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Original Article

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A comparative study evaluating the antioxidant activities of rosemary extracts and commercial products

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Abstract

Background and Aims: *Rosmarinus officinalis* L. (Rosemary) is an important medicinal plant with broad pharmacological activities. Because of their antioxidant activity, products prepared from rosemary extracts are used worldwide. There is no study comparing the antioxidant activities of the commercial products of *R. officinalis* sold in Türkiye. The study aimed to obtain an extract from rosemary with the highest antioxidant capacity that can be evaluated industrially.

Methods: Two commercial products containing rosemary extract available for sale in Türkiye were obtained. Rosemary samples collected from both Trabzon (T) and Aydın (A) were extracted with 100% methanol, 70% methanol, 100% acetone, 100% ethyl acetate, and 30% ethanol. In addition, basic and acidic hydrolysis were used to prepare rosemary extracts with a high phenolic content. The antioxidant capacities of all samples were examined using *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric-reducing antioxidant power (FRAP), and phosphomolibdenum-reducing antioxidant power (PRAP) test systems.

Results: It was demonstrated that the rosemary extracts obtained by one-step base hydrolysis were the most effective in DPPH (IC_{50} values of 12.0 ± 0.1 µg/mL for A and T, respectively), FRAP (A: 500.2 ± 26.4 mg butylated hdroxyanisole equivalents (BHAE)/g extract, T: 487.4 ± 13.5 mg BHAE/g extract, and PRAP (A: 736.6 ± 25.1 mg quercetin equivalents (QE)/g extract, T: 841.8 ± 24.1 mg QE/g extract) assays. These rosemary extracts had the highest total phenolic content (A: 1158.0 ± 11.3 mg gallic acid equivalents (GAE)/g extract, T: 1382.5 ± 11.5 mg GAE/g extract). The rosemary extracts obtained from one-step base hydrolysis were more active than the commercial preparations.

Conclusion: This is the first study to compare the antioxidant properties of rosemary commercial products with different extraction methods. These findings provide a significant contribution to the literature by revealing various extraction methods that can be used in the development of products with high antioxidant effects from rosemary.

Keywords Antioxidant · Commercial product · Extraction techniques · Rosemary · *Rosmarinus officinalis*

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INTRODUCTION

Oxidative stress is a condition that is caused by an imbalance between the production of oxidants and antioxidant and can lead to damage in biological systems. Long-term exposure to free radicals, which are involved in the generation of oxidative stress, can lead to functional and structural damage, ageing, and cell death. Oxidative stress is considered to play a role in the etiology of a wide range of diseases, including atherosclerosis, chronic obstructive pulmonary disease, neurodegenerative diseases, cardiovascular diseases, and cancer (Forman & Zhang, 2021). The term "antioxidant" is defined as a substance or molecule that can delay or prevent cell damage caused by free radicals, thus impeding the occurrence or slowing the progression of oxidative stress-induced diseases (Gulcin, 2020). Medicinal plants are important sources of exogenous natural antioxidant compounds. As is known, medicinal plants with abundant phenolic compounds have a strong antioxidant capacity (Mendonça *et al.*, 2022). *Lamiaceae* plants containing high amounts of polyphenolic compounds have been the focus of antioxidant activity studies (Andrade *et al.*, 2018).

The genus *Rosmarinus* (Lamiaceae) includes three different species, *R. officinalis*, *R. eriocalyx*, and *R. tomentosus*, which are mainly distributed in the western Mediterranean areas. Only *R. officinalis* (syn: *Salvia rosmarinus* Schleid.) grows naturally in the flora of Türkiye (Hernández *et al.*, 2016; Davis *et al.*, 1988). *R. officinalis*, also known as rosemary, is widely used in Türkiye for medicinal purposes and as a spice. The decoction of the above-ground parts is consumed for dyspeptic complaints and as an antihypertensive, vasodilator, and appetiser in Kırklareli and to facilitate digestion, in the treatment of respiratory system diseases and as a wound healer in Izmir (Kültür, 2007; Ugulu *et al.*, 2009). In Balıkesir, the infusion prepared from the flowering branches is consumed internally as a carminative and for treating colds, influenza, migraines, and headaches. The decoction prepared from the seeds is used externally to relieve hair oiliness (Polat *et al.*, 2012). Besides its therapeutic use, it is utilised as a food flavouring and for food production because of its antioxidant as well as antibacterial potential (de Oliveira *et al.*, 2019; Borges *et al.*, 2019).

Phytochemical investigations on *R. officinalis* showed the rich content of the species in flavonoids, phenolic acids, and terpenes (Andrade *et al.*, 2018; Kompelly *et al.*, 2019; Nieto *et al.*, 2018). Rosemary and its various secondary metabolites have broad pharmacological effects, including antitumor, antioxidant, antimicrobial, anti-inflammatory, analgesic, antidiabetic, hepatoprotective, neuroprotective, and antiobesity activities (de Oliveira *et al.*, 2019). The antioxidant activities of various *Rosmarinus* species have been investigated in earlier studies (Yeşil-Çeliktaş *et al.*, 2007; Klancnik *et al.*, 2009; Rocío Teruel *et al.*, 2015). It is crucial to prepare plant extracts in a way that maximises their activity, thereby enhancing their quality and efficacy. Studies assessing the antioxidant activity of rosemary under various extraction conditions have been found in the literature (Bicchi *et al.*, 2000; Pérez *et al.*, 2007; Kasparavičienė *et al.*, 2013; Amaral *et al.*, 2018; Dhouibi *et al.*, 2023; Shen *et al.*, 2023; Hendel *et al.*, 2024). However, the number of studies evaluating the activity of market preparations containing rosemary is quite limited. There is no study comparing the antioxidant activity of the preparations containing rosemary extract available on the market in Türkiye with different extracts obtained from the plant. In our study, we aimed to investigate the techniques that can utilise the herbal resources of Türkiye in the most efficient way to support health and to develop the rosemary extract with the highest capacity in terms of antioxidant capacity. The antioxidant capacities of 20 different extracts, prepared from samples collected from Aydın and Trabzon provinces as well as commercial products using different extraction methods, were evaluated by DPPH, phosphomolibdenum-reducing antioxidant power (PRAP), and ferric-reducing antioxidant power (FRAP) assays.

MATERIALS AND METHODS

Plant Materials and Commercial Products

The aerial parts of *R. officinalis* were obtained from Trabzon (Ortahisar District, Aydınlıkevler; June 2022) and Aydın (Köşk District, Başçayır; June 2022). Plant specimens were identified by Assoc. Prof. Dr. Gülin RENDA. Commercial rosemary products (CPs), which contain only *R. officinalis* extract, were obtained from herbal supplement stores (İstanbul, Türkiye).

Extraction and Sample Preparation

Plant samples collected from both Trabzon and Aydın were dried at room temperature and pulverised using a mill. Samples collected from Aydın and Trabzon were abbreviated as A and T, respectively. Each rosemary extract was prepared using 4 g of the powdered specimen. The sample preparation process was conducted separately for each sample, in accordance with the collection site. Different rosemary extracts were prepared by modifying previous studies (Bicchi *et al.*, 2000; Pérez *et al.*, 2007; Orhan *et al.*, 2008; Kasparavičienė *et al.*, 2013; Li *et al.*, 2020; Dominguez-Rodriguez *et al.*, 2017). Plant samples were macerated with 100 mL each of 70% MeOH (A1, T1), acetone (A2, T2), ethyl acetate (A3, T3), 30% ethanol (A4, T4), and 100% methanol (A5, T5) for 4 h at room temperature. After extraction, the rosemary extracts were filtered using filter

paper (MN 640 m ∅ 110 mm, Macherey–Nagel, Germany) and concentrated using a rotary evaporator (Heidolph G1, Hei-VAP Value) at 40 °C. All dry extracts were weighed, and yields were calculated (A1: 15.5%; T1: 19.75%; A2: 10.25%; T2:12.5%; A3:5.75%; T3:11%; A4:20%; T4:28%; A5:17.5%; T5:29%).

In the single-step acid hydrolysis method, samples were added 120 mL of 2 M HCl, mixed for 60 min under reflux at 40°C, and then filtered. The filtrate was extracted with 120 mL of diethyl ether/ethylacetate (DE/EA, 1:1, v/v). The organic phase was concentrated in a rotary evaporator at 40°C to obtain the crude rosemary extracts (A6, T6). In the single-step base hydrolysis, 120 mL of 2 M NaOH was added to the samples, macerated at room temperature (22°C) for 4 h, and then filtered. The pH of the filtrate was adjusted to 2 with 6 M HCl. Following the extraction with 120 mL of DE/EA (1:1, v/v), crude rosemary extracts were obtained by evaporating the organic phase in a 40°C rotary evaporator (A7, T7) (Li *et al.*, 2020). The samples were extracted with a 120 mL ethanol:water:formic acid (50:50:5) solution for 120 min under reflux at 40°C, and then the extracts were filtered and concentrated at 40°C in a rotary evaporator to obtain crude dry rosemary extracts (A8, T8). According to another preparation method, 120 mL of a mixture of ethanol:water (1:1), pH adjusted to 2 with 0.18 N HCl, was added to the plant sample and extracted for 120 min under reflux at 40°C. At the end of the time, the rosemary extract was filtered and dried in a rotary evaporator at 40°C (A9, T9) (Dominguez- Rodriguez *et al.*, 2017). All methods were applied to samples collected from both Aydın and Trabzon. Commercial products were used in the experiments after evaporation to dryness in a rotary evaporator at a temperature not exceeding 40°C (CP1, CP2). All dry extracts were weighed, and yields were calculated (A6: 19.8%; T6: 17.4%; A7: 13.7%; T7:14.1%; A8:9.4%; T8:10.5%; A9:10.6%; T9:13.5%).

Determination of the Total Phenolic Content in Rosemary Extracts and Commercial Products

The total phenolic content of the rosemary extracts and commercial products was determined using the Folin-Ciocalteu reagent based on the method described by Kahkönen *et al* (1999). Twenty microliters of samples prepared at different concentrations were mixed with 400 µL of Folin-Ciocalteu reagent (0.5 N), 680 µL of distilled water, and 400 µL of 10% (w/v) sodium carbonate, respectively. After 120 min of incubation at room temperature and in the dark, the absorbance of the mixture was measured at 760 nm. Gallic acid was used as the standard. The total phenolic contents of the rosemary extracts were calculated as mg GAE equivalents per g dry weight of the sample.

Antioxidant Activities

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH radical scavenging activities of the rosemary extracts and commercial products were tested using spectrophotometric analysis according to the previous study (Blois, 1958). DPPH solution in methanol (0.2 mM, 200 µL) was added to rosemary extracts prepared at different concentrations, incubated in the dark at room temperature for 30 min, and then absorbance was measured at 517 nm ($A_{extract}$). A mixture without any rosemary extract (50 µL methanol + 200 µL DPPH) was used as a negative control ($A_{control}$). Gallic acid was chosen as a positive control. The percentage free radical scavenging of the rosemary extracts and gallic acid was calculated from the equation as follows:

$$\text{Scavenging effects (\%)} = \frac{A \text{ control} - A \text{ extract}}{A \text{ control}} \times 100$$

The results are presented as half maximal inhibitory concentration (IC_{50}) values.

Phosphomolibdenum-Reducing Antioxidant Power (PRAP) Assay

The ethanol solution of phosphomolybdic acid (10% w/v, 400 µL) was admixed to the rosemary extracts prepared at different concentrations. The mixtures were incubated at 80°C for 30 min, and then the absorbance was read at 600 nm. Quercetin was used as a positive control, while a mixture without rosemary extract (100 µL ethanol + 400 µL phosphomolybdic acid) was chosen as a negative control. PRAP activity was expressed as mg quercetin equivalents (QE)/g extract (Falcioni *et al.*, 2002).

Ferric-Reducing Antioxidant Power (FRAP) Assay

250 µL of phosphate buffer (pH = 6.6; 200 mM) and 250 µL of 1% w/v K_3 [$Fe(CN)_6$] were added to 250 µL of the rosemary extracts prepared at 1 mg/mL and incubated at 50°C for 20 min. At the end of the time, 250 µL of 10% trichloroacetic acid was admixture and centrifuged at 3000 rpm for 10 min. The supernatant (200 µL) was mixed with 200 µL of distilled water and 20 µL of a 0.15% $FeCl_3$ solution. It was incubated again for 30 min at room temperature, and the absorbance was measured at 700 nm. Butylated hydroxyanisole (BHA) was selected as the reference solution. The FRAP activity of rosemary extracts and commercial products was expressed as mg butylated hdroxyanisole equivalents (BHAE)/g extract (Oyaizu, 1986).

Statistical Analysis

The experiments were performed in three replicates. The Microsoft Excel and GraphPad Prism 5.0 programs were used for statistical analysis. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test. P-values < 0.05 were considered statistically significant.

RESULTS

The Total Phenolic Content

The total phenolic contents of the extracts prepared from the aerial parts of rosemary and the commercial products are presented in Table 1. Accordingly, the rosemary extracts with the highest total phenolic content were T7 (1382.5 ± 11.5 mg GAE /g extract), T6 (1351.6 ± 9.5 mg GAE /g extract), A7 (1158.0 ± 11.3 mg GAE/g extract), and A6 (913.7 ± 18.4 GAE mg/g extract) prepared using single-step acid and single-step base hydrolysis methods. It was found that the rosemary extracts with the lowest total phenolic content were A3 (70.2 ± 0.9 mg GAE /g extract) and T3 (115.6 ± 1.8 mg GAE/g extract) prepared using ethyl acetate. Considering the extraction procedures, the total phenol content of the rosemary extracts prepared from the samples collected from Trabzon was higher. CP1 had a higher total phenol content among the commercial products, with a value of 296.1 ± 2.9 mg GAE/g extract. The total phenol content of the commercial products was lower than that of A6, A7, T6, and T7, while it was higher than that of A3 and T3.

Antioxidant Activity Results

The DPPH radical scavenging activities of the rosemary extracts and commercial products are presented in Table 2. Among the rosemary extracts prepared with plant samples collected from Aydın, A7 showed the strongest DPPH radical scavenging effects with IC_{50} value of 12.0 ± 0.1 µg/mL. T7 exhibited the greatest radical scavenging activity of the Trabzon samples with IC_{50} value of 4.8 ± 0.2 µg/mL. A8, A9, and T6 showed remarkable DPPH radical scavenging activity according to IC_{50} values. On the other hand, A3 and T3 were the weakest scavengers of the DPPH radical. The inhibition of DPPH by CP-1 was higher than that by CP-2 when the commercial preparations were compared. The DPPH scavenging activity of the commercial products was lower than that of the A7 and T7 rosemary extracts at all concentrations studied.

The PRAP and FRAP activities of the rosemary extracts and commercial products are presented in Table 3. Among the Aydın samples, A7 had the highest activity with 736.6 ± 25.1 mg QE/g extract. T7 was the most active among the Trabzon samples and all the rosemary extracts, with 841.8 ± 24.1 mg QE/g extract. It was found that the rosemary extracts exhibiting the weakest PRAP activity were A5 and T5. Comparing the commercial products, CP-1 demonstrated stronger PRAP activity than CP-2. These commercial products were less active than A7 and T7. It was shown that both commercial products had higher PRAP activity compared to A5 and T5. The rosemary extracts with the highest FRAP activity were A7 (500.2 ± 26.4 mg BHAE/g extract) and A6 (400.5 ± 24.7 mg BHAE/g extract) for Aydın samples whereas T7 (487.4 ± 13.5 mg BHAE/g extract) and T6 (400.5 ± 25.7 mg BHAE/g extract) for the Trabzon samples. It was determined that the rosemary extract with the weakest FRAP activity was A5 (65.4 ± 2.5 mg BHAE/g extract). CP-2 (180.4 ± 4.9 mg BHAE/g extract) exhibited stronger FRAP activity than CP1 (146.3 ± 6.1 mg BHAE/g extract). Commercial products usually had a lower FRAP activity than most rosemary extracts investigated and BHA, while they had a higher FRAP activity than A3.

DISCUSSION

The use of natural antioxidants to reduce oxidative stress involved in the development of many chronic diseases, to treat various degenerative diseases, and as safe food preservatives is becoming increasingly common. Many studies on

Table 1. Total phenol contents of the rosemary extracts and commercial products

Extract code	Total phenolic content (mg GAE/g extract)
A1	173.6 ± 6.4
A2	132.6 ± 3.6
A3	70.2 ± 0.9
A4	325.3 ± 12.5
A5	191.0 ± 6.3
A6	913.7 ± 18.4
A7	1158.0 ± 11.3
A8	370.2 ± 6.2
A9	488.7 ± 7.3
T1	622.9 ± 8.2
T2	267.8 ± 2.4
T3	115.6 ± 1.8
T4	439.1 ± 7.3
T5	229.9 ± 2.5
T6	1351.6 ± 9.5
T7	1382.5 ± 11.5
T8	449.6 ± 5.2
T9	549.7 ± 2.7
CP-1	296.1 ± 2.9
CP-2	175.9 ± 3.7

A: Samples collected from Aydın, T: Samples collected from Trabzon, CP: Commercial products, GAE: Gallic acid equivalents

Table 2. DPPH radical scavenging effects of rosemary extracts and commercial products

Extract code	IC_{50} (µg/mL)
A1	42.5 ± 2.1
A2	88.5 ± 5.7
A3	133.1 ± 7.2
A4	84.2 ± 4.8
A5	102.4 ± 7.0
A6	18.8 ± 0.3
A7	12.0 ± 0.1^{ns}
A8	39.6 ± 2.4
A9	43.2 ± 4.3
T1	57.2 ± 3.6
T2	83.9 ± 2.6
T3	133.1 ± 9.4
T4	43.3 ± 3.6
T5	99.9 ± 5.3
T6	13.9 ± 0.4^{ns}
T7	4.8 ± 0.2**
T8	43.3 ± 2.5
T9	45.7 ± 4.8
CP-1	90.4 ± 4.7
CP-2	107.2 ± 5.2
GA	12.4 ± 0.4

** p<0.001 compared to GA, ns: not significant compared to GA
A: Samples collected from Aydın, T: Samples collected from Trabzon, CP: Commercial products, GA: Gallic acid, IC50: Half-maximal inhibitory concentration

Table 3. PRAP and FRAP activities of rosemary extracts and their commercial products

Extract code	mg QE/g extract	mg BHAE/g extract
A1	247.9 ± 2.3	75.7 ± 3.5
A2	382.2 ± 4.7	120.4 ± 2.7
A3	288.7 ± 8.4	127.5 ± 12.6
A4	335.6 ± 12.8	200.4 ± 14.6
A5	104.3 ± 10.4	65.4 ± 2.5
A6	668.9 ± 35.7	400.5 ± 24.7
A7	736.6 ± 25.1	500.2 ± 26.4
A8	340.8 ± 16.8	268.3 ± 11.3
A9	314.7 ± 23.5	129.4 ± 6.3
T1	326.2 ± 22.7	200.4 ± 3.7
T2	442.9 ± 15.3	302.5 ± 36.3
T3	258.5 ± 6.2	189.3 ± 11.6
T4	272.0 ± 14.7	147.4 ± 15.6
T5	128.3 ± 11.5	25.2 ± 0.43
T6	703.3 ± 47.2	400.5 ± 25.7
T7	841.8 ± 24.1	487.4 ± 13.5
T8	265.8 ± 6.3	300.4 ± 12.6
T9	348.0 ± 3.4	269.4 ± 6.7
CP-1	171.0 ± 3.5	146.3 ± 6.1
CP-2	140.8 ± 7.3	180.4 ± 4.9

A: Samples collected from Aydın, T: Samples collected from Trabzon, CP: Commercial products, BHAE: butylated hdroxyanisole equivalents, QE: quercetin

antioxidant activity have focused on medicinal plants, which are an important source of natural antioxidants (Parveen *et al*., 2016; Mo *et al*., 2021). The type and polarity of the extraction solvent, the biological test system used, and the geographical and climatic conditions of the region where the herb is obtained are all factors that influence the antioxidant activity of plants. In this regard, it is essential to study the biological activities of medicinal plants obtained from different places using different extraction methods and biological test systems. Commercial products containing various plant extracts are widely sold on the market and widely used in the community, and these products are expected to have high antioxidant activity. There is no study comparing the antioxidant activity of commercial products containing rosemary extract and extracts prepared using different extraction methods in Türkiye. Moreover, the number of studies evaluating the antioxidant activity of commercial products containing rosemary extract is very limited in the literature (Klancnik *et al*., 2009). In this study, the *in vitro* antioxidant activities of two commercial products containing only rosemary extracts available for sale in Türkiye and 18 different extracts prepared from rosemary samples collected from different localities were tested using the DPPH free radical scavenging activity assay, PRAP assay, and FRAP assay. In addition, the regions selected for collecting the plant from nature belong to different climatic zones and different floristic regions. The total phenolic compounds of the samples were also determined. Our study aimed to develop a suitable method for obtaining rosemary extract with high antioxidant activity.

This study demonstrated that rosemary extracts prepared using the one-step base hydrolysis approach scavenged DPPH radicals the most effectively of all the extracts. The rosemary extracts obtained from the one-step base hydrolysis had a stronger DPPH radical scavenging effect than the commercial products. On the other hand, ethyl acetate extracts exhibited weak activity among all the rosemary extracts tested. In the previous study, it was reported that the total phenolic content of 12 different methanol extracts prepared from aerial parts of *R. officinalis* collected from different localities and at different harvest times ranged from 70.3 to 147.3 mg GAE/g extract. In this study, the DPPH free radical scavenging effect of all the rosemary extracts tested was remarkably stronger (Yesil-Celiktas *et al*., 2007a). Bourhia *et al*. reported that rosemary

leaf ethanol extract had a high polyphenolic content (74.15–146.63 µg GAE/mg extract) and showed significant antioxidant activity in the DPPH assay with IC_{50} values ranging from 0.236–0.176 mg/mL (Bourhia *et al*., 2019). Hyun *et al*. assessed the total phenol content of 80% methanol extract and its fractions (*n*-hexane, chloroform, ethyl acetate, butanol, and water) prepared from rosemary sprigs using the Folin-Ciocalteu phenol reagent. The total phenol content of the rosemary extracts and fractions was sorted as follows: ethyl acetate fraction > *n*-BuOH > 80% MeOH > water > *n*-hexane > chloroform. The ethyl acetate fraction had the maximum polyphenol content, with a value of 65.3 mg GAE/g. The scavenging effect of the DPPH radical was ranked as 80% methanol (SC_{50}: 33.8 ± 4.5 µg/mL)> water (SC_{50}: 46.6±12.8 µg/mL)> *n*-hexane (SC_{50}: 61.0 ± 3.4 µg/mL)> *n*-butanol (SC_{50}: 79.2 ± 4.9 µg/mL) > chloroform (SC_{50}: 94.7 ± 1.0 µg/mL)> ethyl acetate (SC_{50}: >100 µg/mL). The 80% methanol extract had the highest activity, whereas the ethyl acetate fraction had the lowest activity (Hyun *et al*., 2015). The DPPH radical scavenging effect of the ethanol extract prepared from rosemary leaves and its dichloromethane, ethyl acetate, and *n*-butanol fractions were evaluated at concentrations ranging from 10–100 µg/mL. The ethanol extract scavenged DPPH radicals more strongly than the ethyl acetate and *n*-butanol fractions at all concentrations studied. It was observed that the dichloromethane and ethyl acetate fractions were more potent than the *n*-butanol fraction at all concentrations. The dichloromethane fraction had a higher DPPH radical scavenging effect than the ethyl acetate fraction at a concentration of 10 µg/mL (Amaral *et al*., 2018). In another study, the IC_{50} values were calculated for the methanol and ethyl acetate extracts prepared from rosemary leaves as 0.510 and 0.719 mg/mL, respectively, in the DPPH test, and the highest radical scavenging effect was detected in the methanol extract (Amar *et al*., 2017). Furthermore, four different commercial products containing rosemary extract, the main active phenolic compounds of which are oil-soluble carnosic acid (V15 and V40) and water-soluble rosmarinic acid (A15 and A40), were analysed for total phenolic content. The total phenolic content was 593 and 915 mg/g extract chlorogenic acid equivalents for A15 and A40, respectively, while it was 198 and 438 mg/g extract chlorogenic acid equivalents for V15 and V40, respectively. In the DPPH test, the EC_{50} values of the A15, A40, V15, and V40 samples were calculated as 0.0132, 0.0074, 0.0227, and 0.0092 mg/ml, respectively. These results showed that rosemary extracts with water-soluble active components have greater antioxidant activity relative to their higher phenolic content (Klancnik *et al*., 2009). In previous studies, methanol was usually the solvent of choice for extraction. In these studies, methanol extracts that were prepared from the leaves and aerial parts of rosemary significantly scavenged DPPH free radicals. In this study, the extracts prepared using 70% methanol from both Aydın and Trabzon samples exhibited moderate activity. On the other hand, the DPPH radical scavenging effect of the 100% methanol extracts was weak. Similar to previous studies, we found that rosemary extracts from Aydın and Trabzon prepared with ethyl acetate had weaker activity in the DPPH assay.

In the present study, it was determined that rosemary extracts obtained by the single-step base hydrolysis approach showed significant activity, whereas extracts prepared with 100% methanol showed the weakest activity in both the PRAP and FRAP assays. Rosemary extracts prepared from the single-step base hydrolysis procedure exhibited higher activity compared to commercial products. Rocío Teruel *et al*. reported that the FRAP activities of powder acetone, liquid methanol, and liquid acetone extracts prepared from rosemary leaves using three different extraction methods were 2063.82 ± 7.00, 1186.54 ± 3.91, and 660.31 ± 4.04 bmM/g, respectively. The potent antioxidant activity of the powder acetone extract was associated with its high phenolic content, calculated at 23.23 mg GAE/100 g extract. Consistent with the FRAP activity findings, the total phenolic content was followed by the liquid methanol extract (20.41 mg GAE/100 g extract) and the liquid acetone extract (18.62 mg GAE/100 g extract), respectively (Rocío Teruel *et al*., 2015). It was determined that the FRAP activities of methanol and ethyl acetate extracts prepared from rosemary leaves were 1.714 ± 0.068 and 1.240 ± 0.052, respectively, and the methanol extract had the highest activity (Amar *et al*., 2017). In the previous study, the metal-reducing power of commercial preparations was evaluated based on the reduction of the Fe^{3+}-ferricyanide complex to the ferrous form (Fe^{2+}). The reducing powers of the A15, A40, V15, and V40 samples were calculated as 69.5, 124, 27.2, and 66.1 mg/mL, respectively. Water-soluble formulations had higher reducing power (Klancnik *et al*., 2009). In previous studies, it was generally stated that the total phenolic compound content and antioxidant activity were directly correlated (Yesil-Celiktas *et al*., 2007b; Klancnik *et al*., 2009; Rocío Teruel *et al*., 2015). Our study supports this finding. The rosemary extracts prepared by single-step base hydrolysis with higher total phenolic content had greater antioxidant activity.

In conclusion, the antioxidant effects of our samples differed depending on the place where the plant was collected, the extraction medium used, and the biological activity assay used. The antioxidant activity of the rosemary extracts prepared from the samples obtained from Trabzon was found to be stronger than that of the rosemary extracts prepared from the samples collected from Aydın. High phenolic content and strong antioxidant activity were found in the rosemary

extracts that were prepared in the extraction medium through a process that involved one-step acid hydrolysis. Commercially available rosemary extracts showed weaker antioxidant activity compared to the one-step base hydrolysis extracts prepared in the present study. Since our study showed that rosemary with better activity can be obtained, the extraction conditions employed in the production of commercially available rosemary extracts should be improved. Based on the findings of our study, it is recommended to use a one-step base hydrolysis extraction procedure to obtain rosemary extracts with high antioxidant activity.

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Conflict of Interest The authors have no conflict of interest to declare.

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