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Comparison of Phytochemical and Antioxidant Properties of *Thymbra Spicata* L. Supplied from Different Geographical Regions

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ABSTRACT

Thymbra spicata L. is an aromatic medicinal plant belonging to the Lamiaceae family, known in traditional medicine for its antimicrobial, anti-inflammatory, and antioxidant properties. Due to its rich chemical composition of phenolic compounds, this species can exhibit significant variations in phytochemical composition depending on the geographical conditions in which it grows. In this study, the phenolic compound profiles, total phenolic (TPC) and total flavonoid (TFC) contents, and antioxidant capacity of *T. spicata* L. samples obtained from Hatay (Turkey) and Yasuj (Iran) were comparatively evaluated. An approach consistent with the principles of green analytical chemistry was adopted in the analytical process, using ethanol as the primary solvent in all extractions and measurements. Quantitative determinations of catechin, esculetin, caffeic acid, coumarin, rutin, ellagic acid, resveratrol, luteolin, and apigenin were performed using a rapid and sensitive HPLC method. As a result of the analyses, the Hatay extract was found to have significantly higher values of compounds such as quercetin (2.138 µg mg^{-1}), esculetin (1.209 µg mg^{-1}), and apigenin (1.232 µg mg^{-1}) compared to Yasuj extract ($p < 0.0001$). In TPC and TFC measurements, Hatay extract showed higher contents with 92.258 ± 3.162 mg GAE g^{-1} and 38.591 ± 0.311 mg RE g^{-1}, respectively (n=3). Antioxidant activities of *Thymbra spicata* L. samples were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), and Cupric Reducing Antioxidant Capacity (CUPRAC) methods; radical scavenging and metal ion reducing capacities of Hatay extract were found to be significantly higher than Yasuj extract. In general, the results indicate that environmental differences depending on the growth region play a determining role on the phytochemical composition and biological activity of *T. spicata*.

Keywords: Antioxidant activity, HPLC, *Thymbra spicata L.,* TFC, TPC.

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Introduction

Oxidative stress occurs when the balance between the production of reactive oxygen species (ROS) and antioxidant defense systems in the body is disrupted. This can trigger many chronic and degenerative conditions, such as cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders [1,2]. Higher levels of ROS can damage cellular macromolecules such as lipids, proteins, and DNA. This can lead to impaired cell function, inflammation, and apoptosis [3]. To prevent such cellular damage, antioxidant compounds strengthen cellular defenses by either directly scavenging ROS or enhancing the activity of antioxidant enzymes. In recent years, natural bioactive compounds of plant origin have become a promising area of research due to their ability to affect multiple molecular targets, their low toxicity, and their broad-spectrum protective effects. [4-6].

Phenolic compounds and flavonoids obtained from plants are the main antioxidant substances that play an important role in coping with oxidative stress in living organisms [7]. These compounds can directly neutralize free radicals thanks to the hydroxyl groups they contain in their aromatic rings. They can also prevent Fenton-type reactions by complexing with transition metals [8,9]. The properties of these phytochemical compounds found in plants are largely determined by their biosynthetic pathways, environmental compatibility, and oxidative stability along with the pharmacokinetic properties of the bioactive compounds [10,11]. The structural diversity and reactivity of phenolic compounds make them potent free radical scavengers and metal chelators. These properties are associated with the antioxidant, anti-inflammatory, and anticancer effects of such compounds [12–14].

Thymbra spicata L., belonging to the Lamiaceae family, is a perennial plant species that grows naturally in the Eastern Mediterranean basin and is known for its aromatic and medicinal properties [15]. Widely found in the Turkish flora, this plant is known among the public as "Zahter," "Dağ thyme," and "Karabaş thyme" and is used in traditional medicine for the treatment of digestive disorders, infections, and inflammatory diseases [16,19]. T. spicata is notable for its rich secondary metabolite content; it is particularly rich in essential oils, phenolic acids, flavonoids, diterpenoids, and tannins [20]. The presence of these components supports the plant's antioxidant, anti-inflammatory, anticancer, antimicrobial, and neuroprotective effects, making it a biologically valuable species [21]. T. spicata, whose antioxidant potential is largely due to phenolic compounds, may exhibit protective

effects against cellular disorders associated with oxidative stress. These properties make it valuable not only for traditional use but also for modern pharmaceutical applications and functional food formulations [22]. Therefore, T. spicata, with its rich phenolic profile and versatile biological effects, is considered an important natural resource for the development of phytopharmaceutical, nutraceutical, and cosmetic products.

Spectrophotometric analysis methods are among the basic techniques frequently used to investigate the antioxidant properties of medicinal and aromatic plants such as *Thymbra spicata* L. colorimetric tests such as DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power) and CUPRAC (Cupric Ion Reducing Antioxidant Capacity) are used to evaluate the free radical scavenging ability and electron transfer capacity of plant extracts [23,24]. The findings obtained through these tests allow to establish a strong relationship between the redox behavior of herbal extracts and their biological effects. In addition, total phenolic content (TPC) and total flavonoid content (TFC) analyses are among the important indicators of antioxidant capacity by determining the overall amounts of phenolic and flavonoid compounds containing hydroxyl groups in the extracts [25]. These analyses reveal the distribution of the amounts of the source components of antioxidant activity, thus contributing to a better understanding of the chemical basis of antioxidant performance.

A detailed and component-specific analysis of the complex structure of plant extracts requires the use of advanced separation techniques that offer high resolution and selectivity, particularly high-performance liquid chromatography (HPLC) [26]. HPLC is a highly sensitive and selective method that allows for the qualitative and quantitative determination of phenolic compounds. This method enables the separation, identification, and quantification of individual phenolic compounds. This allows direct correlations to be established between the chemical profile of the plant and the observed antioxidant activity [27]. Therefore, the combined use of spectrophotometric and HPLC analyses is an important analytical method for comprehensively and scientifically reliable assessment of the effects of plant-derived antioxidants.

The environmental impacts and toxicological profiles of solvents used in analytical processes are being reassessed in line with the principles of Green Analytical Chemistry (GAC), and the use of environmentally friendly and sustainable alternative solvents that are harmless to human health is being encouraged [28]. In this context, ethanol stands out as an ideal solvent that can be used in all stages of the analytical process—including extraction, preparation of antioxidant analysis solutions, and HPLC sample preparation. Its availability from renewable biological sources, low toxicological risk profile, readily biodegradable, and environmental sustainability make it widely accepted as a solvent that meets the GAC criteria [29,30]. These properties of ethanol not only increase the safety and efficiency of chemical analyses but also support the adoption of environmentally responsible approaches in the phytochemical analysis of medicinal and aromatic plants such as *Thymbra spicata* L., contributing to the widespread adoption of green laboratory practices.

In this study, the antioxidant potential of *Thymbra spicata* L. was comparatively evaluated using samples obtained from two different sources. The TPC, TFC and antioxidant capacities of the extracts were determined, and their phenolic compound profiles were also analyzed by chromatographic methods. Ethanol was used in all analytical processes to ensure compliance with green analytical chemistry principles. The findings reveal that the bioactive compound content of *Thymbra spicata* L. varies depending on the geographical source; these differences can directly impact the plant's functional properties and potential pharmaceutical, food, and cosmetic industry.

Materials and Methods

Chemicals and Reagents

Catechin, esculetin, caffeic acid, coumarin, rutin, ellagic acid, resveratrol, quercetin, and apigenin HPLC standards with a chemical purity higher than 98% were obtained by Sigma-Aldrich. Chemicals such as Folin reagent, DPPH, Na_2CO_3, CH_3COONa, $AlCl_3$, TPTZ, $FeCl_3{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$, neocuproine, gallic acid, rutin, trolox, and ascorbic acid were also supplied by the same company with a purity higher than 95%. Ethanol (HPLC-grade) and orthophosphoric acid used in HPLC analyses were provided by Merck Specialities Pvt. Ltd. (Darmstadt, Germany). Before analyses, mobile phase solutions and *Thymbra spicata* L. extracts were filtered using PTFE membrane filters (Sartorius).

Instruments and Conditions

Thymbra spicata L. samples were obtained from local markets in Hatay (Türkiye) and Yasuj (Iran). For extraction, 2 g of ground plant sample was kept in 20 mL of a 70% ethanol-water mixture in an ultrasonic bath for 1 hour. At the end of this period, the filtered extract was separated from the sediment. Then, 20 mL of the new solvent was poured into the same sediment and kept in the ultrasonic bath for another 1 hour. This procedure was carried out three times, and all resulting extracts were combined. The resulting extract was centrifuged at 9500 rpm for 15 minutes. The pellet was then removed from the upper supernatant and filtered. The solvent was then evaporated under vacuum (Heidolph Laborota 4000, Germany). The extract was prepared in ethanol and stored in the dark at 4°C throughout the analyses.

The quantitative determinations of phenolic compounds were performed with the HPLC system (Shimadzu) with the X-Terra column (250 mm, 4.6 mm). HPLC studies were carried out following a technique similar to that described by Bastemur et al. [11]. The mobile phases consisted of deionized water containing 0.1% ortho-phosphoric acid (A) and ethanol (B). The gradient program was applied as follows: 0–7 min., 20–30% B; 7–15 min., 30–

45% B; 15–21 min., 45–60% B; 21–25 min., 60–20% B. The flow rate was 0.85 mL/min, the column temperature was held at 20°C, and the injection volume was set to 20 µL. The analyses were performed using a Diode Array Detector (DAD) with a broad spectral scanning capability, and the signals were monitored at 230 nm. Due to the strong absorbance characteristics of phenolic compounds containing aromatic rings and conjugated systems at this wavelength, simultaneous, selective, and sensitive determination of multiple analytes was achieved.

TPC, TFC, and antioxidant capacities were performed using a microplate UV-Vis spectrophotometer Microplate Reader (EON, BioTek Instruments Inc.). Absorbance measurements were made using 96- and 48-well microplates at wavelengths appropriate for each method.

TPC and TFC

TPC of *Thymbra spicata* L. was measured using the colorimetric method, which was based on the study of Kaura et al., and slightly modified [31]. Gallic acid was chosen as the standard phenolic compound in the analysis, and calibration curves were prepared at eight different concentrations in the range of 0.25–250 µg mL^{-1}. For the assay, 100 µL of plant extract was mixed with 200 µL of 10% (v/v) Folin–Ciocalteu reagent, homogenized, and left at room temperature for 5 minutes to start the reaction. Next, 800 µL of 2% (v/v) Na_2CO_3 solution was added, and the mixture was shaken and incubated at room temperature for 90 minutes. After the reaction period, the absorbance values of the samples were measured at 765 nm. Each analysis was carried out in triplicate, and experiments were repeated on two different days to ensure the reliability of measurements. Total phenolic content was calculated based on the prepared gallic acid standard curve, and results were given as mg GAE g^{-1}.

TFC in the extracts was measured with some modifications to the colorimetric method reported by Chang et al. [32]. The calibration curve was prepared using eight rutin standard solutions in the range of 0.25–250 µg mL^{-1}. For each standard, sample, and blank, 300 µL ethanol, 20 µL of 10% (w/v) $AlCl_3$, 20 µL of 1 M CH_3COONa, and 560 µL distilled water were added to 100 µL of sample solution, bringing the total volume to 1000 µL. After the prepared mixtures were incubated for 20 minutes at room temperature, the absorbance of the reaction mixtures was measured at 415 nm. Total flavonoid content was calculated using the prepared rutin standard curve, and the results were expressed as mg RE g^{-1}. All analyses were conducted in triplicate.

Antioxidant Activity

DPPH is a free radical known for its characteristic purple color and is used to evaluate antioxidant activity. The method relies on an antioxidant reducing a free radical by donating an electron or hydrogen atom, causing a color change. This color change can be monitored by a UV–Vis spectrophotometer in the wavelength range of 515–520 nm [33]. DPPH radical scavenging capacity of *Thymbra spicata* L. extracts was determined by adapting the method suggested by Akpınar et al. [34]. In the study, plant extracts and ascorbic acid, which was used as a positive control, were analyzed. For the application, 40 µL of sample was mixed with 0.2 mM DPPH solution in a total volume of 160 µL in a 96-well microplate. The prepared plates were incubated for 10 minutes at room temperature with shaking, protected from light. Following the reaction, absorbance values were recorded at 520 nm.

CUPRAC is based on the reduction of Cu(II)–neocuproine (Nc) complex to Cu(I)–neocuproine chelate by antioxidants [35]. Analyses were carried out using 48-well microplates. 250 µL of 10 mM $CuCl_2{\cdot}2H_2O$ solution, 250 µL of 7.5 mM ethanolic neocuproine solution, 250 µL of 1.0 M ammonium acetate buffer prepared at pH 7.0, 25 µL of plant extract, and 250 µL of purified water were added to each well, respectively, to make the total volume up to 1.025 µL. The mixtures were incubated in the dark at room temperature for 60 minutes, after which their absorbance was measured at 450 nm. For each extract, the results were expressed as EC_{50} values. All measurements were performed on two independent days, each in triplicate.

FRAP measurement was carried out based on the method reported by Ng et al., with slight modifications. FRAP reagent was freshly made by combining 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ solution, and 20 mM $FeCl_3{\cdot}6H_2O$ solution in a 10:1:1 ratio [36]. During the analysis, 280 µL of FRAP reagent and 20 µL of sample (standard, sample, or blank) were added to each microwell. The mixtures were kept at 37 °C for 30 minutes, after which their absorbance was recorded at 593 nm. To assess antioxidant capacity, a calibration curve was generated using trolox standard solutions prepared in the 4–600 µM concentration range. Results are reported as µmol TE g^{-1}. All analyses were performed in triplicate on two independent occasions to ensure accuracy and reproducibility of the results.

Results and Discussion

HPLC Analysis of Phenolic Compounds

In order to comprehensively determine the phenolic profile in extracts of *Thymbra spicata* L. obtained from Hatay (Turkey) and Yasuj (Iran), the quantitative determination of catechin, esculetin, caffeic acid, coumarin, rutin, ellagic acid, resveratrol, luteolin, and apigenin was analyzed using HPLC. Analyses were carried out by a fast and sensitive HPLC method with a total analysis time of 25 minutes. Calibration curves were generated between 0.05 and 75 µg mL^{-1} for each of the standard phenolic compounds analyzed, and the correlation coefficients (R^2) of the obtained calibration curves ranged from 0.9995 to 0.9999 for all compounds. This indicates that the method accuracy and the reliability of the quantitative analyses are quite high. Peak identification in the samples was achieved by comparing the times of the observed signals with the retention times of the standards. By measuring the peak area of each compound in the chromatograms given in Figure 1, the quantity of phenolic compounds in the samples was calculated in µg mg^{-1} with the help of calibration curves (Table 1).

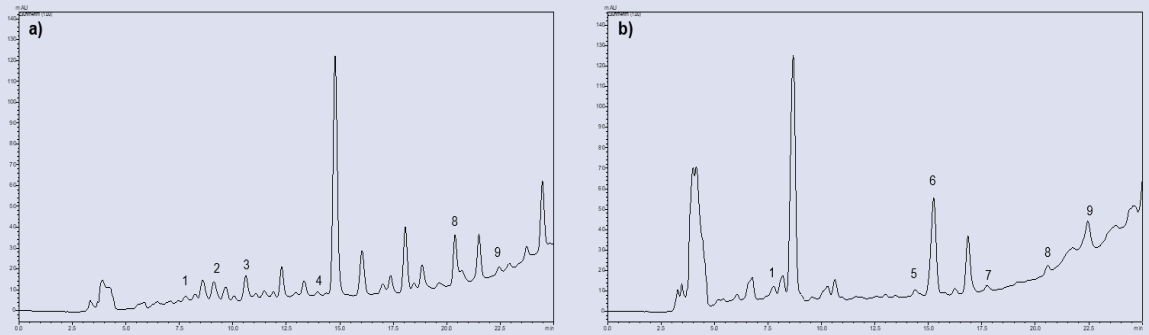

Figure 1. Chromatograms of *Thymbra spicata* L. extract from the Hatay (a) and the Yasuj (b) (1) catechin, 2) esculetin, 3) caffeic acid, 4) coumarin, 5) rutin, 6) ellagic acid, 7) resveratrol, 8) luteolin, and 9) apigenin)

Table 1. The contents of compounds in *Thymbra spicata* L. extracts (µg mg^{-1}).

Compound	Yasuj (Iran) ± SD (n=3)	Hatay (Türkiye) ± SD (n=3)
Catechin	0.175±0.021	0.721±0.010
Esculetin	-	1.209±0.008
Caffeic acid	<LOQ	0.787±0.009
Coumarin	<LOQ	0.322±0.019
Rutin	0.111±0.003	<LOQ
Ellagic acid	1.801±0.064	-
Resveratrol	0.087±0.003	-
Quercetin	0.140±0.005	2.138±0.002
Apigenin	0.400±0.012	1.232±0.061

Quantitatively determined phenolic compound concentrations in *Thymbra spicata* L. extracts from Hatay and Yasuj regions were analyzed using an independent samples t-test to allow comparison between two independent groups for each compound. The significance of inter-regional differences for each compound was assessed using parametric tests, and the results are presented graphically, with corresponding p-values for each compound (Figure 2). All statistical analyses were carried out using GraphPad Prism (version 9.0.2) software, and $p < 0.05$ was accepted significant.

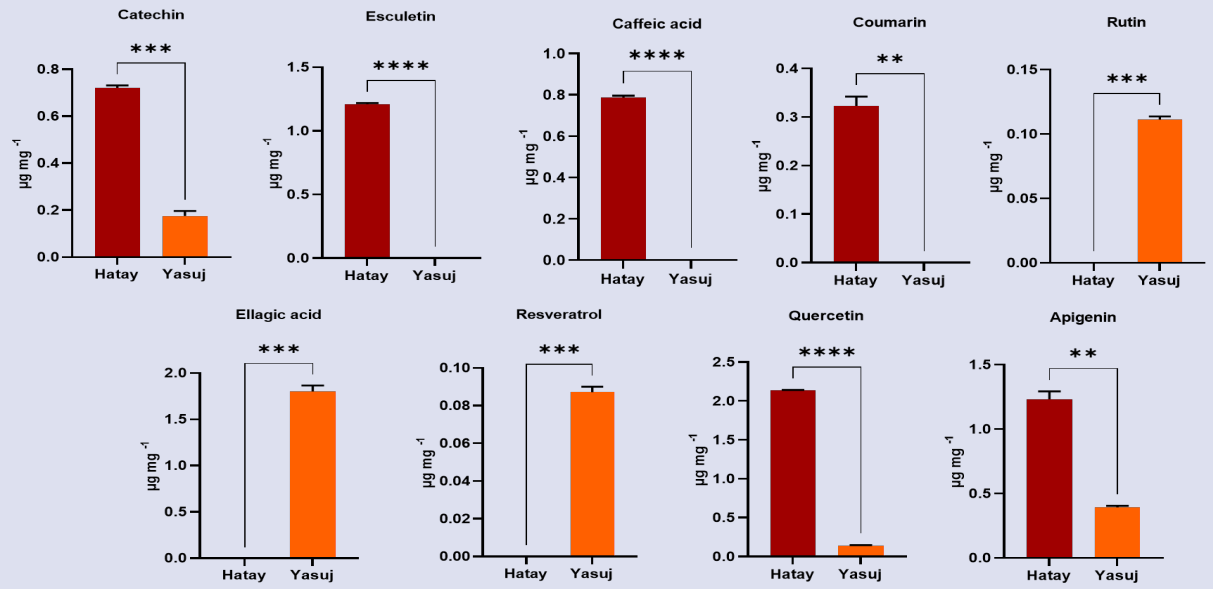

Figure 2. Comparison of phenolic compound amounts determined in extracts of *Thymbra spicata* L. from Hatay and Yasuj regions. Catechin, esculetin, caffeic acid, coumarin, rutin, ellagic acid, resveratrol, quercetin and apigenin contents were analyzed by independent samples t-test. Statistical significance levels: **p < 0.01, ***p < 0.001, ****p < 0.0001.

HPLC analysis results revealed statistically significant differences in phenolic compound contents of *Thymbra spicata* L. samples from Hatay and Yasuj regions. Of the nine phenolic compounds examined, seven (catechin, esculetin, caffeic acid, coumarin, rutin, quercetin, and apigenin) were found at significantly higher concentrations in Hatay samples compared to Yasuj samples ($p < 0.01$–0.0001). Particularly high levels of quercetin (2.138 µg mg^{-1}), esculetin (1.209 µg mg^{-1}), and apigenin (1.232 µg mg^{-1}) were found in Hatay samples, indicating that this region has a richer phytochemical content in terms of these compounds. For these compounds, Hatay samples showed significantly higher concentrations ($p < 0.0001$). Conversely, Yasuj samples had significantly higher concentrations of ellagic acid and resveratrol ($p < 0.001$–0.0001).

The biosynthesis of secondary metabolites in plants is largely shaped by environmental conditions [37,38]. Abiotic (temperature, light intensity, soil pH, humidity) and biotic (microorganisms, herbivore interactions) factors of the growing environment can cause significant changes in the levels of polyphenolic compounds in particular [39–41]. For example, Hatay has a typical Mediterranean climate with hot and dry summers and warm and rainy winters, which can lead to increased secondary metabolite production in plants. On the other hand, Yasuj has a more continental and humid climate, with different altitude and soil structure. In this study, the Hatay sample exhibited higher levels of phenolic compounds (quercetin, apigenin, esculetin) and antioxidant capacity compared to the Yasuj sample; this may be attributed to the longer photosynthetic activity period provided by the Mediterranean climate, the mineral-rich alluvial soil structure, and higher temperature conditions. This may be related to increased polyphenol production in plants as a stress response. In this context, the findings indicate that environmental factors related to the cultivation region have a decisive influence on the phytochemical composition and biological activity of *Thymbra spicata* L. Furthermore, compounds such as ellagic acid and resveratrol, detected at higher levels in the Yasuj samples, suggest that materials from this region may have the potential for different pharmacological effects.

Determination of TPC and TFC

TPC in *Thymbra spicata* L. samples was evaluated by the Folin–Ciocalteu method and the results were given as mg GAE g^{-1}. Calculations were performed using the standard gallic acid calibration curve with the equation $y = 0.0471x + 0.0118$ and the value $R^2 = 0.9994$. According to the results, the sample from Hatay province had the highest total phenolic content with 92.258 ± 3.162 mg GAE g^{-1}, while the Yasuj sample showed the lowest level with 16.412 ± 1.731 mg GAE g^{-1} ($n = 3$). In Figure 3(a), TPC of the Hatay sample was significantly higher than that of the Yasuj sample ($p < 0.01$). This shows that the Hatay sample is much richer in phenolic compounds. Gedikoğlu et al. reported TPC as 13.14 ± 0.135 mg GAE g^{-1} and 13.13 ± 0.249 mg GAE g^{-1} for methanolic and ethanolic extracts of *Thymbra spicata*, respectively [41]. Uğurtay et al. reported the TPC amounts of thyme juice and extract as 628.99 mg GAE kg^{-1} and 766.98 mg GAE kg^{-1}, respectively [42]. Additionally, Tanrıkulu et al. reported the phenolic content of methanol extract of *Thymbra spicata* as 9630 mg GAE kg^{-1} [43]. The TPC values determined in this study were found to be remarkably high compared to previous literature findings.

TFC was analyzed by the aluminum chloride method and the results are given as mg RE g^{-1}. The analyses were based on the rutin standard calibration curve with the equation $y = 0.0115x + 0.0017$ and a correlation coefficient of $R^2 = 0.9993$. As seen in Figure 3(b), the Hatay sample (38.591 ± 0.311 mg RE g^{-1}) showed significantly higher flavonoid content compared to the Yasuj sample (17.136 ± 0.147 mg RE g^{-1}); this difference was highly statistically significant ($p < 0.001$) ($n = 3$). Ertürk et al. reported the TFC amount of *Thymbra spicata* as 0.14 ± 0.001 mg QE g^{-1} [44]. Gedikoğlu et al. reported TFC as 4.36 ± 0.069 mg QUE g^{-1} and 3.24 ± 0.058 mg QUE g^{-1} for methanolic and ethanolic extracts of *Thymbra spicata*, respectively [41]. Tohidi et al. reported TFC values ranging from 1.89 to 8.55 mg QUE g^{-1} for fourteen Thymus extracts from Iran [45]. Total flavonoid content values obtained in this study were determined at higher levels compared to the results reported in the literature.

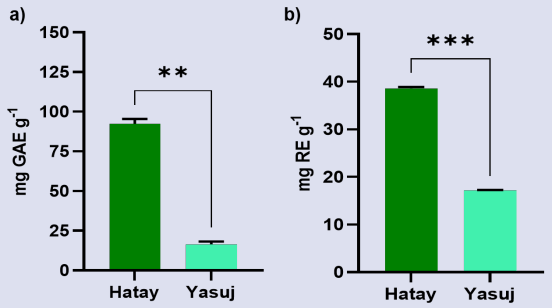

Figure 3. (a) TPC (mg GAE g^{-1}) and (b) TFC (mg RE g^{-1}) of extracts obtained from Hatay and Yasuj regions. Statistical significance levels: **$p < 0.01$, ***$p < 0.001$.

When the obtained data were statistically evaluated, it was determined that the Hatay samples were significantly higher than the other samples in terms of both TPC and TFC ($p < 0.0001$). These results indicate that the *Thymbra spicata* L. plant grown in Hatay province has a richer phenolic and flavonoid profile and therefore may have higher antioxidant potential.

Antioxidant Activity Assay

Due to the complex phytochemical composition of plant extracts, different analysis methods are required for a comprehensive evaluation of their biological activities [34,44]. The antioxidant activities of *Thymbra spicata* L. samples obtained from Hatay (Turkey) and Yasuj (Iran) regions were evaluated using three different colorimetric methods. The antioxidant activity values of both samples are given in Table 2.

Table 2. Antioxidant activities (mean ± standard deviation (n=3)) of *Thymbra spicata* L. samples from Yasuj (Iran) and Hatay (Türkiye) determined by DPPH, FRAP and CUPRAC methods.

Assays	Yasuj (İran)	Hatay (Türkiye)
DPPH (IC_{50}, mg mL^{-1} ±SD)	0.514±0.001	0.0782±0.001
FRAP (µmol troloks g^{-1} ±SD)	89.202±1.009	518.406±0.196
CUPRAC (EC_{50}, mg mL^{-1} ±SD)	18.517±1.233	4.847±0.212

The IC_{50} is defined as the amount of extract required to reduce the DPPH radical concentration by 50% for free radical scavenging activity. At higher IC_{50} values, free radical scavenging activity is lower [46]. In the DPPH radical scavenging assay, the Hatay sample provided 50% inhibition at a much lower concentration with an IC_{50} value of 0.0782 ± 0.001 mg mL^{-1} compared to the Yasuj sample (Yasuj: 0.514 ± 0.001 mg mL^{-1}; n = 3) (Figure 4a). This result indicates that the radical scavenging capacity of the Hatay sample was approximately 6.5 times higher than that of the Yasuj sample. As a result of the analysis, this difference was found to be highly significant ($p < 0.0001$). Khalil et al. reported that the IC_{50} values of ethanolic and water extracts of *Thymbra spicata* were similar (24.5 ± 1.09 µg mL^{-1} and 28.5 ± 1.28 µg mL^{-1}, respectively) [47]. Gedikoğlu et al. reported the IC_{50} for methanolic and ethanolic extracts of *Thymbra spicata* as 35.28 ± 0.45 µg mL^{-1} and 43.9 ± 0.845 µg mL^{-1}, respectively [41]. When the IC_{50} values found in this study are compared with the literature, it is observed that the values are comparable or even higher.

The FRAP (Ferric Reducing Antioxidant Power) method evaluates the ability of antioxidant compounds to reduce Fe^{3+} ions to Fe^{2+} ions, and the data are usually expressed as µmol trolox g^{-1} [48]. The findings obtained from the FRAP analysis revealed that the antioxidant capacity of the Hatay sample was not limited to radical scavenging, but that its reducing power toward ferric ions was also quite high (Figure 4b). While the measured value in the Hatay sample was 518.406 ± 0.196 µmol trolox g^{-1}, this value was only 89.202 ± 1.009 µmol trolox g^{-1} in the Yasuj sample, and the difference between the two samples was highly statistically significant ($p < 0.0001$; n = 3). A p-value at this level indicates that the observed difference is extremely unlikely to have arisen by chance and provides strong statistical support for the high reducing capacity of the Hatay sample. Because the FRAP assay is sensitive to the electron-donating ability of phenolic compounds, the higher values observed in the Hatay sample may be associated with higher concentrations of phenolic compounds with strong reducing potential, particularly quercetin, esculetin, and apigenin. The effectiveness of these compounds in converting ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions suggests that the significant difference between the two samples is not solely due to total phenolic content, but also to differences in the structural properties and redox capacities of individual phenolic compounds. Ertürk et al. reported a FRAP value for *Thymbra spicata* of 15.106 ± 0.180 µmol trolox g^{-1} [44]. Alizadeh et al. reported values of 24.23–26.45 µM QE g^{-1} for a closely related species, *Thymus daenensis* [49]. These reported values were higher than those obtained in the present FRAP analysis.

The CUPRAC method is based on the reduction of Cu^{2+} to Cu^{+} by antioxidant compounds, and the reducing capacity is generally expressed as the effective concentration (EC_{50}) value [23]. In the CUPRAC analysis, antioxidant capacity was evaluated based on the reduced Cu^{+} ion, and the results are given as EC_{50} values. The Hatay sample exhibited an effective concentration value of 4.847 ± 0.212 mg mL^{-1}, which was approximately 3.8 times lower than that of the Yasuj sample (18.517 ± 1.233 mg mL^{-1}) (Figure 4c; n = 3). This indicates that the Hatay sample also possesses a stronger reducing capacity in the CUPRAC system. As a result of the analysis, this difference was found to be highly significant ($p < 0.0001$). Kırkan et al. reported the EC_{50} value for *Thymbra spicata* as 138.38 ± 1.91 mg L^{-1} [50]. Küçükaydın et al. reported CUPRAC activities of *Thymus cariensis* and *T. cilicicus*, both closely related species, as 29.54 ± 0.05 µg mL^{-1} and 24.79 ± 0.02 µg mL^{-1}, respectively [51]. The number of CUPRAC activity studies conducted on *T. spicata* in the literature is limited, and lower values than those obtained in the present study have generally been reported.

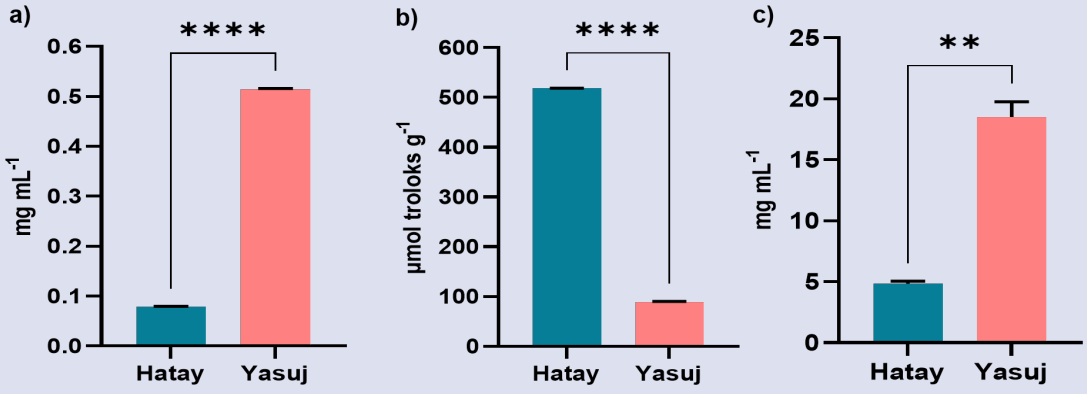

Figure 4. Antioxidant activities of Hatay (Turkey) and Yasuj (Iran) samples determined by different methods: a) DPPH radical scavenging capacity (IC_{50}, mg mL^{-1}, ascorbic acid equivalent), b) Ferric reducing capacity by FRAP method (µmol trolox/g), c) Copper (II) reducing capacity by CUPRAC method (EC_{50}, mg mL^{-1}, trolox equivalent). According to the statistical analysis results: ****$p < 0.0001$, **$p < 0.01$.

These analyses, which measure different mechanisms, revealed significant differences between the antioxidant potentials of the samples. Overall, the Hatay sample demonstrated significantly higher antioxidant capacity than the Yasuj sample across all antioxidant methods. The differences observed across all three methods are consistent both in absolute terms and in statistical analyses. The low IC_{50} and EC_{50} values and the high FRAP value demonstrate that the Hatay sample has strong effects on both radical scavenging and metal ion reduction mechanisms.

Conclusion

In this study, *Thymbra spicata* L. obtained from the Hatay region was found to have higher values in phenolic compounds, TPC, TFC, and antioxidant capacity compared to the Yasuj sample. The high amounts of quercetin, esculetin, and apigenin in the Hatay sample, along with low IC_{50} and EC_{50} values and high FRAP values, demonstrate that plants from this region possess strong antioxidant properties in both free radical scavenging and metal ion reduction mechanisms. The high activity level, particularly in antioxidant analyses, supports the potential for this sample to be considered a natural antioxidant source. Furthermore, the use of ethanol as the solvent in the analysis process helped avoid the use of toxic chemicals and ensured that the study was conducted with minimal environmental impact. In conclusion, we highlight the potential for significant differences in the phytochemical profile and biological activity of *Thymbra spicata* L. depending on the growing region, and these data support its potential for use in food, pharmaceutical, and nutraceutical applications. It is considered that quercetin, in particular, may stand out in the pharmaceutical field with its anti-inflammatory and cardiovascular protective effects; apigenin in nutraceutical products with its cell-protective properties; and esculetin in cosmetic formulations with its skin-protective and free radical-suppressing effects. In future studies, detailing the metabolomic profile of this species with advanced techniques such as LC-MS/MS may contribute to a more comprehensive understanding of the phytochemical diversity.

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Conflicts of interest

There are no conflicts of interest in this work.

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