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RESEARCH ARTICLE

WILEY Phytochemical Analysis

Classification of barberry genotypes by multivariate analysis of biochemical constituents and HPLC profiles

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Abstract

Introduction: Recently, there has been a growing interest in the use of edible barberry and their extracts as a source of natural antioxidants in food and pharmaceutical industries.

Objective: The aim of this study was to evaluate the biochemical constituents of 18 samples of barberry fruits and classification of barberry genotypes by multivariate analysis.

Methods: Total phenolic, total flavonoid, total anthocyanin, total tannin, total carbohydrate contents and antioxidant activity were determined using Folin–Ciocalteu, aluminum chloride, colorimetric, vanillin, anthron and DPPH (2,2′-diphenyl-1-picrylhydrazyl) assays, respectively. High-performance liquid chromatography (HPLC) system is used for quantitative determination of phytochemical constituents. The multivariate data analysis (principal component analysis and hierarchical cluster analysis) and heat map data visualisation techniques were performed to classify barberry genotypes using Minitab and GraphPad Prism software, respectively.

Results: The highest amounts of total phenolics and flavonoids were obtained in fruit extracts of G3 (*Berberis vulgaris*). The highest total anthocyanin content and antioxidant activity were observed in G8 (*B. vulgaris*) and G16 (*B. vulgaris*), respectively. HPLC analysis of phytochemicals (gallic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, cinnamic acid, rutin, apigenin, and quercetin) revealed that gallic acid and *p*-coumaric acid were found as the most abundant phytochemical compounds. Based on multivariate analysis and heat map visualisation techniques, *Berberis* genotypes were classified into three main clusters.

Conclusions: These results showed that barberry species (especially *B. vulgaris* and *B. carataegina*) are promising sources of natural antioxidants and biochemical compounds beneficial to be used in the food industry and that the multivariate analysis was a suitable approach to classify the barberry samples.

KEYWORDS

anthocyanin, antioxidant, barberry, chromatography, flavonoids, phenolic

1 | INTRODUCTION

Recently there has been much interest in the use of edible barberry and their extracts as a source of natural antioxidants in food and pharmaceutical industries.[1] Reports indicate that in recent decades the medicinal value of wild edible fruits is growing in different regions of the world.[2] Also, these reports suggest that the cultivation and consumption of these species have high economic value.[3,4] The genus *Berberis*, with over 500 species, is one of the most important members of the family Berberidaceae. Genus *Berberis* is geographically spread in Asia, Europe, East Africa, and North and South America. Some species of this genus are also found in Iran such as, *B. crataegina*, *B. integerriam*, *B. khorasanica*, *B. orthobotrys*, *B. vulgaris*, and *B. thunbergii*.[5] In traditional folk medicine, roots, stems, leaves, flowers, and fruits of this plant have been used in various treatments of diseases such as analgesic, antibacterial, antipyretic, antidiabetic, and anti-itching. Recent studies show that extracts of barberry have beneficial effects on the vascular and nervous systems.[6] Also, barberry fruits have been used as medicinal remedies for acute and chronic inflammation treatment,[7] antihistaminic and anticholinergic activity,[8] antioxidant properties[9] and effect on heart contractility.[10] Several studies have shown that extracts of barberry fruit have useful effects on the heart and also blood circulation.[11]

Various anthocyanins, flavonoids, proteins, terpenoids, antioxidants, lignans, vitamins, carotenoids, tannins, organic, and phenolic acids are the main active substances of *Berberis* species.[12] Furthermore, barberry fruits have been determined as the main source of antioxidants, due to the high content of phenolic compounds such as, apigenin, rutin, quercetin, chlorogenic acid, caffeic acids, and anthocyanins.[13,14] Genetic diversity among different genotypes, environmental factors such as temperatures, soil, weather, light intensity, time of harvest, and post-harvest management, are critical elements that affect the antioxidant capacity and phenolic compounds in plants.[15,16] Previous studies on different organs and genotypes of genus *Berberis* revealed that their antioxidant capacity and phytochemicals are various.[13,14,17,18] Yildiz et al.[19] in a study on 19 barberries genotypes of *B. vulgaris* collected from north-eastern Anatolia concluded that, physicochemical properties, composition, and antioxidant capacity were varied. Also, Ozgen et al.[20] reported that total phenolic content (TPC), total anthocyanin content (TAC), and antioxidant activity by the FRAP (ferric reducing antioxidant power) assay were various among 30 barberry fruit accessions. Although many studies have been conducted on the antioxidant capacity and phytochemical compounds of stems,[21] roots,[22] flowers,[23] barks,[14] leaves[13] of the barberry species, few studies exist on the fruits.

In order to recognise the antioxidant and phytochemical potential of barberry fruits, many researchers in Iran are currently exploring the fruits of different Iranian species for use in the food and pharmaceutical industries. Phenolic compounds such as flavonoids, phenolic acids, anthocyanins are recognised for their value as antioxidants.[17] Northwest Iran is one of the main sources of genetic variation of genus *Berberis*; however, few researches have been carried out on phytochemicals of this genus in northwest Iran. The aim of this study was to evaluate the characterisation of phytochemicals and antioxidant activities of 18 samples of barberry fruits (including three species) in the northwest of Iran and to classify barberry genotypes by multivariate analysis.

2 | EXPERIMENTAL

2.1 | Plant materials

The barberry fruits (including 18 genotypes) were collected in full ripening stage from northwest Iran (Table 1 and Figure 1) in October and November 2016. The fruits of *Berberis* genotypes after sampling were transported to the laboratory and were stored under frozen conditions until analysis.

2.2 | Preparation of fruit extracts

For this purpose, 0.5 mL of pure fruit juice was mixed with 4.5 mL methanol/water (80%, *v*/*v*). Then the juice of the fruits was centrifuged at 11000×*g* for 15 min and filtered. The extracts were stored at 4°C until analysis.

2.3 | Total phenolic content (TPC)

The Folin–Ciocalteu method was used with some modification.[24] Briefly, 100 μL of methanolic extract was shaken for 1 min with 1 mL of diluted (1:10) Folin–Ciocalteu reagent. Then, 800 μL of 10% sodium carbonate (Na_2CO_3) was added and the final volume was made up to 5.0 mL with distilled water. After the mixture was left to stand for 2 h at room temperature, the absorbance at 760 nm was measured using a UV-visible spectrophotometer (model: UV2100 PC). The results of TPC were estimated using a standard curve prepared using gallic acid and were expressed as milligrams of gallic acid equivalents (GAEs) per 100 mL of extract, Sigma Aldrich Chemie (Steinheim, Germany).

2.4 | Total flavonoid content (TFC)

Total flavonoid content (TFC) was determined using aluminium chloride ($AlCl_3$) reagent according to a known method, by quercetin as a standard.[25] The methanolic extract (400 μL) was added to 0.3 mL distilled water followed by 5% sodium nitrite ($NaNO_2$) (0.03 mL). After 5 min at 25°C, $AlCl_3$ (0.03 mL, 10%) was added. After a further 5 min, the reaction mixture was mixed with 0.2 mL of 1mM sodium hydroxide (NaOH). Finally, the reaction mixture was diluted to 1 mL with distilled water and the absorbance was measured at 420 nm. The results were expressed as milligrams of quercetin equivalent (QUE) per 100 mL of extract.

TABLE 1 Sampling locations of the different barberry genotypes studied

Code	Province	Species	Height (m)	Latitude	Longitude
G1	West Azerbaijan	*B. carataegina*	1404	37°20′26.27″	45° 8′41.29″
G2	West Azerbaijan	*B. vulgaris*	1357	38° 0′5.75″	45° 5′0.17″
G3	West Azerbaijan	*B. vulgaris*	1506	36°53′10.60″	45°27′35.76″
G4	East Azerbaijan	*B. vulgaris*	1278	38°15′55.02″	45°21′13.67″
G5	West Azerbaijan	*B. vulgaris*	1328	38°14′17.63″	44°45′27.11″
G6	East Azerbaijan	*B. vulgaris*	1427	38°22′25.53″	45°21′33.08″
G7	East Azerbaijan	*B. vulgaris*	1326	38°15′40.87″	45°56′47.73″
G8	East Azerbaijan	*B. vulgaris*	1390	38°19′20.16″	45°56′3.59″
G9	East Azerbaijan	*B. integerima*	1321	38°49′31.44″	45°48′21.76″
G10	East Azerbaijan	*B. integerima*	980	38°54′19.18″	45°50′12.06″
G11	East Azerbaijan	*B. integerima*	645	38°52′6.04″	46° 0′27.42″
G12	East Azerbaijan	*B. integerima*	539	38°51′45.25″	46°13′55.82″
G13	East Azerbaijan	*B. integerima*	397	39° 9′51.10″	47° 1′45.61″
G14	East Azerbaijan	*B. integerima*	410	38°48′39.42″	47° 3′48.58″
G15	East Azerbaijan	*B. integerima*	1379	38°53′36.22″	46°54′59.04″
G16	East Azerbaijan	*B. vulgaris*	1815	37°38′23.81″	45°57′38.91″
G17	East Azerbaijan	*B. vulgaris*	1140	37°29′2.33″	46°10′30.00″
G18	Ardebil	*B. vulgaris*	1450	38°23′35.34″	47°33′12.56″

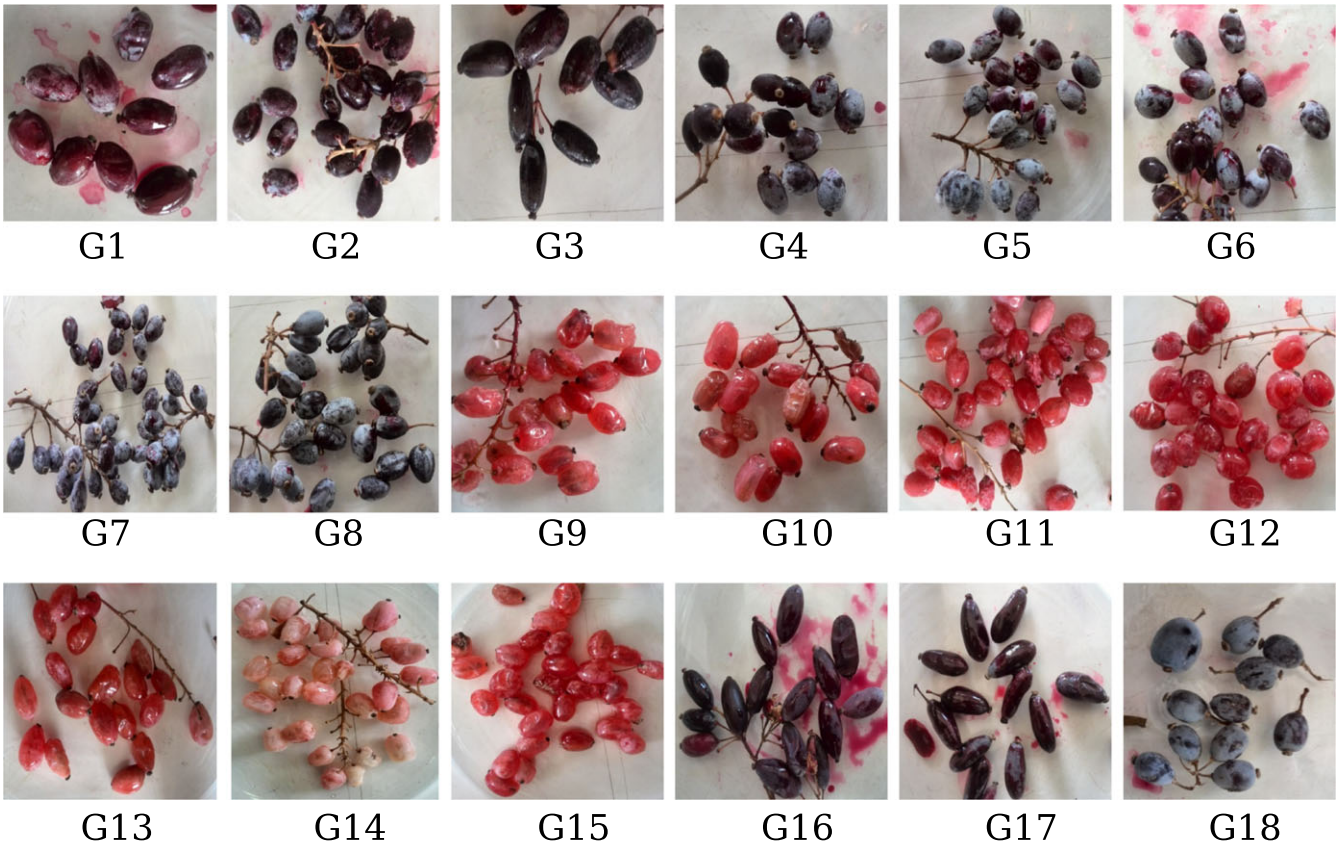

FIGURE 1 Fruits of different barberry genotypes [Colour figure can be viewed at wileyonlinelibrary.com]

2.5 | Antioxidant activity

The antioxidant activity was measured through the DPPH (2,2′-diphenyl-1-picrylhydrazyl) free radical scavenging assay for every one of the methanolic extracts.[26] Next, a dilution 1 M of DPPH was prepared. We calculated the absorbance of 500 μL mixture of the extract and 1 mL of the DPPH solution calculated at 517 nm. Based on the following equation, free radical scavenging activity was measured:

Percentage of radical scavenging activity = [(Absorbance of control – Absorbance of sample)/Absorbance of control] × 100.

2.6 | Total carbohydrate

To determine water-soluble carbohydrate contents (total carbohydrate content, TCHC), 0.5 mL of fruit juice were mixed with 5 mL of ethanol 95%. Then the solution was filtered and 5 mL of 70% ethanol was added to the solution. The mixture was centrifuged at 3500 × g for 15 min. Then ethanol extract (13 μL) added to 3 mL anthron [150 mg/100 mL sulfuric acid (H_2SO_4)] extraction and incubated at 100°C for 10 min. Absorbance was read at 625 nm. The TCHC was calculated from the calibration curve of glucose.[27]

2.7 | Total tannin content (TTC)

The total tannin content (TTC) was determined by the Luthar and Kreft[28] method. In short, methanol extract (400 μL) was mixed with 2.5 mL vanillin solution [Vanillin 1%, hydrochloric acid (HCl) 8%, relativity 50:50% *v*/*v*] for 20 min at 28°C. For the blank, 4% HCl instead of vanillin reagent was added to the extract, and TT was determined using a spectrophotometer at 765 nm (model: UV2100 PC). The standard curve was prepared by 0.0–2.0 mg/mL solutions catechin and was used in calculating tannin amounts.

2.8 | Total anthocyanin content (TAC)

The TAC was determined according to the method of Connor et al.[29] 1 ml of fruit juice was added to acidified methanol (methanol/HCl, 99:1, *v*/*v*) and centrifuged at 12000 × g for 10 min. Then acidified methanol (1.5 mL) was added to the supernatant (0.5 mL) and absorbance was read at 530 nm. The TAC was calculated using the following formula:

$$E^{1cm}_{1\%}(530) = 98.2$$
$$1\% = (\text{Density of } 1\text{gr}/100\text{ml})$$
$$\text{Thickness of the cuvette} = 1\text{cm}$$
$$\text{Total anthocyanin content} = \frac{(\text{solution absorbance}(530\text{ nm})) \times (\text{dilution factor} \times 100)}{98.2}$$

2.9 | HPLC analysis of the extracts

Methanolic extract of barberry fruits were centrifuged (7 min at 8000 × g) and supernatants were collected and filtered for analysis. The separations and detection of individual phenolic acids (gallic acid, caffeic acid, *p*-coumaric acid, chlorogenic acid, cinnamic acid) and flavonoids (rutin, quercetin, apigenin) were performed with a high-performance liquid chromatography (HPLC) 1100 series model (Agilent Technologies, Santa Clara, CA, USA). Extracts of barberry fruits are analysed by UV detection at 250 nm for quercetin and chlorogenic acid, 272 nm for gallic acid, cinnamic acid and apigenin and 310 nm for caffeic acid, rutin and *p*-coumaric acid with a diode-array detector (DAD). The separation was achieved using a Zorbax Eclipse XDB C18 column (4.6 mm × 250 mm, 5 μm particle size) at 25°C. Data acquisition and integration were performed with chemstation software. The injection volume was 20 mL and flow rate of the mobile phase was maintained at 1 mL/min. Solvent A was water containing 1% acetic acid, and solvent B was acetonitrile. The gradient conditions were as follows: 0–5 min, 10% B; 5–10 min, 25% B; 10–15 min, 65% B.

2.10 | Preparation of standard solutions

The stock solution of concentration 1 mg/mL was prepared by dissolving 1 mg standard of each phenolic compound in 0.5 mL HPLC methanol. For calibration curves, the standard solutions were diluted to six concentrations (5, 10, 20, 30, 40, 60), each of which was separately injected into the HPLC system, and the calibration curve is obtained by plotting the peak region against the concentration of each sample with a square correlation coefficient $R^2 < 0.99$ representing the linearity measurements.

2.11 | Statistical analysis

All data analyses were performed using SAS 9.1.3 software. The Duncan's multiple-range test was used for means comparison and determination of statistical significance at the $P < 0.05$ probability level. To classify different genotypes based on phytochemical compounds principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed among the variables analysed using Minitab 16 software. Also, Heat-maps were used to visualise studied traits in each genotype by GraphPad Prism 7 software.

3 | RESULTS AND DISCUSSION

3.1 | Total phenolic content (TPC)

The TPC values of fruit extracts of barberry genotypes are presented in Table 2. The amount of TPC in the extracts obtained from fruits varied from 25.98 mg GAE/100 mL extract in G15 (*B. integerima*) to 94.04 mg GAE/100 mL extract in G3 (*B. vulgaris*). Results showed that TPC of extracts was influenced significantly by both the genotype and sampling location. The TPC of *Berberis* fruits were reported previously between 2.56 and 3.62 [mg GAE/L dry weight (DW)] among barberry species collected from Turkey.[20] Motalleb et al.[9] reported the average TPC in barberry fruits as 34.5 [mg GAE/g fresh weight (FW)]. Yildiz et al.[19] reported the TPC in barberry fruit juice ranging from 2565 to 3629 (mg GAE/L FW). Also, Hassanpour and Alizadeh[30] reported the total phenol content of some Iranian barberry between 261.68 and 623.07 mg 100/g FW. Zovko Končić et al.[14] reported the TPC for barberry fruit from 7.29 to 52.54 mg/g. The present study, however, performed an extensive evaluation of 18 different barberry genotypes native to Iran, in order to provide valuable data and new insights useful for planning breeding strategies, as well as for selecting species with high phenolic contents for producing natural antioxidants for

TABLE 2 Fruit phytochemical characteristics of barberry genotypes

Code	TPC (mg GAE/100 mL extract)	TFC (mg QUE/100 mL extract)	TAC (%)	TCHC (μg/mL extract)	TTC (μg mL extract)
G1	70.95b	40.50b	2.85i	0.91hi	7.92f
G2	39.14 ghf	28.60d	21.41b	8.20b	8.65e
G3	94.04a	76.70a	16.53e	4.50 cd	18.50a
G4	37.28 ghi	24.96ef	19.43c	4.62 cd	7.99f
G5	40.66 fg	24.00ef	9.17 h	5.68c	5.92 h
G6	49.98e	33.10c	20.75b	4.48 cd	9.33d
G7	38.81fgh	22.16f	13.82f	10.69a	8.07f
G8	56.81 cd	26.10de	22.81a	5.79c	12.16c
G9	36.33hi	24.20ef	2.11i	3.91def	2.48i
G10	35.09ij	13.36 g	0.94j	4.41cde	1.01 k
G11	32.14jk	14.70 g	0.97j	4.68 cd	1.07jk
G12	30.08 k	9.46 h	0.61jk	2.29fgh	1.47j
G13	28.68kl	14.60 g	0.14 k	0.41i	1.10jk
G14	36.71hi	2.96i	0.50jk	0.63hi	0.80 k
G15	25.98 l	4.16i	0.80jk	3.94def	1.07jk
G16	53.76d	32.40c	18.11d	1.11ghi	8.53e
G17	60.42c	34.90c	19.50c	4.13cde	13.68b
G18	41.14f	24.66ef	12.59 g	2.77efg	7.23 g

Note: Means with the same letter are not significantly different from each other ($P > 0.05$). TPC, total phenolic content; TFC, total flavonoid content; TAC, total anthocyanin content; TCHC, total carbohydrate content; TTC, total tannin content.

pharma food products. Some researchers suggest that the TPC of plants is influenced by various factors such as genetic background, longitude, sampling location, pre-harvest and post-harvest conditions and maturity stage at harvest.[31-33]

3.2 | Total flavonoid content (TFC)

TFC of barberry fruit extracts are shown in Table 2. A significant difference among genotypes was recorded and TFC ranges were from 2.96 mg QUE/100 mL extract to 76.70 mg QUE/100 mL extract. The highest amount of TFC was obtained in the fruit extracts of G3 (*B. vulgaris*), whereas the lowest TFC level was found in G14 (*B. integerima*). The *B. vulgaris* had the highest TFC level among the studied species fruit extracts. The TFC is influenced by the interaction between genotype and sampling location. Hassanpour and Alizadeh[30] reported the TFC in barberry from 1.32 to 2.8 mg/g FW. Zovko Končić et al.[14] reported the TFC for extracts of barberry from 0.12 to 4.23 mg/g.

3.3 | Total anthocyanin content (TAC)

The TAC is widely varied in genotypes of *Berberis* and ranges from 0.14 to 22.81% (Table 2). The highest and lowest values of TAC were observed in G8 (*B. vulgaris*) and G13 (*B. integerima*), respectively. Recent studies have shown that barberry fruits in addition to TPC and TFC are an excellent source of anthocyanin.[34,35] Similar results have been reported in terms of the TAC for *Berberis* species, i.e. 506–803 mg/L as cy-3-glycoside,[21] 16.32 to 91.66 mg/100 g FW,[30] and 0.93 mg/g FW.[34] Yildiz et al.[19] reported that TAC in fruits of *B. vulgaris* was between 271 and 1004 mg/L as cy-3-glycoside. Variations in TAC were probably due to climatic and geographical locations,[36,37] different extraction methods,[38] and genotypes.[19] The maturation of berries at the time of harvest is the main factor that affects the amount of anthocyanins. Breeding programmes focused on the increase in the concentration of anthocyanins in berry fruits.[39] Anthocyanins can have positive therapeutic properties like anti-inflammatory, antioxidant, anti-bacterial, antiviral and anticarcinogenic affects.[40] Previous studies have revealed that cyanidin, peonidin, petunidin, malvidin, delphinidin, and pelargonidin are the major anthocyanin components of *Berberis* species.[19]

3.4 | Total carbohydrate content (TCHC)

The results for TCHC in the studied fruit extracts are presented in Table 2. The TCHC values of the different genotypes ranged from 0.41 to 10.69 μg/mL extract. The highest TCHC was found in G7 and a low level of TCHC was found in G13 extracts. Total soluble carbohydrate of fruits was significantly different between genotypes, which could be attributed to different genetic background and climatic conditions.[41] The amount of soluble carbohydrates is an important factor in determining the sensory quality of ripe fruit.[42]

3.5 | Total tannin content (TTC)

Tannins as phenolic compounds are common in most berries.[43] These compounds have protective effects against biotic and abiotic

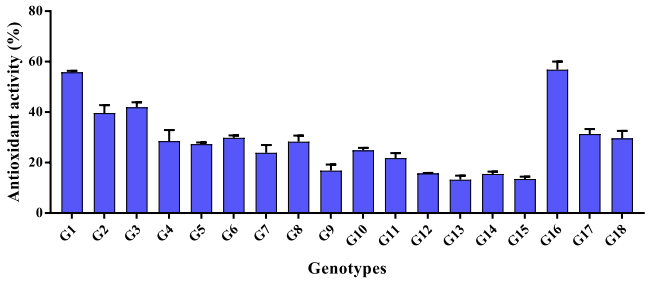

FIGURE 2 Antioxidant activity of different genotypes of barberry by DPPH assay [Colour figure can be viewed at wileyonlinelibrary.com]

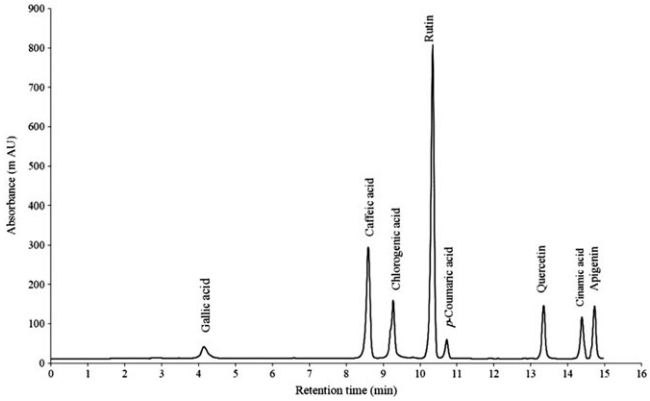

FIGURE 3 HPLC chromatograms of eight biochemical standards

stresses.[44] The concentrations of TTC in fruit extracts ranged from 0.8 to 18.50 μg/mL extract (Table 2). The highest TTC was identified in G3 fruit extracts and the lowest TTC was observed in G14 genotype. Tannin levels like other phenolic compounds are affected by the environment and genetics. In berries rich in anthocyanin pigments, tannins stabilise and protect anthocyanins by binding to them to form copolymers.[45]

3.6 | Antioxidant activity

Fruit extracts of *Berberis* genotypes were evaluated for antioxidant capacity by DPPH assay. As shown in Figure 2, antioxidant activity of extracts was influenced by both the genotypes and sampling location. The highest antioxidant activity was obtained in G16 fruit extract (56.84%) and lowest antioxidant activity was observed in G13 fruit extract (13.20%). In the present study, *B. vulgaris* genotypes and *B. carataegina* showed higher antioxidant activities than *B. integerima* genotypes.

Hassanpour and Alizadeh[30] reported the amount of antioxidant activity by DPPH assay in different barberry genotypes from 20.47% to 74.72% which is in agreement with our present results. The evaluation of antioxidant capacity of *Berberis* genotypes showed that these genotypes possess considerable antioxidant potential due to the presence of simple phenolics, anthocyanins, phenolic acids and flavonoids. β-Carotene, vitamin C, butylated hydroxy toluene, phenolic compounds were suggested to be the compounds with strong radical-scavenging activity in fruit extracts of barberry.[9,19,46-49] Phenolic compositions of barberry extract included rutin, caffeic acid, chlorogenic acid, and apigenin have a high correlation with antioxidant activity. Rutin and caffeic acid strongly correlated with antioxidant activity in this study in agreement with other reports.[50,51] Castelluccio

TABLE 3 Content of biochemical compounds in fruits of different barberry genotypes

	Biochemical compounds of fruit extracts of barberry (mg/L)							
Code	Apigenin	Rutin	Quercetin	Gallic acid	Caffeic acid	Chlorogenic acid	*p*-Coumaric acid	Cinnamic acid
G1	1.96 h	7.61a	4.80jk	52.33c	51.78a	119.53a	48.02i	0.35d
G2	3.94c	2.73 k	3.20 m	29.95 l	ND	16.84e	9.99p	ND
G3	3.47d	5.71d	4.20 kl	ND	15.72b	11.89 g	14.30n	ND
G4	4.23b	3.45 h	4.10 l	ND	4.49j	11.37i	23.34 l	ND
G5	2.75f	3.39i	37.20a	48.04f	3.92 m	11.92f	29.64 k	ND
G6	2.21 g	3.48 g	9.80e	36.04i	4.27 k	11.92f	12.70o	ND
G7	4.44a	5.44c	13.80c	51.29e	5.28 g	18.25d	38.73j	0.43c
G8	1.85i	5.71b	6.60 h	51.61d	4.93 h	34.95c	20.10 m	0.46b
G9	ND	ND	4.60jkl	43.82 h	ND	11.66 h	257.09a	ND
G10	1.85i	ND	4.20 kl	30.20 k	5.81e	7.89j	99.04e	ND
G11	1.41 k	ND	9.40ef	ND	ND	ND	119.12c	0.33e
G12	1.39 l	4.65e	7.60 g	ND	3.97 l	3.05 m	85.76f	0.33e
G13	1.06 m	3.93f	5.20j	16.13 m	3.67n	3.17 l	112.68d	0.33e
G14	1.07 m	3.20j	16.20b	334.82a	4.68i	2.01n	171.11b	0.34de
G15	ND	ND	8.90f	ND	5.76f	4.45 k	85.14 g	ND
G16	ND	ND	5.90i	35.72j	9.93c	41.39b	ND	0.57a
G17	3.02e	ND	11.90d	45.86 g	ND	ND	80.04 h	ND
G18	1.68j	ND	6.80 h	54.38b	8.56d	ND	4.10q	ND

Note: Means with the same letter are not significantly different from each other ($P > 0.05$). ND, not detected.

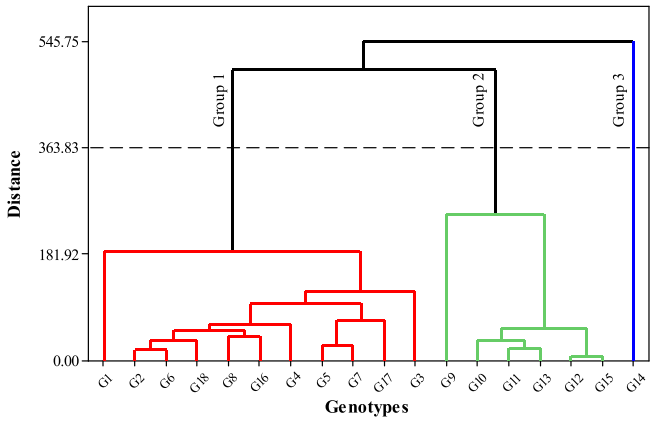

FIGURE 4 Hierarchical cluster analysis (HCA) of barberry genotypes based on the 14 main traits [Colour figure can be viewed at wileyonlinelibrary.com]

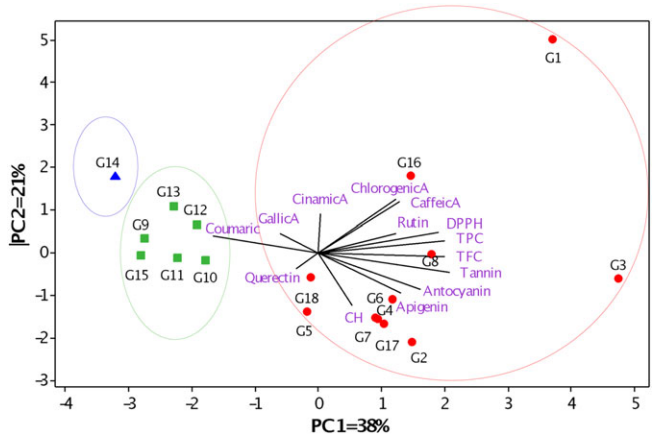

FIGURE 5 Principal component analysis (PCA) of barberry genotypes based on the 14 main traits. The red, green, and purple shapes represent the first, second and third groups of the cluster, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

et al.[52] reported that chlorogenic acid and caffeic acid were the most active compound in antioxidant capacity in comparison with *p*-coumaric acid. Furthermore, Cos et al.[53] noted that, caffeic acid had the highest scavenging activity in berry fruits.

3.7 | HPLC analysis of the extracts

Figure 3 shows the chromatograms of eight standards injected into HPLC. The amounts of individual phenolic acids (gallic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, cinnamic acid) and flavonoids (rutin, apigenin and quercetin) content were significantly variable amongst the 18 genotypes analysed in this study (Table 3). Gallic acid and *p*-coumaric acid were found as the most abundant phenolic compounds in the extracts of barberry fruits. The high concentrations of gallic acid (334.82 mg/L), caffeic acid (51.78 mg/L), chlorogenic acid (119.53 mg/L), *p*-coumaric acid (257.09 mg/L), cinnamic acid (0.57 mg/L), rutin (7.61 mg/L), apigenin (4.44 mg/L), and quercetin (37.20 mg/L) were obtained in G14, G1, G1, G9, G16, G1, G7, and G5 fruit extracts, respectively. In most genotypes, cinnamic acid was not detected or detected at a very low level. The highest concentrations of rutin, caffeic acid and chlorogenic acid were observed in *B. carataegina* (G1) fruit extracts grown in west Azerbaijan.

The content of apigenin, rutin, chlorogenic acid and cinnamic acid in fruit extracts of *B. crataegina* was reported as 20.08 mg/kg, 27.09 mg/kg, 70.24 mg/kg and 1.10 mg/kg, respectively.[13] The biosynthesis of phenolic composition of berry fruits can be endogenously-regulated during different developmental variations,[54] which can be affected by exogenous agents. Various exogenous factors such as environmental conditions (temperature, biotic and abiotic stress, light intensity, humidity) and agricultural practices (soil fertility, irrigation) influence phenolic compound biosynthesis and accumulation in medicinal plants.[15,55-57] Amino acid phenylalanine, produced through the shikimic acid pathway, is a precursor for several phenolic compounds. They are formed as a result of deamination of the phenylalanine by the enzyme phenyl alanine-ammonia lyase (PAL).[58] Environmental factors, especially light, is one of the most effective factors in the phenolic metabolism. Light with an impact on PAL, stimulates the synthesis of phenolic compounds.[59]

Wang et al.[60] reported that the content of phenolic compounds increased significantly with increasing of temperature and carbon dioxide (CO_2) concentration. The diversities found in this study among the genotypes in phenolic compounds could be related to environmental conditions such as geographical variations (altitude, latitude and height), light intensity and temperature.

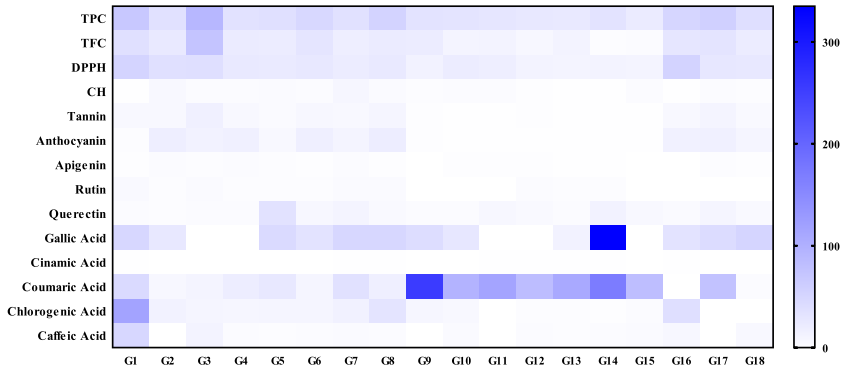

FIGURE 6 Biochemical compounds distribution in fruits of barberry genotypes with heat map visualisation. From white for low concentrations to blue for high concentrations (TPC, total phenolic content; TFC, total flavonoid content; CH; water-soluble carbohydrate) [Colour figure can be viewed at wileyonlinelibrary.com]

3.8 | Classification of barberry genotypes

HCA, PCA and heat-map visualisation were performed to classify the barberry genotypes regarding the 14 main traits (TPC, TFC, DPPH, TCHC, TTC, TAC, gallic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, cinnamic acid, rutin, apigenin, and quercetin). The cluster analysis was carried out by the Ward linkage method (Figure 4). Based on this analysis, the *Berberis* genotypes were classified into three main clusters. In the first cluster, genotypes of *B. vulgaris* and *B. carataegina* species (G1, G2, G3, G4, G5, G6, G7, G8, G16, G17, and G18) were designated as the genotypes with the highest antioxidant activity and phenolic compound content (except for gallic acid and *p*-coumaric acid). In the second cluster, genotypes of *B. integerima* species (G9, G10, G11, G12, G13 and G15) were determined due to high amounts of *p*-coumaric acid. The third cluster comprised genotype G14 (*B. integerima*) due to high levels of gallic acid.

PCA classification confirmed the results of cluster analysis (Figure 5). A PCA was performed, reducing the multidimensional structure of the data and providing a two-dimensional map to explain the variance observed. The first two components of the PCA explained 59% of the total variance (38% for component 1 and 21% for component 2). The first component (PC1) has strong positive correlation with TPC, TFC, DPPH, TTC, TAC, and *p*-coumaric acid. The second principal component (PC2) separates the samples according to TCHC, TAC, apigenin, cinnamic acid, caffeic acid, and chlorogenic acid. The heat map summarises the quantitative data on phytochemical characteristics and antioxidant activity distribution in fruit extracts of *Berberis* species (Figure 6). A colour was associated with the amount of compounds: from white for low concentrations to blue for high concentrations.

PCA and HCA were applicable approaches to check for classification among *Berberis* genotypes. Results of HCA and PCA revealed that genotypes of *B. vulgaris* and *B. carataegina* species possess considerable antioxidant potential due to the presence of TFC and TPC, TAC, TTC and individual phenolic compounds (caffeic acid, chlorogenic acid, cinnamic acid, rutin, apigenin and quercetin). A number of phytochemical studies have been reported from different barberry genotypes which prove their antioxidant properties. Anthocyanins,[19] phenolic acids and flavonoids,[17,61] present in fruits of barberry genotypes have antioxidant capacity as reported by various research groups.

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