic acid was used as a positive control. Antioxidant activity was reported based on milligrams of ascorbic acid (mg AA) per gram of fresh weight (g FW). The inhibition percentage of DPPH by antioxidant compound was calculated according to the following formula [19].

DPPH inhibition (%) = [A0-A1/A0] ×100

where A0 is the absorbance of control, A1 is the he absorbance of sample.

### Total Carbohydrate Content

Ethanol extract was used to measure total carbohydrate content (TCH). For this purpose, in the first step, 1 g of plant matter was extracted with 5 ml of ethanol (96% v/v). After filtering and transferring the supernatant to a separate tube, 5 ml of ethanol (70% v/v) was added to residual plant material in the tube. In the second step, all the solution was centrifuged at 4000 rpm for 10 minutes (ROTINA 380/380R, Hettich, Germany). The reaction components in the final step included 50 µl of the ethanolic extract of each sample and 3 ml of the prepared anthrone reagent (150 mg in 100 ml of H2SO4) inside the tubes. Then the tubes were transferred to Bain-Marie at 100 °C for 10 minutes. After cooling, the absorbance of the sample was read at 625 nm. Glucose was used as a standard to draw the calibration curve [20].

### Extraction and Analysis of Phenolic Compounds by HPLC

Quantities of phenolic chemicals were isolated, identified, and determined using the samples' methanol extract. The extracts were filtered by a 25 mm Nylon syringe filter 25mm before being injected into the HPLC. The Agilent 1100 (HPLC) was used to analyze nine phenolic compounds, including gallic acid, caffeic acid, chlorogenic acid, rutin, *p*-coumaric acid, rosmaric acid, quercetin, cinnamic acid, and apigenin. Its features included a 20µL injection loop, a degasser, a diode-array detector (HPLC-DAD) which was adjusted at 250, 272, and 310 nm, a four-solvent gradient pump, an Octadecylsilane column, and Chemstation software for data processing. To appropriate isolate the compounds, the elution process was applied as follow: the mobile phase initially at a rate of 10% acetonitrile and 90% acetic acid (1% solution) at a flow rate of 1 ml/min was performed, then, in five minutes reached 25% acetonitrile and 75% acetic acid at a flow rate of 1 ml/min. Finally, in 10 min, 65% acetonitrile and 35% acetic acid at the same flow rate was achieved. 15 minutes was the isolation time [21]. Micrograms of phenolic compounds per gram of fresh weight (µg/g FW) were used for recording.

### Statistical Analysis

Analysis of all factors were performed in triplicate with an experiment in CRD (completely randomized design). SPSS software was used for data analysis. The least significant difference (LSD) test was used for means comparison and determination of significance level ($P < 0.05$). HCA (Hierarchical cluster analysis) and PCA (Principal component analysis) were done among the traits using Minitab software. Also, R software based on Pearson's method was used for correlation between data.

## RESULTS

### Total Carotenoid Content and beta-carotene

Comparing the average data of total carotenoid and beta-carotene content indicates a significant difference of these compounds in 27 ecotypes ($P < 0.05$). As Figure 4.A shows, G19 (16.17 µg/g FW) and G17 (6.17 µg/g FW) have the highest and lowest amount of total carotenoid content, respectively. The results of beta-carotene content measurement showed that G19 (0.024 mg/g FW) had the maximum as well as G17 (0.009 mg/g FW) had the minimum amount of beta-carotene content (Fig. 4.B).

### Total Phenol Content

The quantitative data analysis revealed that the level of TPC ranged between 5.42 to 22.60 mg GAE/g FW (Fig. 5). TPC was found significant ($P \leq 0.05$) among ecotypes. The maximum and

minimum values of TPC were recorded in G25 and G13, respectively.

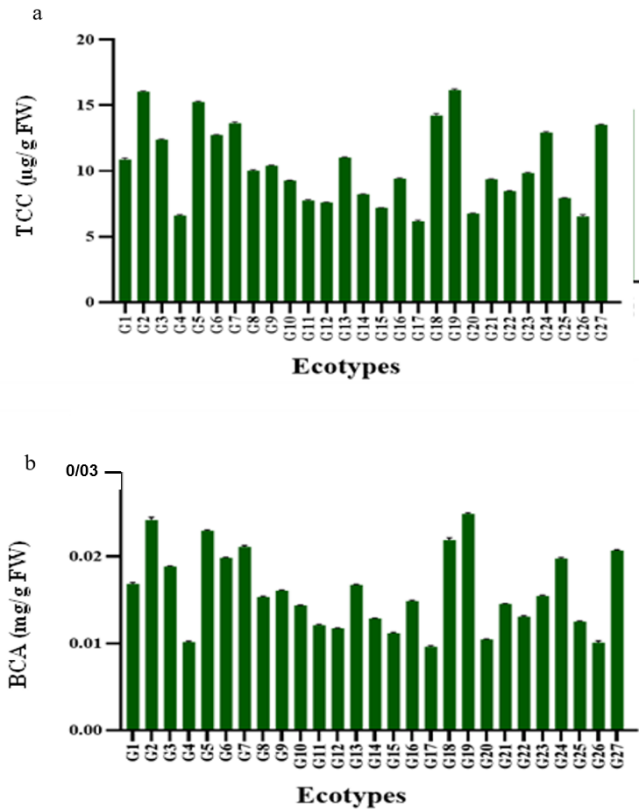


**Fig. 4** (a) Total carotenoid content and (b) beta-carotene content of *E. ritro* ecotypes.

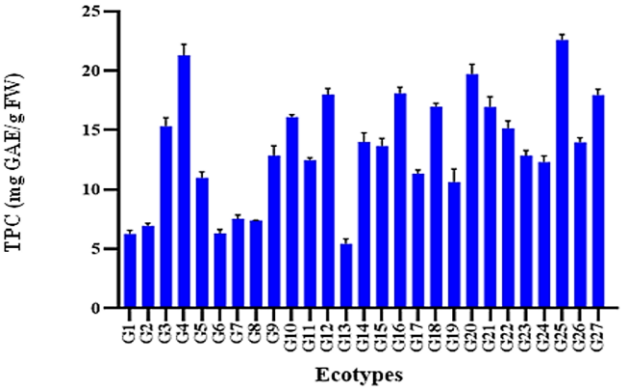


**Fig. 5** Total phenol content of *E. ritro* ecotypes.

## Total flavonoid Content

The TFC is displayed in Figure 6. According to the TFC analysis results in this research, there is a significant variation ($P < 0.05$) in this compound amongst the 27 *Echinops* ecotypes. The range of TFC varied between 1.56 and 4.21 mg QUE/g FW. The maximum and minimum content of TFC was found in G12 and G19, respectively.

## Antioxidant Activity

Figure 7 shows the inhibition rate of DPPH free radical in methanolic extracts of 27 *Echinops* ecotypes. As the results show, different ecotypes show various antioxidant ability. Among them, G4 (16.52 mg AA/g FW) and G13 (2.30 mg AA/g FW) had the highest and lowest DPPH radical inhibition levels, respectively. As among the 27 studied *Echinops* ecotypes, almost the lowest TPC and TFC were reported in G13, in the same ecotype, the minimum antioxidant activity was obtained. These findings show that there is a very strong relationship between bioactive compounds and antioxidant activity.

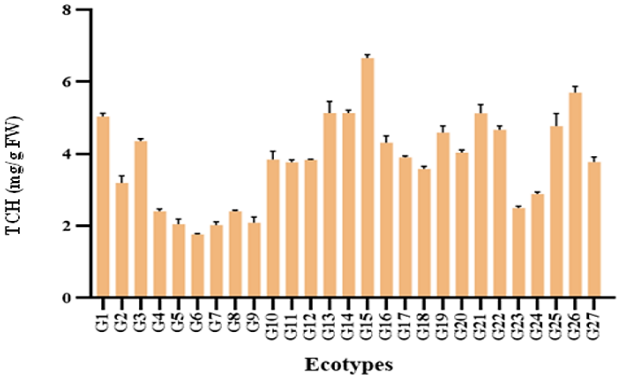


**Fig. 6** Total flavonoid content of *E. ritro* ecotypes.

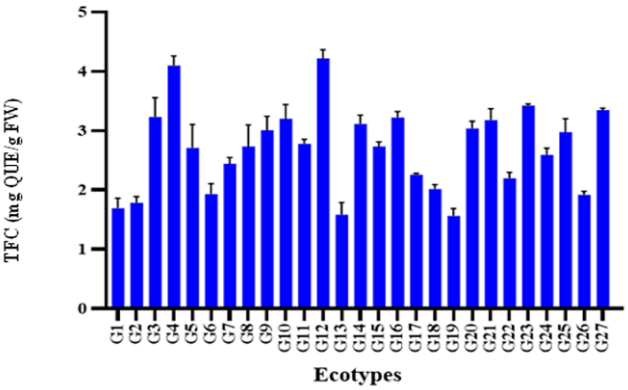


**Fig. 7** Antioxidant activity of *E. ritro* ecotypes by DPPH assay.

## Total Carbohydrate Content

The results of data analysis presented that there is a significant difference in the level of TCH of 27 studied ecotypes. The amount of TCH ranged between 1.76 and 6.66 mg/g FW. The maximum and minimum of TCH were obtained in G15 and G6, respectively (Fig. 8).

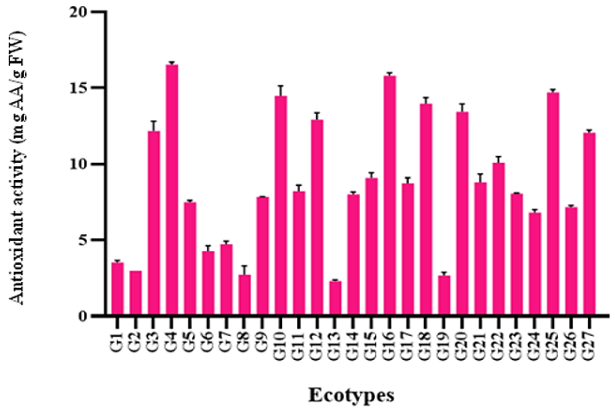


**Fig. 8** Total Carbohydrate content of *E. ritro* ecotypes.

## Phenolic Compounds Analysis

In this research, the measurement of nine phenolic compounds including gallic acid, caffeic acid, chlorogenic acid, rutin, *p*-coumaric acid, rosmaric acid, quercetin, cinnamic acid and

apigenin in the methanolic extract of *E. ritro* ecotypes were performed by HPLC. The results showed that there is a significant difference between the ecotypes in terms of the quantity of phenolic compounds ($P < 0.01$). The chromatogram of G4 is presented in Figure 9. This figure shows the detection of different phenolic compounds at different times in G4 (Fig. 9). Rutin, apigenin and *p*-coumaric acid were detected as main compounds in *E. ritro* extracts that *p*-coumaric acid was the most abundant compound overall. The concentration of evaluated phenolic compounds is reported in Table 2. The results demonstrated that the level of phenolic compound varied from 0.22-7.40, 0.20-3.02, 0.78-10.88, 0.12-28.31, 0.59-538.29, 0.10-4.72, 0.20-5.29, 0.10-8.70 and 0.51-17.09 µg/g FW for gallic acid, caffeic acid, chlorogenic acid, rutin, *p*-coumaric acid, rosmaric acid, quercetin, cinnamic acid and apigenin, respectively. Maximum value of gallic acid, caffeic acid, chlorogenic acid, rutin, *p*-coumaric acid, rosmaric acid, quercetin, cinnamic acid and apigenin had appeared in G15, G26, G15, G27, G4, G21, G14, G1 and G20 samples, respectively (Table 2).

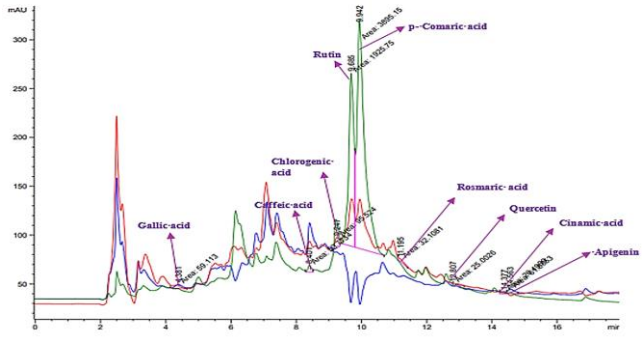


**Fig. 9** Chromatogram of phenolic compounds in G4.

## Hierarchical Clustering and Principal Component Analysis

Hierarchical clustering analysis (HCA) and principal component analysis (PCA) are two statistical techniques whose purpose is to classify similar objects and highlight the similarities between variables, respectively [58,59]. In this work, Ward's linkage method was used for cluster analysis (Fig. 10A). Four classes are observed in the output dendrogram. The main classification was performed based on the antioxidant activity, phytochemical and phenolic compounds of *Echinops* ecotypes. In the first class, G27 and G4 have the highest amount of rutin and coumaric acid. In the second class, G15, G14, G11, G9, G17, and G5 are similar in terms of quantity of most phytochemical and phenolic compounds as well as antioxidant activity. In the third class, G20, G12, G10, G16, G18, and G3 contain the highest antioxidant activity and total phenol content. In the fourth class, there are a large number of ecotypes studied (i.e., G25, G22, G13, G7, G26, G23, G8, G6, G24, and G19) that have low values of cinnamic acid, rutin, apigenin, chlorogenic and coumaric acid. PCA grouping is consistent with the results of cluster analysis (Fig. 10B). The first and second components covered 33 and 17% of the total variance, respectively (50% in total). The first component has a strong correlation with gallic acid, antioxidant activity, TPC, and TFC. The second component performs the separation of ecotypes based on coumaric acid, beta-carotene and carotenoids.

## Correlation Analysis

Figure 11 shows the correlation results between phytochemical traits and phenolic compounds in 27 *Echinops* ecotypes. Blue and red colors express positive and negative correlation, respectively. Correlation coefficients are proportional to the square size and color intensity (Dark red, weak correlation; Dark blue, strong correlation). Based on the results, the strongest correlation was found between rutin, *p*-coumaric acid, total phenol, and flavonoid content with antioxidant activity, followed by rosmaric acid and quercetin, $\beta$-carotene and total carotenoid content, and apigenin and cinnamic acid (Fig. 11). Also in this figure, the relationship between TCH and other parameters appears weak.

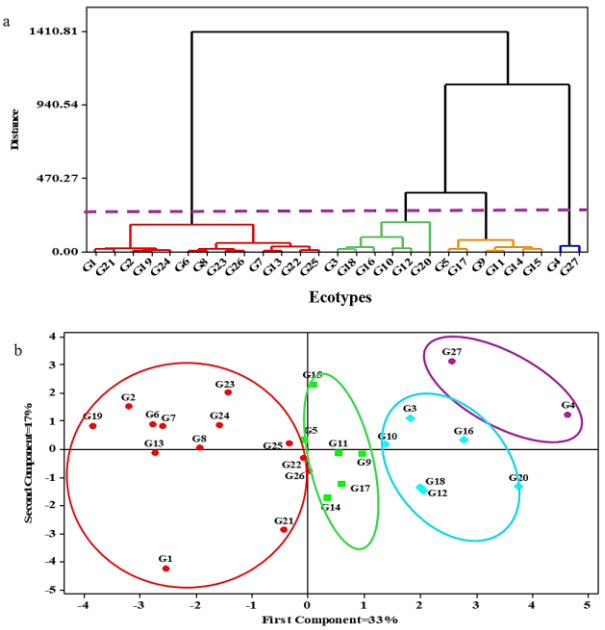


**Fig. 10** (a) Hierarchical cluster analysis (HCA) and (b) principal component analysis (PCA) of *E. ritro* ecotypes based on phytochemical composition, antioxidant activity and phenolic compounds data.

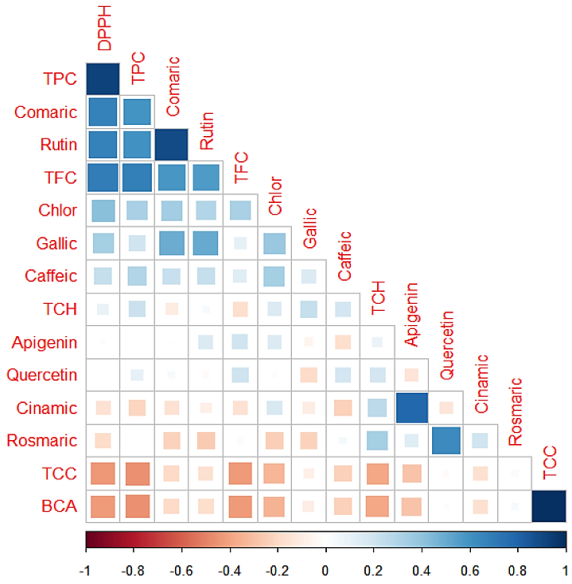


**Fig. 11** Correlation between phytochemical composition, antioxidant activity, and phenolic compounds of *E. ritro* ecotypes: BCA: $\beta$-carotene content, TCC: Total carotenoid content, TPC: Total phenolic content, TFC: Total flavonoid content, DPPH: Antioxidant activity by DPPH assay, TCH: Total carbohydrate content, Gallic: gallic acid, Caffeic: caffeic acid, Chlor: chlorogenic acid, Comaric: *p*-coumaric acid, Rosmaric: rosmaric acid, and Cinamic: cinnamic acid.

**Table 2** Phenolic compounds in aerial parts of *E. ritro* ecotypes (µg/g FW)

| Code | Gallic acid | Caffeic acid | Chlorogenic acid | Rutin | *p*-Coumaric acid | Rosmarinic acid | Quercetin | Cinnamic acid | Apigenin |
|---|---|---|---|---|---|---|---|---|---|
| G1 | 0.30 u ± 0.02 | 0.40 lmno ± 0.02 | 5.89 i ± 0.02 | 0.20 v ± 0.02 | 5.30 v ± 0.05 | 1.51 d ± 0.06 | 0.50 i ± 0.03 | 8.70 a ± 0.07 | 16.83 b ± 0.02 |
| G2 | 5.40 c ± 0.04 | 1.21 fgh ± 0.03 | 1.19 t ± 0.02 | 0.19 v ± 0.02 | 4.01 w ± 0.07 | 1.20 f ± 0.03 | 0.49 ij ± 0.03 | 0.40 l ± 0.03 | 3.81 i ± 0.03 |
| G3 | 3.30 I ± 0.01 | 1.89 bcd ± 0.02 | 8.49 c ± 0.03 | 16.20 c ± 0.01 | 228.26 d ± 0.04 | 0.50 h± 0.02 | 2.90 b ± 0.02 | 0.29 m ± 0.02 | 3.39 j ± 0.02 |
| G4 | 3.90 f ± 0.04 | 1.19 fgh ± 0.02 | 8.05 d ± 0.06 | 15.79 d ± 0.04 | 538.29 a ± 0.08 | 0.50 h ± 0.02 | 1.40 d ± 0.01 | 0.20 n ± 0.02 | 2.60 m ± 0.03 |
| G5 | 0.80 q ± 0.02 | 1.49 def ± 0.01 | 6.01 h ± 0.03 | 4.30 l ± 0.02 | 95.49 l ± 0.03 | 0.22 k ± 0.01 | 0.50 i ± 0.03 | 0.20 n ± 0.01 | 2.70 l ± 0.03 |
| G6 | 0.80 q ± 0.04 | 0.61 jklmn ± 0.04 | 3.40 n ± 0.02 | 2.39 p ± 0.02 | 49.89 q ± 0.03 | 0.30 j ± 0.03 | 0.79 f ± 0.02 | 1.40 e ± 0.02 | 3.20 k ± 0.04 |
| G7 | 0.32 u ± 0.03 | 0.90 hijk ± 0.02 | 2.30 s ± 0.02 | 2.19 q ± 0.01 | 39.20 r ± 0.05 | 0.31 j ± 0.03 | 1.02 e ± 0.03 | 1.30 f ± 0.02 | 5.71 g ± 0.03 |
| G8 | 1.00 p ± 0.02 | 0.20 no ± 0.03 | 2.30 s ± 0.02 | 2.69 o ± 0.02 | 52.00 p ± 0.01 | 0.20 k ± 0.03 | 0.30 l ± 0.05 | 0.40 l ± 0.03 | 7.62 e ± 0.04 |
| G9 | 0.40 t ± 0.03 | 0.92 hij ± 0.01 | 6.89 g ± 0.01 | 2.21 q ± 0.03 | 119.20 k ± 0.03 | 0.30 j ± 0.03 | 0.70 g ± 0.05 | 1.21g± 0.04 | 6.10 f ± 0.02 |
| G10 | 2.06 l ± 0.07 | 0.80 hijkl ± 0.02 | 3.89 k ± 0.03 | 11.39 g ± 0.03 | 176.10 g ± 0.03 | 0.59 g ± 0.02 | 0.22 m ± 0.01 | 1.30 f ± 0.03 | 5.02 h ± 0.03 |
| G11 | 2.69 k ± 0.03 | 1.69 cde ± 0.03 | 4.02 j ± 0.03 | 7.52 j ± 0.02 | 119.80 k ± 0.02 | 0.30 j ± 0.02 | 0.20 m ± 0.02 | 1.02 I ± 0.04 | 3.29 k ± 0.04 |
| G12 | 0.59 r ± 0.03 | ND | 3.02 p ± 0.03 | 15.10 e ± 0.01 | 154.70 h ± 0.02 | 0.40 I ± 0.01 | 0.69 g ± 0.03 | 2.19 d ± 0.02 | 14.40 c ± 0.02 |
| G13 | 3.02 j ± 0.02 | 0.49 klmn ± 0.03 | 0.78 v ± 0.02 | 1.19 s ± 0.03 | 38.60 r ± 0.01 | 0.50 h ± 0.03 | 0.39 k ± 0.02 | 0.69 j ± 0.02 | 2.43 n ± 0.02 |
| G14 | 1.09 o ± 0.01 | 1.50 def ± 0.04 | 3.50 m ± 0.01 | 5.42 k ± 0.02 | 139.50 i ± 0.03 | 3.69 b ± 0.03 | 5.29 a ± 0.04 | 0.49 k ± 0.03 | 2.39 n ± 0.02 |
| G15 | 7.40 a ± 0.04 | 1.02 ghij ± 0.03 | 10.88 a ± 0.03 | 9.19 I ± 0.02 | 129.73 j ± 0.03 | 0.52 h ± 0.03 | 0.59 h ± 0.04 | 2.23 d ± 0.01 | 5.64 g ± 0.04 |
| G16 | 5.61 b ± 0.03 | 1.39 efg ± 0.02 | 10.30 b ± 0.04 | 9.70 h ± 0.03 | 201.30 f ± 0.04 | 0.32 j ± 0.01 | 0.23 m ± 0.01 | 1.13 h ± 0.01 | 5.62 g ± 0.03 |
| G17 | 3.30 i ± 0.02 | 0.42 lmno ± 0.01 | 3.92 k ± 0.03 | 3.33 n ± 0.03 | 85.51 m ± 0.02 | 0.10 l ± 0.04 | 0.30 l ± 0.03 | 1.31 f ± 0.03 | 3.29 k ± 0.03 |
| G18 | 3.81 g ± 0.03 | 0.72 ijklm ± 0.04 | 2.70 q ± 0.02 | 12.79 f ± 0.02 | 216.22 e ± 0.02 | 0.32 j ± 0.04 | 0.60 h ± 0.03 | 0.29 m ± 0.02 | 1.22 q ± 0.01 |
| G19 | 0.30 u ± 0.02 | 0.40 lmno ± 0.02 | 1.02 u ± 0.01 | 0.30 u ± 0.04 | 3.32 w ± 0.01 | 2.09 c ± 0.03 | 1.40 d ± 0.03 | 0.12 o ± 0.01 | 0.60 s ± 0.04 |
| G20 | 4.01 e ± 0.01 | 1.92 bc ± 0.04 | 6.89 g ± 0.02 | 21.72 b ± 0.02 | 323.63 c ± 0.02 | 0.39 i ± 0.03 | 0.42 k ± 0.01 | 2.84 b ± 0.02 | 17.09 a ± 0.02 |
| G21 | 0.29 u ± 0.02 | 1.12 fghi ± 0.01 | 2.72 q ± 0.01 | 0.72 t ± 0.03 | 6.62 u ± 0.04 | 4.72 a ± 0.03 | 1.82 c ± 0.02 | 2.43 c ± 0.05 | 9.32 d ± 0.03 |
| G22 | 1.41 n ± 0.02 | 1.54 cdef ± 0.05 | 3.21 o ± 0.02 | 3.33 n ± 0.02 | 32.03 s ± 0.01 | 0.22 I ± 0.01 | 0.44 jk ± 0.04 | 0.42 l ± 0.02 | 2.12 o ± 0.01 |
| G23 | 1.72 m ± 0.04 | 2.13 b ± 0.01 | 3.81 l ± 0.03 | 2.69 o ± 0.04 | 56.82 o ± 0.05 | 1.30 e ± 0.02 | 1.02 e ± 0.02 | 0.32 m ± 0.01 | 3.21 k ± 0.02 |
| G24 | 0.50 s ± 0.02 | 0.31 mno ± 0.01 | 7.79 e ± 0.03 | 0.12 w ± 0.04 | 0.59 x ± 0.02 | 0.20 k ± 0.02 | 1.04 e ± 0.04 | 0.33 m ± 0.03 | 1.70 p ± 0.02 |
| G25 | 0.23 v ± 0.03 | 0.94 hij ± 0.01 | 2.64 r ± 0.05 | 1.32 r ± 0.02 | 25.92 t ± 0.02 | 0.13 l ± 0.01 | 0.70 g ± 0.05 | 0.11 o ± 0.02 | 0.51 t ± 0.03 |
| G26 | 0.22 v ± 0.03 | 3.02 a ± 0.02 | 7.23 f ± 0.01 | 4.12 m ± 0.02 | 57.89 n ± 0.03 | 0.31 j ± 0.01 | 0.80 f ± 0.05 | 0.42 l ± 0.01 | 2.10 o ± 0.02 |
| G27 | 4.42 d ± 0.01 | 1.40 efg ± 0.02 | 3.40 n ± 0.05 | 28.31 a ± 0.04 | 505.50 b ± 0.1 | 0.30 j ± 0.05 | 0.30 l ± 0.06 | 0.10 o ± 0.01 | 0.98 r ± 0.05 |
| Sig. | ** | ** | ** | ** | ** | ** | ** | ** | ** |

ND: Not Detected

## DISCUSSION

Beta-carotenes are a member of the carotenoid family, which have a range of yellow, orange, and red pigments [22]. The human body is not able to synthesize carotenoid compounds [23], while many plants have the potential to produce these compounds [24]. Among the biological effects of these pigments on the human body, it can be mentioned that they are precursors for the synthesis of vitamin A, enhance Immunity, and prevent chronic-degenerative diseases such as cancer and cardiovascular disease [25]. Based on the above evidence, it can be said that carotenoids and beta-carotene can play an important role in human health [26]. Therefore, it seems necessary to supply these compounds through the consumption of plants [27].

Phenolic compounds are one of the largest groups of natural products that can be found in a wide range of plants [28]. These compounds are natural antioxidants that can play an important role in free radical scavenging [29]. In a study, the level of TPC in *E. emiliae* was found to be 23.52, 1.87, and 29.93 mg GAE/g DW in methanolic, chloroform, and hexane extracts, respectively [30]. In another paper, the amount of TPC in the methanolic extract of *E. spinosissimus* root was reported to be 95.31 mg GAE/g DW [31]. The concentration of TPC was detected to be 575.5 mg GAE/g DW in the root of *E. polyceras* [27]. In the study of two species of *E. ritro* and *E. tournefortii*, the amount of TPC in the methanolic extract was 83.45 and 76.81 mg GAE/100 g DW, respectively [32]. Based on the studies mentioned above, it can be said that presence of TPC in plants strongly depends on the type of extraction solvent, geographic origin, and ecotype [30,33]. One of the most common polyphenol compounds is flavonoids, which are found in almost all plant organs [34]. Flavonoids are made through the phenylpropanoid pathway, and their activity depends on the structure [35,36]. In the research of Sytar *et al.*, the TFC in *E. ritro* was recorded as mg QE/mg DW [37]. Al-Assaf and Khazem reported that level of TFC in methanolic, ethanolic (50% v/v), and distilled water extracts of *E. polyceras* root was 130, 119, and 103 mg QE/g DW, respectively [27]. In *E. spinosus* and *E. hussoni*, the concentration of TFC was detected as 44.6 and 23.8 mg CE/g DW, respectively [38]. In another study, the TFC in *E. spinosus* Roxb was presented at 0.004% [39]. In general, the results of this paper and previous studies suggest that the concentration of secondary metabolites, including TFC, is influenced by ecotype and altitude [40].

Recently, various methods have been used to evaluate antioxidant activity in the field of plant sciences [41]. Among them, the DPPH assay is one of the easiest and most suitable methods to evaluate the antioxidant potential and scavenge free radicals [42]. Previous reports have recorded 78.35% of antioxidant activity by DPPH assay in *E. ritro* [37]. In another work, the level of antioxidant activity in the methanolic extract of *E. antalyensis* was recorded as 86.36 µg/ml [43]. The evaluation of antioxidant activity in *E. spinosissimus* with DPPH and ABTS revealed that its amount was 87.72 and 161.66%, respectively [44]. Mohseni et al. stated that methanol/ethyl acetate (Soxhlet method) in *E. persicus* has a high ability to scavenge DPPH free radicals (89.14%) [45].

In the last few decades, the bioactive ingredients and medicinal effects of most medicinal plants have been explained by many researchers [46]. Plant carbohydrates are a large group of these compounds [47]. Carbohydrates, in addition to providing an energy source, play a role in the structure of organic compounds (as a carbon skeleton) and the molecular signal cascade during biotic and abiotic stresses [48]. In previous studies, the amount of carbohydrates in the aerial part of *E. spinosissimus* was recorded as 23.23% [49]. The results of this study revealed that the quantity of carbohydrates is different among *Echinops* ecotypes. Therefore, it seems that the storage and accumulation of bioactive ingredients, including carbohydrates, can be controlled by harvest time [50], growth seasons, and plant organs [51].

Currently, investigating the protective role of phenolic compounds against plant stresses has become an exciting challenge [52]. In addition, antioxidants and antibacterials are other applications of these compounds in plants [53]. The antioxidant effect in plants of the Asteraceae family is exerted by the presence of various bioactive compounds, especially polyphenols. [67]. In research, the concentration of chlorogenic acid, caffeic acid, and cinnamic acid in *E. kebericho* root extract was recorded as 138.16, 2.21, and 413.14 µg/g DW, respectively [54]. The values of coumaric acid, caffeic acid, catechin, vanillic acid, cinnamic acid, rutin, quercetin, and kaempferol in the ethanolic extract of *E. spinosissimus* root were obtained as 8.59, 2.94, 5.96, 2.08, 4.68, 7.63, 15.23, and 30.37 mg/kg DW, respectively [55]. Abd El-Moaty identified luteolin-6-arabinose-8-glucose (25.34 mg/100g), apigenin-6-arabinose-8-glactose (23.04 mg/100g), apigenin-6-glucose-8-rhamnose (20.08 mg/100g), and hesperidin (34.5 mg/100g), hespirtin (39.23 mg/100g) as major compounds in *E. spinosissimus* aerial parts [49]. Based on our results and the above documents, it seems that there is a great variation between the type and amount of phenolic compounds in different *Echinops* ecotypes. In general, the type of distribution area, geographical and environmental conditions, genetic diversity of species, as well as genes involved in the biosynthesis of phenolic compounds, can be the reasons for this diversity [56, 57]. Huang *et al.* documented that antioxidant activity in plants is positively correlated with the amount of polyphenolic compounds [60]. Several studies explained that there is a strong correlation between total phenolic and flavonoid content with antioxidant activity [61-66], which was consistent with the results of our study.

## CONCLUSION

In this paper, twenty-seven ecotypes of *E. ritro* were investigated for phytochemical compounds, antioxidant activity, and polyphenolic compounds. For the first time, we reported that the aerial parts of *Echinops* ecotypes are a potential source for some phytochemical compounds, including carotenoid (G19), beta-carotene (G2 and G19), total phenol (G25), total flavonoid (G12), total carbohydrates (G15), and antioxidant activity (G4). *P*-coumaric acid, rutin, and apigenin were identified as major phenolic compounds. According to the results obtained from hierarchical clustering and principal component analysis, there was significant variation between *Echinops* ecotypes for phytochemical compounds, antioxidant activity, and phenolic compounds. Overall, the data of this work provide the possibility of superior ecotype screening in terms of natural compounds and antioxidant activity. Therefore, it can be of great help in breeding programs as well as pharmaceutical, food, and cosmetic industries.

### Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

This study and the research behind it would not have been possible without the special support of Shahid Bakeri Higher Education Center of Miandoab.

## REFERENCES


1. Bouzabata A., Mahomoodally F., Tuberoso C. Ethnopharmacognosy of *Echinops spinosus* L. in North Africa: a mini review. Journal of Complementary and Alternative Medical Research. 2018; 8(1):40-52. https://doi.org/10.5455/jcmr.20180318051853.
2. Sánchez-Jiménez I., Lazkov G.A., Hidalgo O., Garnatje T. Molecular systematics of *Echinops* L. (Asteraceae, Cynareae): A phylogeny based on ITS and trnL-trnF sequences with emphasis on sectional delimitation. Taxon. 2010; 59(3):698-708. https://doi.org/10.2307/25677662.
3. Rechinger KH. Flora Iranica, Compositae III-Cynareae. Graz, Austria. 1979; 139:218-30.
4. Montazerolghaem S., Rahiminejad M.R., Mozaffarian V., Susanna A. Taxonomic notes on the genus *Echinops* (Compositae, Cardueae–Echinopsinae) in Iran. Phytotaxa. 2016; 263(2):81. https://doi.org/10.11646/phytotaxa.263.2.1.
5. Sharafzadeh S., Alizadeh O. Some medicinal plants cultivated in Iran. Journal of Applied Pharmaceutical Science. 2012; 30(Issue):134-7.
6. Bitew H., Hymete A. The genus Echinops: Phytochemistry and biological activities: A review. Frontiers in Pharmacology. 2019; 10:1234. https://doi.org/10.3389/fphar.2019.01234.
7. Asadi M., Hadinedoushan H., Mirghanizadeh S.A., Karimollah A., Dashti F., Malek-hosseini S. The effect of *Echinops Lasiolepis* extracts, native plant of Yazd province, on peripheral blood mononuclear cell proliferation and IFN-γ secretion. International Journal of Medical Laboratory. 2014; 1(1):7-14.
8. Abdallah H.M., Ezzat S.M., El Dine R.S., Abdel-Sattar E., Abdel-Naim A.B. Protective effect of *Echinops galalensis* against CCl4-induced injury on the human hepatoma cell line (Huh7). Phytochemistry Letters. 2013; 6(1):73-8. https://doi.org/10.1016/j.phytol.2012.10.012.
9. Kevin K., John K., Carolyn N., Derrick S., Lubega A. *In vitro* anti-tuberculosis activity of total crude extract of *Echinops amplexicaulis* against multi-drug resistant Mycobacterium tuberculosis. Journal of Health Sciences. 2018; 6:296-303. https://doi.org/10.17265/2328-7136/2018.04.008.
10. Kiyekbayeva L., Mohamed N.M., Yerkebulan O., Mohamed E.I., Ubaidilla D., Nursulu A., Assem M., Srivedavyasasri R., Ross S.A. Phytochemical constituents and antioxidant activity of *Echinops albicaulis*. Natural Product Research. 2018; 32(10):1203-7. https://doi.org/10.1080/14786419.2017.1323213.
11. Li L.B., Xiao G.D., Xiang W., Yang X., Cao K.X., Huang R.S. Novel substituted thiophenes and sulf-polyacetylene ester from *Echinops ritro* L. Molecules. 2019; 24(4):805. https://doi.org/10.3390/molecules24040805.
12. Mohebat R., Bidoki M.Z. Comparative chemical analysis of volatile compounds of *Echinops ilicifolius* using hydrodistillation and headspace solid-phase microextraction and the antibacterial activities of its essential oil. Royal Society Open Science. 2018; 5(2):171424. https://doi.org/10.1098/rsos.171424.
13. Karimov U.T., Aisa H.A. Phytochemical study of the plant *Echinops integrifolius* growing in the Altai (XUAR PRC). Chemistry of Natural Compounds. 2012; 48(5):903-5. https://doi.org/10.1007/s10600-012-0419-6.
14. Mahmood A.A., Khadeem E.J. Phytochemical investigation of flavonoids glycoside in the Iraqi *Echinops heterophyllus* (Compositae). Pharmacie Globale. 2013; 4(9):1.
15. Dong L.I., Ning L., Wan X.I., Peng Z.H., Zhong-jun M., Xian L. Chemical constituents of the root of *echinops grijisii* Hance. Shenyang Yao Ke Da Xue Xue Bao. 2008; 8(007).
16. Lightenthaler H.K. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology. 1987; 148:350-82. https://doi.org/10.1016/0076-6879(87)48036-1.
17. Slinkard K., Singleton V.L. Total phenol analysis: automation and comparison with manual methods. American Journal of Enology and Viticulture. 1977; 28(1):49-55. https://doi.org/10.5344/ajev.1977.28.1.49.
18. Silva M.C., Paiva S.R. Antioxidant activity and flavonoid content of *Clusia fluminensis* Planch. & Triana. Anais da Academia Brasileira de Ciências. 2012; 84:609-16. https://doi.org/10.1590/S0001-37652012000300004.
19. Nakajima J.I., Tanaka I., Seo S., Yamazaki M., Saito K. LC/PDA/ESI-MS profiling and radical scavenging activity of anthocyanins in various berries. Biomedicine Research International. 2004; 2004(5):241-7. https://doi.org/10.1155/S1110724304404045.
20. Carroll N.V., Longley R.W., Roe J.H. The determination of glycogen in liver and muscle by use of anthrone reagent. Journal of Biological Chemistry. 1956; 220(2):583-93. https://doi.org/10.1016/S0021-9258(18)65284-6.
21. Gholizadeh-Moghadam N., Hosseini B., Alirezalu A. Classification of barberry genotypes by multivariate analysis of biochemical constituents and HPLC profiles. Phytochemical Analysis. 2019; 30(4):385-94. https://doi.org/10.1002/pca.2821.
22. Bogacz-Radomska L., Harasym J. β-Carotene—properties and production methods. Food Quality and Safety. 2018; 2(2):69-74. https://doi.org/10.1093/fqsafe/fyy004.
23. Bakan E., Akbulut Z.T., İnanç A.L. Carotenoids in foods and their effects on human health. Akademik Gıda. 2014; 12(2):61-8.
24. Khoo H.E., Prasad K.N., Kong K.W., Jiang Y., Ismail A. Carotenoids and their isomers: color pigments in fruits and vegetables. Molecules. 2011; 16(2):1710-38. https://doi.org/10.3390/molecules16021710.
25. González-Peña M.A., Ortega-Regules A.E., Anaya de Parrodi C., Lozada-Ramírez J.D. Chemistry, occurrence, properties, applications, and encapsulation of carotenoids—A review. Plants. 2023; 12(2):313. https://doi.org/10.3390/plants12020313.
26. Fiedor J., Burda K. Potential role of carotenoids as antioxidants in human health and disease. Nutrients. 2014; 6(2):466-88. https://doi.org/10.3390/nu6020466.
27. Al-Assaf I., Khazem M. Antioxidant Activity of Total phenols and Flavonoids extracted from *Echinops* polyceras roots grown in Syria. Iraqi Journal of Pharmaceutical Sciences. 2021; 30(2):261-8. https://doi.org/10.31351/vol30iss2pp261-268.
28. Bhattacharya A., Sood P., Citovsky V. The roles of plant phenolics in defence and communication during Agrobacterium and Rhizobium infection. Molecular Plant Pathology. 2010; 11(5):705-19. https://doi.org/10.1111/j.1364-3703.2010.00625.x.
29. Hassanpour S.H., Doroudi A. Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. Avicenna Journal of Phytomedicine. 2023; 13(4):354. https://doi.org/10.22038/AJP.2023.21774.
30. Şapcı H., Vural C., Özcan S. Antimicrobial and Antioxidant activity of *Echinops emiliae* (Asteraceae). International Journal of Secondary Metabolite. 2017; 4(3):400-5. https://doi.org/10.21448/ijsm.375102.
31. Zitouni-Nourine S.H., Belyagoubi-Benhammou N., El-Houaria Zitouni-Haouar F., Douahi O., Chenafi F., Fetati H., Chabane Sari S., Benmahieddine A., Zaoui C., Mekaouche F.Z., Atik Bekkara F. *Echinops spinosissimus* turra root methanolic extract: Characterization of the bioactive components and relative wound healing, antimicrobial and antioxidant properties. Plants. 2022; 11(24):3440. https://doi.org/10.3390/plants11243440.
32. Aydın Ç., Özcan G.T., Turan M., Mammadov R. Phenolic contents and antioxidant properties of *Echinops ritro* L. and E. tournefortii Jaup. Et. Spach extract. International Journal of Secondary Metabolite. 2016; 3(2):74-81. https://doi.org/10.21448/http-ijate-net-index-php-ijsm.243309.
33. Shameh S., Alirezalu A., Hosseini B., Maleki R. Fruit phytochemical composition and color parameters of 21 accessions of five *Rosa* species grown in North West Iran. Journal of the Science of Food and Agriculture. 2019; 99(13):5740-51. https://doi.org/10.1002/jsfa.9842. Epub 2019 Jul 16.
34. Kumar S., Pandey A.K. Chemistry and biological activities of flavonoids: an overview. The Scientific World Journal. 2013; 2013(1):162750. https://doi.org/10.1155/2013/162750.
35. Heim K.E., Tagliaferro A.R., Bobilya D.J. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. The Journal of Nutritional Biochemistry. 2002; 13(10):572-84. https://doi.org/10.1016/s0955-2863(02)00208-5.
36. Falcone Ferreyra M.L., Rius S.P., Casati P. Flavonoids: biosynthesis, biological functions, and biotechnological applications. Frontiers in Plant Science. 2012; 3:222. https://doi.org/10.3389/fpls.2012.00222/
37. Sytar O., Hemmerich I., Zivcak M., Rauh C., Brestic M. Comparative analysis of bioactive phenolic compounds composition from 26 medicinal plants. Saudi Journal of Biological Sciences. 2018; 25(4):631-41. https://doi.org/10.1016/j.sjbs.2016.01.036.

38. Al-Harbi K.B., El-Tigani-Asil E.A., Ahmed A.F, El-Ashmawy I.M., Al-Wabel N.A. Wound healing potential of methanolic extracts of some plants native to Al-Qassim Region, Saudi Arabia. Journal of Food, Agriculture and Environment. 2016; 14:3-4.
39. Samir H., Abbas M.S., Soliman A.S., Lotfy R.A. Phytochemical screening, antioxidant and cytotoxic activities of some plants species derived from the northwestern coast of Egypt. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2018; 9(6):82-94.
40. Sampaio B.L., Bara M.T., Ferri P.H., Santos S.D., Paula J.R. Influence of environmental factors on the concentration of phenolic compounds in leaves of Lafoensia pacari. Revista Brasileira de Farmacognosia. 2011; 21:1127-37. https://doi.org/10.1590/S0102-695X2011005000177.
41. Salazar R., Pozos M.E., Cordero P., Perez J., Salinas M.C., Waksman N. Determination of the antioxidant activity of plants from Northeast Mexico. Pharmaceutical Biology. 2008; 46(3):166-70. https://doi.org/10.1080/13880200701498952.
42. Kedare S.B., Singh R.P. Genesis and development of DPPH method of antioxidant assay. Journal of Food Science and Technology. 2011; 48(4):412-22. https://doi.org/10.1007/s13197-011-0251-1.
43. Şapcı H., Vural C. Bioactivity researching on New Species of *Echinops* L. *Asteraceae):* Antimicrobial and Antioxidant Activity of Antalyensis. Anadolu University Journal of Science and Technology C-Life Sciences and Biotechnology. 2018; 7(1):68-73. https://doi.org/10.18036/aubtdc.333248.
44. Al Masoudi L.M., Hashim A.M. Morphological Features and Biological Activity of Different Extracts of *Echinops spinosissimus* Grown in Saudi Arabia. Agronomy. 2023; 13(2):573. https://doi.org/10.3390/agronomy13020573.
45. Mohseni S., Sani A.M., Tavakoli M., Raoufi AM. Effect of extraction conditions on antioxidant activities of *Echinops persicus*. Journal of Essential Oil Bearing Plants. 2017; 20(6):1633-44. https://doi.org/10.1080/0972060X.2017.1399088.
46. Deore Sonali V., Kadam Vasant B. Estimation of total carbohydrates content in different parts of *Trigonella foenum*-graecum Linn. Natural Medicine. 2016; 4: 72-75.
47. Tharanathan R.N., Muralikrishna G., Salimath P.V., Rao M.R. Plant carbohydrates—an overview. Proceedings of Indian Academy of Sciences. 1987; 97(2):81-155. New Delhi: Springer India. https://doi.org/10.1007/BF03053322.
48. Trouvelot S., M.C., Héloir B., Poinssot A., Gauthier F., Paris, C., Guillier, M., Combier, L., Trdá X., Daire M., Adrian. Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. Frontiers in Plant Science. 4 (2014) 592. https://doi.org/10.3389/fpls.2014.00592.
49. Ibrahim H., Moaty A. Chemical Constituents of *Echinops Spinosissimus* Turra, International Journal of Advanced Research. 2016; 4:1129-36. https://doi.org/10.21474/IJAR01/1178.
50. Qaderi M.M., Martel A.B., Strugnell C.A. Environmental factors regulate plant secondary metabolites. Plants. 2023; 12(3):447. https://doi.org/10.3390/plants12030447.
51. Li Y., Kong D, Fu Y., Sussman M.R., Wu H. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiology and Biochemistry. 2020; 148:80-9. https://doi.org/10.1016/j.plaphy.2020.01.006.
52. Zhang Y., Cai P., Cheng G., Zhang Y.A. brief review of phenolic compounds identified from plants: Their extraction, analysis, and biological activity. Natural Product Communications. 2022; 17(1):1934578X211069721.https://doi.org/10.1177/1934578X211069721.
53. Kumar S., Abedin M.M., Singh A.K., Das S. Role of phenolic compounds in plant-defensive mechanisms. InPlant phenolics in sustainable agriculture. 1:517-532. Singapore: Springer Singapore. https://doi.org/10.1007/978-981-15-4890-1_22.
54. Dessalegn E., Rupasinghe H.V. Quantification of phenolic compounds by HPLC-DAD and in vitro antioxidant activity of root extract of *Echinops kebericho* Mesfin (Asteraceae). Chemical and Process Engineering Research. 2020; 31(62):6-13. https://doi.org/10.7176/CPER/62-02.
55. Khedher O., Rigane G., Riguene H., Ben Salem R., Moussaoui Y. Phenolic profile (HPLC-UV) analysis and biological activities of two organic extracts from *Echinops spinosissimus* Turra roots growing in Tunisia. Natural Product Research. 2021; 35(24):5786-93. https://doi.org/10.1080/14786419.2020.1837812.
56. Pourhosseini S.H., Mirjalili M.H., Ghasemi M., Ahadi H., Esmaeili H., Ghorbanpour M. Diversity of phytochemical components and biological activities in *Zataria multiflora* Boiss. (Lamiaceae) populations. South African Journal of Botany. 2020; 135:148-57. https://doi.org/10.1016/j.sajb.2020.08.024.
57. Ghorbani N., Chamani E., Shokoohian A.A., Ramezanpour S.S. Biochemical diversity of yellow flag (Iris pseudacorus L.) at various geographical locations. International Journal of Horticultural Science and Technology. 2023; 10(4):421-32. https://doi.org/10.22059/ijhst.2022.346841.580.
58. Sfidari E., Kadkhodaie-Ilkhchi A., Najjari S. Comparison of intelligent and statistical clustering approaches to predicting total organic carbon using intelligent systems. Journal of Petroleum Science and Engineering. 2012; 86:190-205. https://doi.org/10.1016/j.petrol.2012.03.024.
59. Huang J., Ding L., Tian W., Zhi H., Chen J., Wu L., Wang L., Xie J., Bai J., Fan H., Zhao S. Polyphaenolic profiling, antioxidant properties, and inhibition of α-glucosidase of *Mesona chinensis* benth from Southern China. Microchemical Journal. 2021; 168:106399. https://doi.org/10.1016/j.microc.2021.106399.
60. Jolliffe I.T., Cadima J. Principal component analysis: a review and recent developments. Philosophical transactions of the royal society A: Mathematical, Physical and Engineering Sciences. 2016; 374(2065):20150202. https://doi.org/10.1098/rsta.2015.0202.
61. Kumar S., Sandhir R., Ojha S. Evaluation of antioxidant activity and total phenol in different varieties of Lantana camara leaves. BMC Research Notes. 2014;7(1):560. https://doi.org/10.1186/1756-0500-7-560.
62. Pandey B., Rajbhandari M. Estimation of total phenolic and flavonoid contents in some medicinal plants and their antioxidant activities. Nepal Journal of Science and Technology. 2014; 15(1):53-60.
63. Mello L.D., Quadros GP. Correlation between antioxidant activity and total phenolic content with physicochemical parameters of blended extracts of *Camellia sinensis*. Acta Scientiarum. Health Sciences. 2014;36(1):97-103. https://doi.org/10.4025/actascihealthsci.v36i1.12615.
64. Osman M.A., Mahmoud G.I., Shoman S.S. Correlation between total phenols content, antioxidant power and cytotoxicity. Biointerface Res. Appl. Chem. 2020; 11(3):10640-53. https://doi.org/10.33263/BRIAC113.1064010653.
65. Modareskia M., Fattahi M., Mirjalili M.H. Thymol screening, phenolic contents, antioxidant and antibacterial activities of Iranian populations of Trachyspermum ammi (L.) Sprague (Apiaceae). Scientific Reports. 2022;12(1):15645. https://doi.org/10.1038/s41598-022-19594-7.
66. Vafaeian A., Fathi S., Jaberian H., Nazarideljou M.J. Comparing the characteristics of lemongrass plant in different harvesting time under soil and soilless cultivation systems. Journal of Medicinal plants and By-products. 2025; 14(1):20-9. https://doi.org/10.22034/jmpb.2024.365417.1676.
67. Kazeminia M., Mehrabi A., Mahmoudi R. Chemical composition, biological activities, and nutritional application of Asteraceae family herbs: A systematic review. Trends in Phytochemical Research. 2022;6(3): 187-213. https://doi.org/10.30495/tpr.2022.1954612.1248.