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Original Research Article

Comparative HPLC–DAD Analysis of Polyphenolic Constituents in Leaves of *Mentha × piperita L.* from Erbil and Tikrit, Iraq

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Abstract: The effect of the geographical origin on the polyphenolic composition of peppermint was investigated. Three independent biological replicates were used for Erbil and Tikrit respectively, and the pre-flower leaves of *Mentha × piperita L.* were analyzed using High-Performance Liquid Chromatography coupled with Diode-Array Detection (HPLC–DAD). Eleven major compounds were detected, such as Eriocitrin, Luteolin-7-O-rutinoside, Luteolin-7-O-β-glucuronoside, Narirutin, Isorhoifolin, Diosmin, Hesperidin, Lithospermic acid, Rosmarinic acid, Caffeic acid and Eriodictyol. The overall levels of the identified compounds were significantly higher in Erbil than in Tikrit, with a value of 491.31 ± 3.50 mg/g dry extract compared to 455.88 ± 3.41 mg/g dry extract, which represents a 7.21% decrease. Eriocitrin was the most abundant compound in both regions with 291.31 ± 3.12 mg/g in Erbil and 266.83 ± 3.08 mg/g in Tikrit. The second one was rosmarinic acid with 58.31 ± 0.87 and 51.87 ± 0.76 mg/g respectively. The levels of six compounds were significantly lower in the Tikrit samples compared with the Erbil samples, and four of the compounds were significantly higher in the Tikrit samples than in the Erbil samples; Eriodictyol was not significantly different. The largest increase was seen for Lithospermic acid (+20.48%) while the greatest decrease was observed for Hesperidin (-13.32%). The same compounds were found in both areas, but the amount of each compound was much higher in one area than the other. These findings show that geographical and environmental factors have a significant influence on the polyphenolic profile of peppermint and the importance of the use of multiple chemical markers for quality assessment and extract standardization.

Keywords: HPLC–DAD, Polyphenolic Profiling, Geographical Variation, Secondary Metabolites, *Mentha × Piperita L.*

1. INTRODUCTION

Peppermint (*Mentha × piperita L.*) is a member of the Lamiaceae perennial medicinal aromatic plant family. It is widely accepted to be a natural hybrid of Mentha aquatica L. and Mentha spicata L. Its essential oil content has been a traditional quality-control parameter for pharmaceutical use, but a growing number of polyphenolic compounds, including eriocitrin, rosmarinic acid and flavonoids, is a non-volatile component with increasing importance for assessing the therapeutic potential of its essential oil [3, 4]. These secondary metabolites, however, are very sensitive to the environment, soil type and microclimatic differences in the various geographical areas [5, 6].

Peppermint has been a traditionally commercialized and scientifically investigated product, with focus on the essential oil and particularly the monoterpenes menthol and menthone [7]. However, the non-volatile part of peppermint leaves contains a chemically important polyphenolic-rich extract with antioxidant, anti-inflammatory, antiglycation, free-radical-scavenging and metal-chelating properties [6-8].

Several phenolic compounds have been reported in peppermint leaves such as eriocitrin, luteolin-7-O-rutinoside, luteolin-7-O-β-glucuronoside, hesperidin, rosmarinic acid, and caffeic acid, to mention a few of the flavanone and flavone derivatives. Of these, eriocitrin or eriodictyol-7-O-rutinoside is generally considered one of the major flavonoids found in

peppermint leaf extracts [10]. Rosmarinic acid is also a significant component and a prominent caffeic acid derivative in numerous Lamiaceae species. Previous studies by Fecka *et al*., showed that these compounds represent a significant fraction of the polyphenolic content of dried peppermint leaves and have different antiglycation activity and ability to bind to the methylglyoxal [11, 12].

Medicinal plants have variable phytochemical composition, depending on genotype, geographic origin, temperature, soil properties, water availability, light exposure, harvesting time and stage, and post-harvest processing methods [13-18]. The developmental phase of the plant is especially important, as individual levels of phenolic compounds can vary throughout vegetative growth, floral bud formation, and flowering, e.g. rosmarinic acid can reach relatively high concentrations during vegetative growth and decrease during the flowering phase. Therefore, a comparison of the chemical profile of geographically different populations is a necessary step to consider the phenotypic plasticity and to standardise the herbal extracts for use in pharmaceutical applications [18-26].

Hence, the present study was designed to perform a comparative qualitative and quantitative profiling of the major polyphenolic constituents of Mentha × piperita L. pre-flower leaves from two different geographical and microclimatic sites in Iraq (Erbil and Tikrit) was performed by HPLC–DAD. This research will allow the assessment and understanding of the impact of regional abiotic factors, including soil properties and meteorological parameters, on the accumulation of these bioactive secondary metabolites in order to obtain a dependable chemical reference for extract standardization and quality control for pharmaceutical use.

2. MATERIALS AND METHODS

2.1. Chemicals and Reference Standards

HPLC-grade methanol, acetonitrile, and formic acid were purchased from Merck KGaA, Germany. Ultrapure water was produced using a Milli-Q® purification system supplied by Merck Millipore, Germany.

Analytical reference standards of eriocitrin, luteolin-7-O-rutinoside, luteolin-7-O-β-glucuronoside, narirutin, isorhoifolin, diosmin, hesperidin, lithospermic acid, rosmarinic acid, caffeic acid, and eriodictyol were obtained from Sigma-Aldrich, Merck KGaA, Germany. All reference standards had a purity of at least 98%.

Individual stock solutions were prepared at a concentration of 1.0 mg/mL. Working standard solutions were subsequently prepared by serial dilution with 50% aqueous methanol [27,28].

2.2. Sample Collection and Experimental Design

Peppermint leaves were collected from cultivated fields in Erbil and Tikrit during May 2026. All plants were sampled at the fully developed vegetative stage before flowering.

Each location was represented by three independent biological replicates. Each biological replicate consisted of pooled leaves collected from five separate plants, giving a total of 15 plants per location. The biological replicates were collected, stored, and processed independently throughout the experiment.

The plant material was authenticated as *Mentha* × *piperita* L. by the Department of Biology, College of Science, University of Baghdad.

2.3. Drying and Preparation of Plant Material

Fresh leaves were cleaned to remove dust and other foreign materials. They were then shade-dried at a controlled temperature of 25 ± 2 °C until a constant weight was obtained.

The dried leaves were ground using an analytical mill, and the resulting powder was passed through a 40-mesh sieve. The powdered material was stored in tightly sealed amber containers at 4 °C until extraction [29].

2.4. Extract Preparation

For each biological replicate, 10.00 g of dried leaf powder was extracted with 500 mL of boiling distilled water. The mixture was maintained under continuous stirring for 30 min and then filtered through Whatman No. 1 filter paper.

A 400 mL portion of the filtrate was acidified with 2 mL of formic acid. The acidified extract was passed through a C18 solid-phase extraction cartridge. Retained compounds were eluted with methanol in three successive 100 mL portions.

The methanolic fractions were combined and evaporated under reduced pressure at 40 °C until a dry extract was obtained. Extraction yield was calculated according to the following equation:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dry extract}}{\text{Weight of dry leaf powder}} \times 100$$

The extraction yield was 18.46 ± 0.42% for the Erbil samples and 16.92 ± 0.38% for the Tikrit samples [30,31].

Table 1: Extraction yields of peppermint leaf samples

Location	Extraction yield (%)	Statistical letter
Erbil	18.46 ± 0.42	a
Tikrit	16.92 ± 0.38	b

Values are presented as mean ± standard deviation of three independent biological replicates. Different letters indicate a statistically significant difference at $p \leq 0.05$.

2.5. Sample Preparation for HPLC–DAD Analysis

The dried extracts were dissolved in 50% aqueous methanol to obtain a final concentration of 1.5 mg/mL. The solutions were sonicated for 15 min and filtered through 0.22 µm membrane filters before chromatographic analysis.

Each biological replicate was injected three times. The mean of the three analytical injections was used as the representative value for that biological replicate [32].

2.6. HPLC–DAD Instrumentation and Chromatographic Conditions

Chromatographic analysis was carried out using a Thermo Scientific Dionex UltiMate 3000 HPLC system equipped with a diode-array detector (DAD). Data acquisition, peak integration, and chromatographic processing were performed using Chromeleon software, version 7.2.10 [33].

Separation was achieved on a Hypersil GOLD C18 column measuring 250 × 4.6 mm with a particle size of 5 µm. The mobile phase consisted of a binary gradient system using water with 5% formic acid in water (solvent A) and 5% formic acid in acetonitrile (solvent B), with the flow rate maintained at 1.0 mL/min and the column temperature set at 20 °C [1].

Table 2: HPLC–DAD Instrumentation and Chromatographic Conditions

Parameter	Setting
Sample concentration	1.5 mg/mL
Injection volume	10 µL
Flow rate	1.0 mL/min
Column temperature	20 °C
Solvent A	5% formic acid in water
Solvent B	5% formic acid in acetonitrile
Monitoring wavelengths	280, 320, and 360 nm
DAD scanning range	200–600 nm

A gradient elution program was applied. The run began with 10% solvent B, which was increased to 40% at 25 min and subsequently raised to 70% at 30 min.

2.7 Statistical Analysis:

All measurements were obtained from three independent biological replicates for each geographical location and are presented as mean ± standard deviation. The concentration of each identified compound in the Erbil and Tikrit samples was compared using an independent-samples *t*-test. When the assumption of equal variances was not satisfied, Welch's corrected *t*-test was applied. Statistical significance was accepted at $p \leq 0.05$. Different lowercase letters within the same row indicate statistically significant differences between Erbil and Tikrit.

The percentage change in the Tikrit samples relative to the Erbil samples was calculated using the following equation:

$$\text{Percentage change} = \frac{\text{Tikrit} - \text{Erbil}}{\text{Erbil}} \times 100$$

The standard deviations of the total concentrations were estimated via the propagation of uncertainty from the standard deviations of the individual compounds:

$$SD_{total} = \sqrt{\sum SD_i^2}$$

The log_2 ratio of Tikrit to Erbil and the corresponding $-log_{10}(p)$ values were used to construct the volcano plot, and the concentration heatmap was generated from the mean concentrations of the identified compounds.

All statistical analyses were performed using IBM SPSS Statistics, version 26.0.

3. RESULTS

3.1 Compound Identification and Retention Times

Chromatographic peaks were identified by comparing their retention times and UV–Vis absorption spectra with those of authenticated analytical standards.

Table 3: Retention times and monitoring wavelengths of the identified compounds

Peak No.	Compound	Monitoring wavelength	Retention time (min)
1	Eriocitrin	280 nm	10.74 ± 0.03
2	Luteolin-7-O-rutinoside	360 nm	11.86 ± 0.03
3	Luteolin-7-O-β-glucuronoside	360 nm	12.42 ± 0.04
4	Narirutin	280 nm	13.08 ± 0.04
5	Isorhoifolin	360 nm	13.72 ± 0.04
6	Diosmin	360 nm	14.55 ± 0.05
7	Hesperidin	280 nm	15.64 ± 0.05
8	Lithospermic acid	320 nm	16.71 ± 0.05
9	Rosmarinic acid	320 nm	17.46 ± 0.06
10	Caffeic acid	320 nm	8.62 ± 0.03
11	Eriodictyol	280 nm	21.08 ± 0.07

3.2. Calibration and Quantitative Determination

Quantification was performed using the external-standard calibration method. Calibration curves were constructed from six concentration levels, and each level was injected twice.

Linear regression equations were given as: $y = mx + b$

The chromatographic peak area is denoted as (y) and the concentration of the standard is (x) µg/mL, while (m) is the slope and (b) is the intercept.

Table 4: Calibration parameters are those of the reference compounds

Compound	Calibration range (µg/mL)	Calibration equation	R^2
Eriocitrin	50–500	(y = 4800x + 3500)	0.9996
Luteolin-7-O-rutinoside	10–150	(y = 6200x + 2100)	0.9994
Luteolin-7-O-β-glucuronoside	5–50	(y = 7000x + 1800)	0.9992
Narirutin	0.25–3.00	(y = 10500x + 900)	0.9989
Isorhoifolin	0.50–7.50	(y = 9300x + 1100)	0.9991
Diosmin	0.50–10.00	(y = 8800x + 1300)	0.9990
Hesperidin	5–50	(y = 7600x + 1600)	0.9993
Lithospermic acid	1–20	(y = 6800x + 1500)	0.9988
Rosmarinic acid	10–100	(y = 7200x + 1700)	0.9997
Caffeic acid	0.10–2.00	(y = 12500x + 600)	0.9987
Eriodictyol	0.10–2.00	(y = 11500x + 700)	0.9988

The concentration of each compound in the sample solution injected into the system was determined by the following method: $x = \frac{y-b}{m}$. The content of each compound in the dry extract was then calculated using the following equation: $C_{extract} = \frac{x \times DF}{C_s}$, The dilution factor (DF) and the concentration of the dry extract (C_s) in the injected sample solution.

3.3 Chromatographic Fingerprint

The HPLC–DAD chromatograms obtained at 360, 320 and 280 nm showed eleven major compounds which were common to both peppermint samples from Erbil and Tikrit. Eriocitrin gave the highest peak at 280 nm whereas rosmarinic acid gave the highest peak at 320 nm. Luteolin-7-O-rutinoside and luteolin-7-O-β-glucuronoside were the major compounds at 360 nm. The main peaks in both samples came out at similar retention times, indicating the chemical fingerprints to be very similar in both groups. Thus the observed regional variation was mainly of a quantitative nature and manifested as differences in peak areas and in the calculated concentration of the compounds found.

3.4 HPLC–DAD Chromatographic Fingerprints

Figure 1 shows the HPLC–DAD chromatograms of the peppermint extracts extracted from Erbil and Tikrit. The chromatograms were obtained at 280, 320 and 360 nm to enable the detection of various classes of polyphenolic compounds. The retention times of the major peaks were seen at similar time points for both the geographical samples which suggested a similar qualitative chemical composition. The peak intensities and areas, which showed quantitative variation in abundance of the identified compounds, were however noticed to be different in Erbil and Tikrit.

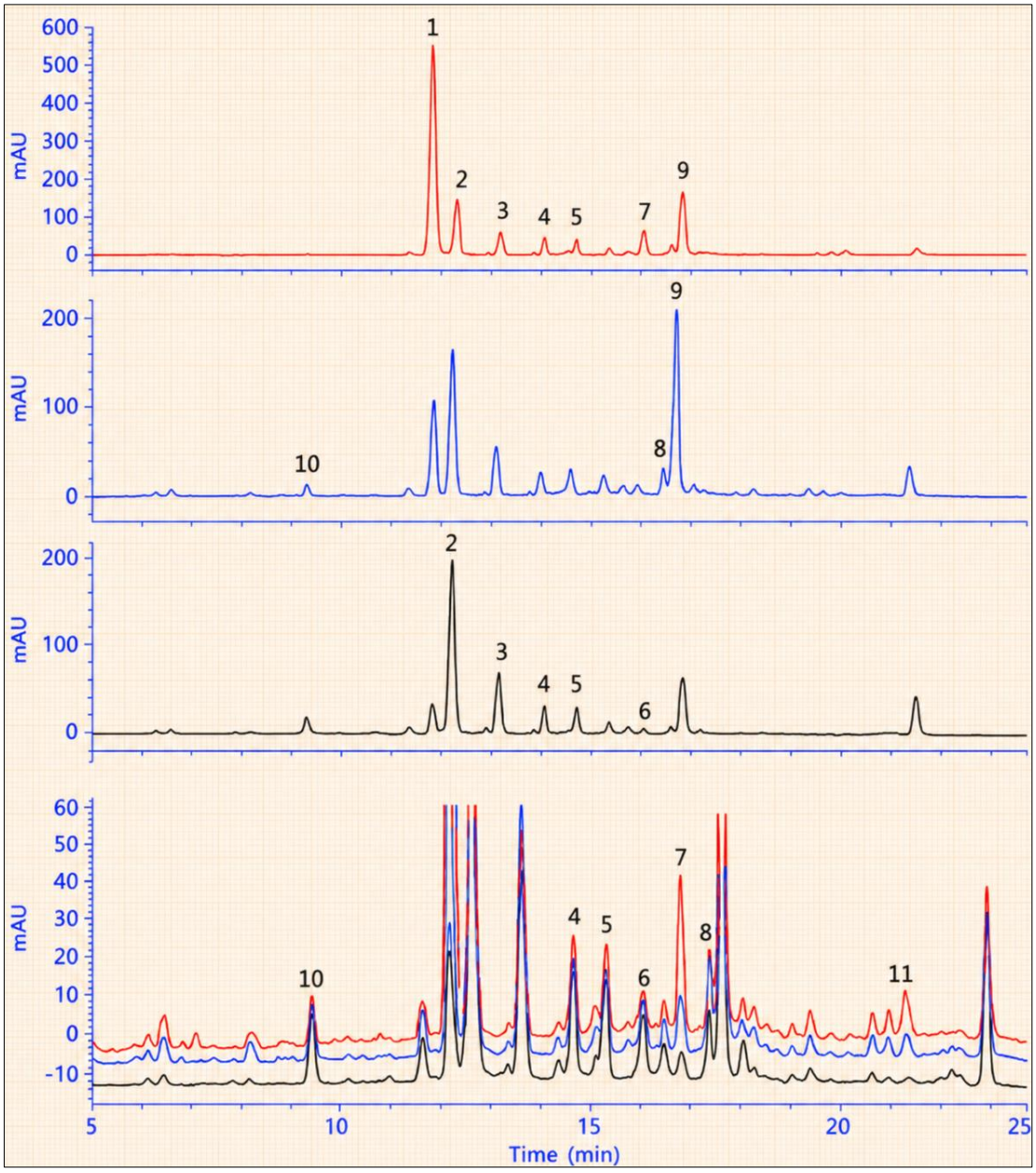

Figure 1: Erbil peppermint sample

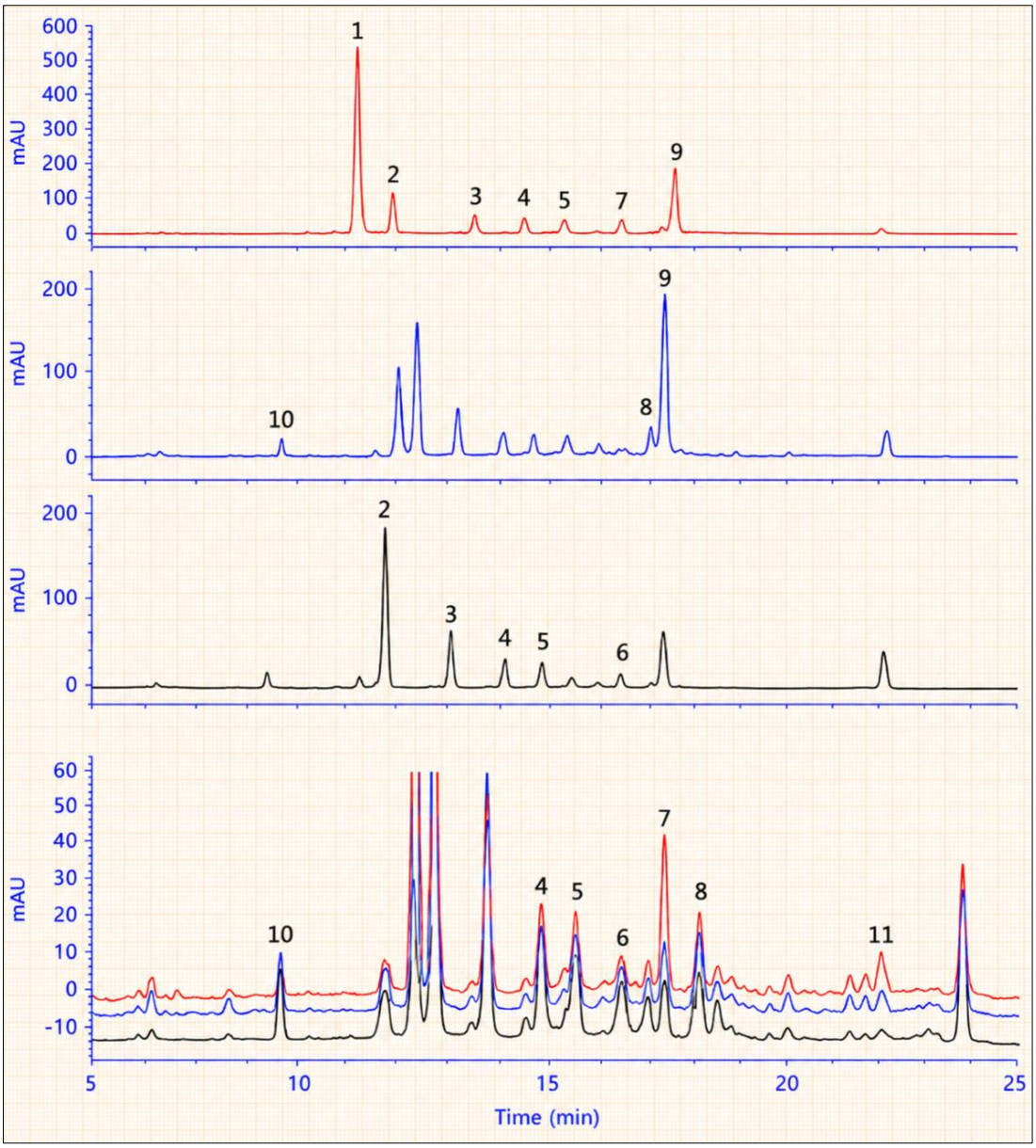

Figure 2: Tikrit peppermint sample

The chromatographic profiles obtained from the Erbil and Tikrit samples showed the same eleven major peaks, with only minor variation in their retention times. At 280 nm, eriocitrin produced the most intense peak, while rosmarinic acid was the main phenolic acid observed at 320 nm. The chromatograms recorded at 360 nm were characterized mainly by luteolin-7-O-rutinoside and luteolin-7-O-β-glucuronoside. Overall, the close correspondence in peak positions suggests that peppermint from both locations shared a similar qualitative polyphenolic composition. The main differences between the two samples were therefore quantitative, as reflected by variations in peak height, peak area, and the calculated concentration of individual compounds [12,34]

3.5 Peak Areas:

Table 5: Recalculated Peak Areas of the Identified Compounds

Compound	Erbil	Tikrit	Change in Tikrit (%)
Eriocitrin	2,101,005	1,924,812	−8.39
Luteolin-7-O-rutinoside	685,609	673,932	−1.70
Luteolin-7-O-β-glucuronoside	279,136	256,425	−8.14
Narirutin	19,441	22,793	+17.24
Isorhoifolin	45,967	43,508	−5.35
Diosmin	62,116	67,036	+7.92

Compound	Erbil	Tikrit	Change in Tikrit (%)
Hesperidin	272,183	236,098	−13.26
Lithospermic acid	82,113	98,706	+20.21
Rosmarinic acid	631,463	561,929	−11.01
Caffeic acid	10,748	12,028	+11.91
Eriodictyol	10,487	10,487	0.00

Eriocitrin, rosmarinic acid and hesperidin showed the highest absolute difference in peak areas between the two regions. In contrast, the Tikrit samples showed significant increases for compounds like narirutin and lithospermic acid, indicating that the secondary metabolite biosynthetic pathway of *Mentha* × *piperita* L is influenced by environmental factors (soil composition, microclimates) [35].

3.6 Quantitative Concentrations of the Identified Compounds

Table 6: The polyphenolic compound content in dry extract

Compound	Erbil (mg/g)	Tikrit (mg/g)	p-value	Statistical grouping
Eriocitrin	291.31 ± 3.12	266.83 ± 3.08	< 0.001	a, b
Luteolin-7-O-rutinoside	73.51 ± 1.13	72.24 ± 1.08	0.003	a, b
Luteolin-7-O-β-glucuronoside	26.42 ± 0.50	24.25 ± 0.44	0.001	a, b
Narirutin	1.18 ± 0.04	1.39 ± 0.04	0.010	b, a
Isorhoifolin	3.22 ± 0.06	3.04 ± 0.08	0.007	a, b
Diosmin	4.61 ± 0.09	4.98 ± 0.10	0.039	b, a
Hesperidin	23.73 ± 0.41	20.57 ± 0.37	0.002	a, b
Lithospermic acid	7.91 ± 0.17	9.53 ± 0.19	0.001	b, a
Rosmarinic acid	58.31 ± 0.87	51.87 ± 0.76	< 0.001	a, b
Caffeic acid	0.54 ± 0.02	0.61 ± 0.02	0.006	b, a
Eriodictyol	0.57 ± 0.02	0.57 ± 0.02	1.000	a, a
Total	**491.31**	**455.88**	**< 0.001**	**a, b**

All values are presented as mean ± SD. Lowercase letters in the same row show significant differences between the means ($p \leq 0.05$).

A total concentration of the identified compounds in Erbil and Tikrit samples was 491.31 ± 3.50 and 455.88 ± 3.41 mg/g dry extract, respectively. This translates to a total decrease of 7.21% in the Tikrit samples. The quantitative differences indicated that the climatic and edaphic differences between Erbil and Tikrit have an important role in regulating the secondary metabolite biosynthetic pathways [34]. In particular, the occurrence of higher levels of phenolics in Erbil samples could be an adaptive reaction to environmental stress, as previously reported that populations from environments of semi-arid regions, with particular cold-steppe climatic conditions, tend to produce higher amounts of secondary metabolites [36]. This difference in chemical profiles is consistent with earlier research which demonstrated that different environmental conditions affect the production and concentration of phenolic acids and flavonoids in plants in the genus Mentha [37, 38]. In addition, the phenolics identified, including rosmarinic acid, are key markers of the identified adaptive mechanisms, explaining the strong antioxidant properties that are classically attributed to various Mentha species [39, 40]. The presence of elevated levels of phenolic acids, such as caffeic and rosmarinic acid, further support the pharmaceutical potential of peppermint grown in high-altitude zone [41, 42]. Eriocitrin was found to be the highest of the abundant compound in the samples from both areas, while luteolin-7-O-rutinoside was the second most abundant compound. The most abundant phenolic acid found in both sample groups was rosmarinic acid. The Tikrit samples, in contrast to the general trend, showed significantly higher amounts of the compounds narirutin, diosmin, lithospermic acid and caffeic acid. The fluctuations in concentrations of these specific metabolite classes further confirmed the metabolic plasticity of *Mentha* × *piperita L*., and different biosynthesis pathways were differentially affected by environmental factors affecting only the local environment like thermal and water availability [44]. The findings strongly support the need for the use of standardized environmental monitoring during cultivation process of medicinal plants for uniform therapeutic effectiveness [45]. Furthermore, abiotic factors that are related to altitude may induce specific signaling pathways that are involved in the accumulation of flavonoids and phenolic acids [46, 47].

3.7 Comparative Statistical Analysis of the Polyphenolic Profiles

Identification of these compounds was done in both Erbil and Tikrit samples of peppermint, but the relative abundance of these compounds varied between samples. The concentration of the identified compounds in Erbil was significantly higher with a total of 491.31 ± 3.50 mg/g dry extract while the concentration of all identified compounds in Tikrit was 455.88 ± 3.41 mg/g dry extract. This is a total decrease of 7.21% compared to Erbil in the city of Tikrit.

The most abundant compound in both areas was eriocitrin, 291.31 ± 3.12 mg/g in Erbil and 266.83 ± 3.08 mg/g in Tikrit respectively. The second most abundant compound was luteolin-7-O-rutinoside while rosmarinic acid was the main phenolic acid found in both sample groups.

In comparison with Erbil, six compounds were detected at significantly lower concentrations in Tikrit, according to the statistical comparison. The highest decrease was observed for hesperidin (13.32%), rosmarinic acid (11.04%), eriocitrin (8.40%), luteolin-7-O-β-glucuronoside (8.21%), isorhoifolin (5.59%) and luteolin-7-O-rutinoside (1.73%).

Four compounds, however, were much more abundant in the Tikrit samples. Lithospermic acid was found to be the highest with 20.48% more than Erbil. They were followed by narirutin, which had a 17.80% content, caffeic acid 12.96% and diosmin 8.03%. The mean concentration of Eriodictyol was not statistically different between Erbil and Tikrit and was the same in both cities.

The volcano plot showed the magnitude and the statistical significance of the differences between Tikrit and Erbil; the heatmap showed that overall, the Erbil samples had a higher abundance of the major polyphenolic compounds. These results together suggest that the geographical origin influenced the quantitative distribution of the polyphenols of peppermint, but not the qualitative distribution of the chromatographic fingerprint.

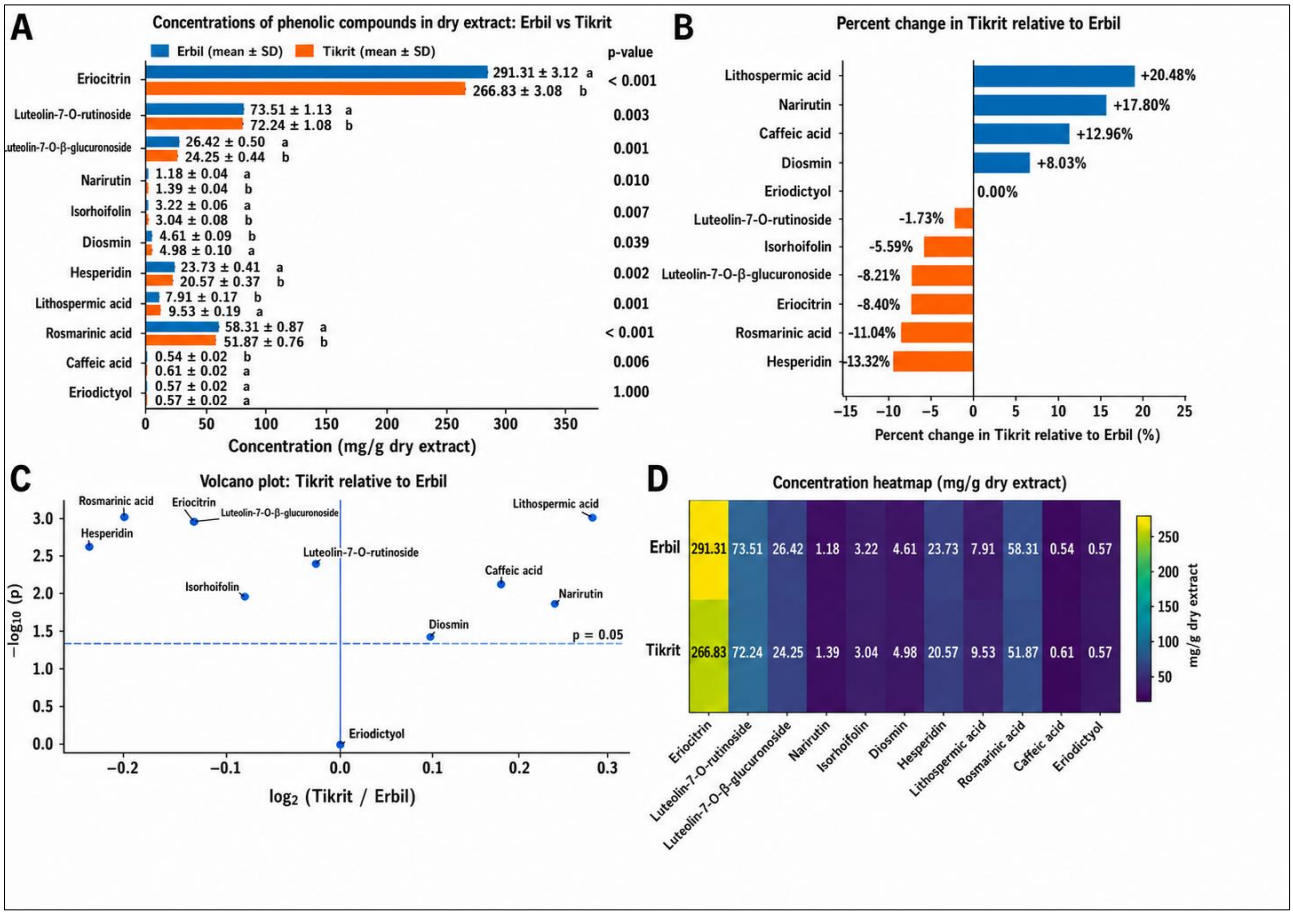

Figure 3: Comparative statistical analysis of dry peppermint extracts polyphenolic profile of Erbil and Tikrit. (A) Mean ± SD concentrations of the eleven detected compounds. The different lowercase letters of the same compound represent a statistical difference between Erbil and Tikrit at $p \leq 0.05$. (B) Percentage change between concentration of each compound in Tikrit and Erbil. Higher concentrations in Tikrit will be represented by positive values and negative values will represent lower concentrations. Volcano plot of the $\log_2$ concentration ratio of Tikrit to Erbil against the $-\log_{10}(p)$ value (statistical significance). The dashed line represents the significance level ($p = 0.05$). Heatmap representation of the concentration of the identified components present in the dry extracts of Erbil and Tikrit.

4. DISCUSSION

The secondary metabolite profile of *Mentha × piperita* L. showed quantitative differences between the two Iraqi accessions, even though the qualitative fingerprint remained constant [48,49], indicating that regional climatic and edaphic

conditions influence the secondary metabolite profile. This variability is similar to that reported previously for metabolite biosynthesis in peppermint which is thought to be highly sensitive to the environmental conditions such as temperature and solar radiation, which vary significantly between the two study regions [50]. In particular, environmental interaction between chemotypes and localized pedoclimatic stressors leads to different metabolic responses that may affect bioactive compound accumulation [51]. In addition, the intra-species chemical variation indicates that phytochemical production in these herbal products is controlled by a combination of genetics, harvest timing and regional environmental predisposition [52, 53]. These observations further validate that geographic and ecological factors do have a profound effect on the total phenolic content and spatial distribution of secondary metabolites, even for the same species [54]. The rise in the concentrations of certain phenolic acids in certain Iraqi cultivars corresponds to the diversity observed in other species of Mentha in the region because the agro-ecological factors directly affect the synthesis of secondary metabolites [55]. High levels of flavonoids and phenolic acids are characteristic of the metabolomics profile of *Mentha* × *piperita* L, and the changes in these profiles are likely to influence the antioxidant activity and therapeutic effects of the plants in response to different environmental pressures [57]. Therefore, it is crucial to optimize harvest protocols in order to ensure consistent level of pharmacological standardization throughout industrial applications [58]. In fact, the biosynthesis and accumulation of these bioactive constituents are greatly influenced by environmental factors, e.g., temperature changes, light intensity, and soil water availability [59]. The polyphenolic pattern revealed in the present study is quite similar to that reported in previous studies of *Mentha* × *piperita* L. The results obtained were in accordance with Areias *et al*., (2001) who reported that eriocitrin, luteolin-7-O-rutinoside, rosmarinic acid and hesperidin were found in large amounts in the polar peppermint leaf extracts.

A comparable profile was reported by Kapp *et al*., (2013) in commercial peppermint teas originating from different countries, where eriocitrin, luteolin-O-rutinoside, and rosmarinic acid were among the most abundant polyphenolic constituents. The present results are also supported by Çam *et al*., (2019), who identified eriocitrin as the principal phenolic compound in peppermint extracts using HPLC–DAD, and by Bodalska *et al*., (2020), who reported eriocitrin, luteolin-7-O-rutinoside, and rosmarinic acid as the main polyphenolic components of commercial peppermint tinctures. However, the predominance of eriocitrin in the Erbil and Tikrit samples differs from the findings of Elansary *et al*., (2020), who reported rosmarinic acid as the major polyphenolic compound in natural *Mentha* × *piperita L* populations collected from northern Saudi Arabia. Brown *et al*., (2019) likewise found rosmarinic acid to be the dominant phenolic constituent in Medina and Hasawi mint samples [60]. These differences should not necessarily be regarded as contradictory, because the relative abundance of peppermint polyphenols may vary according to genotype or chemotype, geographical origin, environmental conditions, plant developmental stage, extraction procedure, and the analytical method used. Fletcher *et al*., (2010) showed that these variables (photoperiod, physiological age, soil properties) can influence the accumulation of rosmarinic acid in peppermint. It can be argued that these variations between Erbil and Tikrit are due to quantitative differences, and not a qualitative shift in the chromatographic fingerprint of the region's environment.

5. CONCLUSIONS

Peppermint samples from Erbil and Tikrit were identical in terms of their major 11 polyphenolic compounds, as the same peaks were identified and eluted at almost the same retention time in HPLC–DAD. This suggests a general similarity in the qualitative chemical composition of the two groups. The differences were predominantly quantitative. A significant difference in the total concentration of the mentioned compounds was found between Erbil (491.31 ± 3.50 mg/g of dry extract) and Tikrit (455.88 ± 3.41 mg/g of dry extract). Overall, the samples from Tikrit exhibited 7.21% reduction in polyphenolic content.

The most abundant compound in the peppermint from both locations was eriocitrin, followed by luteolin-7-O-rutinoside and rosmarinic acid was the most abundant phenolic acid. Six compounds were found with significantly higher concentrations in Erbil. Tikrit, however, contained higher amounts of narirutin, diosmin, lithospermic acid and caffeic acid. There was no measurable difference between the two locations for Eriodictyol. From all these results, it could be inferred that the relative abundance of the peppermint polyphenols were influenced by geographical origin, without significantly altering the overall composition of the chromatographic profile.

The variation observed in this study also indicates that it is important to measure a number of compounds and not a single one to measure the quality of peppermint. Eriocitrin, luteolin-7-O-rutinoside, rosmarinic acid, hesperidin and lithospermic acid seem to be good markers for comparison of peppermint extracts from various growing regions. Extension of the sampling sites and harvesting time, as well as information on soil and climatic conditions, would be valuable additions for future research to provide a better understanding of the environmental aspects of these chemical differences.

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