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Anatomic and Phytochemical Investigation of Herbal Tea Bags Sold as Lemon Balm (*Melissa officinalis* L.) in the Market

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ABSTRACT

Melissa officinalis L., an ethnobotanically valuable plant, has been used for the treatment of several diseases since ancient times. However, different plants with the same name are sold instead of lemon balm in markets that sell herbal products in Türkiye. For this purpose, 15 different brands of tea bags in crushed form, sold as lemon balm (*Melissa officinalis* L.) in markets, were analyzed. The total phenolic, flavonoid, and antioxidant capacities of the hydroalcoholic extracts of these tea samples along with the stomatal structure were investigated. Additionally, chemical compositions and rosmarinic acid contents were determined by the high-performance thin-layer chromatography method. Among the samples examined, S1, S2, S9, and S12 tea samples were found to meet the eligibility criteria. The leaves of these samples had diacytic stomata and the rosmarinic acid ratio in their phytochemical composition was over 2%. Furthermore, caffeic acid was detected in these samples.

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Piyasada Tıbbi Melisa (*Melissa officinalis* L.) Olarak Satılan Bitkisel Poşet Çayların Anatomik ve Fitokimyasal Olarak İncelenmesi

ÖZET

Etnobotanik olarak değerli bir bitki olan melisa eski çağlardan beri birçok hastalığın tedavisinde kullanılmaktadır. Buna karşın, ülkemizde aynı isme sahip farklı bitkiler piyasada tıbbi melisa (*Melissa officinalis* L.) yerine satılmaktadır. Bu amaçla, piyasada *M. officinalis* olarak satılan öğütülmüş formda 15 farklı markalı poşet çay analiz edildi. Bu örneklerin hidroalkolik ekstrelerinin toplam fenol, flavonoid ve antioksidan kapasiteleri ile birlikte stoma yapıları incelendi. Buna ek olarak, kimyasal birleşimleri ve yapılarında bulunan rosmarinik asit miktarları Yüksek Performanslı İnce Tabaka Yöntemi ile belirlendi. İncelenen örnekler arasında, S1, S2, S9 ve S12 kodlu örneklerin uygunluk kriterlerini karşıladığı bulundu. Buna göre bu örneklerin yaprakları diasitik stoma yapısına sahip olduğu ve kimyasal yapılarında rosmarinik asit oranlarının %2'nin üzerinde olduğu belirlendi. Ayrıca bu örneklerde kafeik asidin de olduğu tespit edildi.

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INTRODUCTION

Lemon balm (*Melissa officinalis* L.), known as "Melisa, oğulotu" in Türkiye, is a perennial herb belonging to the Lamiaceae family. It typically grows to a length of about 30-120 cm, and its stem and leaf parts are covered with hairs. *Melissa officinalis*, which grows naturally in Southern Europe, the Caucasus, Northern Iran, Northern Iraq, and Türkiye, is extensively cultivated worldwide (Mill, 1982; Güner et al., 2012; Shakeri et al., 2016).

The medicinal use of the plant dates back centuries. Tea prepared with the leaf part of *M. officinalis* is especially beneficial for sleep and gastrointestinal problems (Carnat et al., 1998). In addition, clinical

studies have shown that it possesses antioxidant, antiviral, antifungal, antibacterial, antitumor, antianxiety, and antihyperlipidemic properties (Mimica-Dukic et al., 2004; Ferreira et al., 2006; Nolkemper et al., 2006; Cases et al., 2011). *M. officinalis* contains various phytochemicals in its structure. Aerial parts contain flavones such as luteolin, apigenin, flavanol (catechin, epicatechin), flavanone (naringenin, hesperidin), and essential oil compounds like citral. It also contains triterpenes (ursolic and oleanolic acid) and intense phenolic acids (rosmarinic acid, caffeic acid, chlorogenic acid) (Patora & Klimek, 2002; Dastmalchi et al., 2008; Pereira et al., 2014; Miraj et al., 2017). In particular, its pharmacological activity is thought to be due to rosmarinic acid, and its standardization in leaves and extracts is based on this phenolic compound according to the European Pharmacopoeia (Ramanauskiene et al., 2016; Anonymous, 2023; Ulgen et al., 2023).

There are different plants with the name "Melisa" in Türkiye. The leaves of beebrush (*Aloysia* L. sp.), which have a similar odor, are sold in many markets instead of *M. officinalis* (Saskara et al., 2010). However, no studies have been conducted on grounded-type teas so far. This study aimed to compare *M. officinalis* tea bags purchased from 15 different markets as anatomical and phytochemically. For this purpose, the stomata type of the samples was examined. Additionally, total phenolic, and flavonoid contents as well as antioxidant activities were determined. Furthermore, rosmarinic acid content in the structure of the samples was quantitatively determined by the high-performance thin layer chromatography (HPTLC) method.

MATERIALS and METHODS

Plant Materials and Chemicals

15 different brands of tea bags (coded S1-S15) sold as "*M. officinalis* leaves" in the markets were purchased and stored at room temperature throughout the experiment (Figure 1). *Melissa officinalis* standardized extract was obtained from Martin Bauer (Germany) for comparison with other samples in a thin-layer chromatography study. Other chemicals used in experiments were purchased from Sigma.

Preparation of Hydroalcoholic Extract

250±1 mg of herbal tea was extracted with 25 ml of 75% ethanol in an ultrasonic bath for 20 minutes. It was filtered with a 0.45 µm syringe filter and the liquid portion was evaporated using a rotary evaporator under 45°C and 50 mbar pressure conditions. After calculating the extract yields, stock solutions of 5 mg ml^{-1} were prepared for each sample to be used in the experiments (Table 1). These prepared stock solutions were stored in the refrigerator at +4°C throughout the study.

Figure 1. The general appearance of the herbal material used in the experiments. (Sample 3)

Şekil 1. Çalışmalarda kullanılan bitkisel materyalin genel görünümü. (Örnek 3)

Anatomical Analysis

The type of stomata, stomatal length, width, and outline dimensions (Figure 2) of the samples were observed and photographed at 40x magnifications using the Zeiss (Oberkochen, Germany), Axio Lab A1 microscope with 50% chloral hydrate solution according to the microscopic examination of the Melissae folium section in the European Pharmacopoeia (Anonymous, 2023).

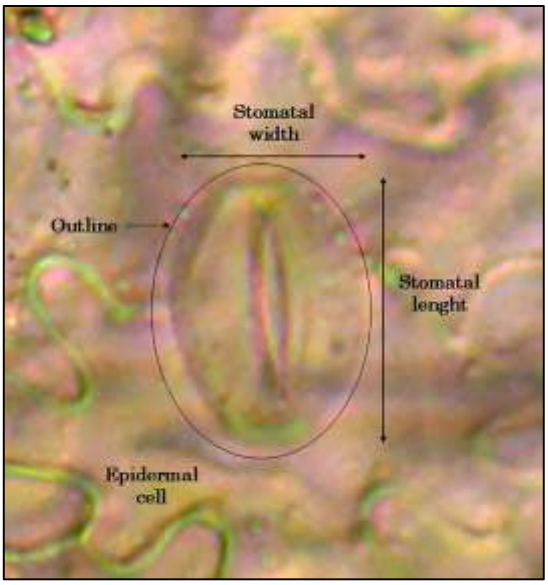

Figure 2. The stomatal sections were examined at 40x magnification using a 50% chloral hydrate solution during anatomical analysis

Şekil 2. Anatomik analizde %50 kloral hidrat solüsyonu ile 40x büyütmede incelenen stoma bölümleri.

Table 1 Extract Yields and Dilution Volumes
Çizelge 1. Ekstre Verimleri ve Seyreltme Oranları

Samples	Sample weights (mg)	Extract (mg)	Yield (%)	Volume used to prepare 5 mg ml^{-1} stock solution (ml)
S1	250.4	53.2	21.2	10.64
S2	250.0	50.1	20.0	10.02
S3	250.5	56.8	22.7	11.36
S4	250.0	63.1	25.2	12.62
S5	250.8	38.9	15.5	7.78
S6	250.9	41.6	16.6	8.32
S7	250.6	49.5	19.8	9.9
S8	250.9	49.3	19.7	9.86
S9	250.1	36.5	14.6	7.3
S10	250.6	78	31.2	15.6
S11	250.5	55.8	22.3	11.16
S12	249.7	34.1	13.6	6.82
S13	250.0	57.5	23.0	11.5
S14	249.8	66.8	26.7	13.36
S15	250.8	59.2	23.6	11.84

Total Phenolic Content

25 µl of 0.5 mg ml^{-1} sample or blank (water), 25 µl of distilled water, 125 µl of 10% Folin Ciocalteu reagent, and 100 µl of 7.5% sodium carbonate were added directly into the 96-well plate and incubated at 37°C for 30 minutes (Singleton & Rossi, 1965). After incubation, the absorbance of the solutions was measured with a Thermo Scientific (Massachusetts, ABD) Varioskan Lux microplate reader at a wavelength of 760 nm. The total phenolic content of tea samples was expressed as mg gallic acid (4-125 µg ml^{-1}; y =66.656x-0.0303, R^2 = 0.9974) equivalent per g of extract.

Total Flavonoid Content

$AlCl_3$ Colorimetric assay in the absence of $NaNO_2$

30 µl of 5 mg ml^{-1} sample or blank (ethanol), 60 µl of 70% ethanol, 30 µl of 10% $AlCl_3$, 30 µl of 1 M sodium acetate, and 150 µl of methanol were added into the wells. After the 15-minute incubation period, the absorbance of the reactions was measured at a wavelength of 415 nm (Degirmencioglu et al., 2019). Results were shown as mg quercetin (4-250 µg ml^{-1}; 37.126x+0.0069, R^2=0.9999) equivalent to total flavonoid content per g Of extract.

$AlCl_3$ Colorimetric assay in the presence of $NaNO_2$

200 µl of 0.5 mg ml^{-1} sample, 200 µl of water, and 30 µl of 5% sodium nitrite were mixed and kept for 5 minutes. Afterward, 30 µl of 10% $AlCl_3$ was added, mixed by a vortex again, and left at room temperature for 6 minutes. Lastly, 200 µl of 1 M NaOH and 340 µl water were added to the solution, mixed, and kept for 15 minutes (Zhishen et al., 1999). Absorbances were defined at a wavelength of 510 nm and results were specified as mg catechin (16-500 µg ml^{-1}; y=15.33x-0.0118, R^2=0.9976) equivalent per g of extract.

Total Anthocyanin Content

40 µl of 5 mg ml^{-1} sample and 160 µl of pH1 and Ph4.5 buffer solutions (pH1 buffer was prepared with 25 mM of potassium chloride and pH4.5 buffer was prepared with 400 mM of sodium acetate, pH of both buffer solutions was adjusted using diluted hydrochloric acid) were mixed separately. 15 minutes later absorbances were observed at 520 and 700 nm wavelengths (Lee et al., 2005). The absorbance of anthocyanin was determined according to Equation 1. The total anthocyanin content of the examined tea sample was given as mg cyanidin 3-glucoside (4-125 µg ml^{-1}; y=29.296x+0.0067, R^2= 0.9999) equivalent per g of extract.

Anthocyanin absorbance= $(A_{520}-A_{700})_{pH1}$ - $(A_{520}-A_{700})_{pH4.5}$ (Eq. 1)

Total Antioxidant Contents

DPPH

280 µl of 0.1 mM ethanolic DPPH solution (2,2-Diphenyl-1-picrylhydrazyl, absorbance ~0.7) was added to 20 µl of 0.5 mg ml^{-1} sample or blank (ethanol) and left in the dark environment for half an hour at room temperature. The absorbance of the formed color changes was measured at a wavelength of 520 nm (Blois, 1958). The results were expressed as mg Trolox (8-125 µg ml^{-1}; y=79.756x-0.0047, R^2=0.9998) equivalent to total antioxidant content per g of extract.

ABTS Radical scavenging assay

20 µl sample or blank (methanol) and 280 µl ABTS reagent (7 × 10^{-3} M of ABTS and 2.45 × 10^{-3} M of potassium persulfate were mixed in equal volumes and kept for 12-16 hours at room temperature in a dark

environment, then the solution was diluted at a ratio of 1:10 with methanol and adjusted to an absorbance of approximately 0.7) were added into the wells and incubated for 6 minutes at room temperature. The absorbance of solutions was read at 734 nm wavelength (Re et al., 1999). Results were defined as mg Trolox (8-125 µg ml^{-1}; y=84.011x-0.0047, R^2 = 0.9931) equivalent to total antioxidant content per g of extract.

Ferric-reducing antioxidant power assay (FRAP)

20 µl of sample or blank (water) and 280 µl of freshly prepared FRAP reagent (2×10^{-2} M $FeCl_3$, 1×10^{-2} M TPTZ, and pH 3.6 sodium acetate buffer were mixed in a ratio of 1:1:10, respectively) were mixed and kept for 6 minutes at room temperature. Then, absorbances of the reaction were determined at a wavelength of 595 nm (Benzie & Strain, 1996). Results were stated as mg Trolox (8-250 µg ml^{-1}; y=102.5x+0.061, R^2 = 0.9992) equivalent to total antioxidant content per g of extract.

CUPRAC

The Cupric Reducing Antioxidant Capacity assay was determined according to the method of Apak et al. (2004) with minor modifications. Initially, 20 µl sample or blank (water) solutions were added well plate, then 280 µl of reagent consisting of $1x10^{-2}$ M copper (II) chloride, $7.5x10^{-3}$ M neocuproine and 1 M ammonium acetate (pH 7) was added onto solution and kept in the dark at room temperature for half an hour. The absorbance of the yellow color formed at the end of the reaction was measured at a wavelength of 450 nm. Results of the tea samples were given as mg Trolox (16-250 µg ml^{-1}; y=67.53x-0.0029, R^2=0.9998) equivalent to the total antioxidant content per g of extract.

Qualitative and Quantitative Analysis by High-Performance Thin Layer Chromatography

The chromatographic separations of rosmarinic acid, caffeic acid, and verbascoside compounds in the tea extracts were visually determined by the HPTLC method, qualitatively. References including 200 µg ml^{-1} of standardized *M. officinalis* extract, rosmarinic acid, and caffeic acid with 5 mg ml^{-1} of samples were implemented 5 µl on a glass-backed plate coated with silica gel 60 F_{254} as an 8 mm wide band by an automatic Camag Limonat V sample spotter (Muttenz, Switzerland). For the development process, two mobile phase systems were utilized. For detection of rosmarinic acid and caffeic acid; chloroform: ethyl acetate: formic acid (5:4:1, *v:v:v*), for the verbascoside; ethyl acetate: water: formic acid: acetic acid (15:2:1:1, *v:v:v:v*) systems were used. After development, the plates were heated at 105 °C for 3 min and then derivatized with NP and PEG reagents respectively. Derivatized plate image was captured at 254 and 366 nm wavelengths by HPTLC imaging device.

The quantitative determination of rosmarinic acid content in the tea extracts was performed using the HPTLC method. A solution containing 0.2 to 1 µg of rosmarinic acid and 0.5 mg ml^{-1} of sample extracts was applied on the silica plate in triplicate by a sample applicator equipped with a 100 µl syringe (Hamilton, Bonaduz, Switzerland) connected to a nitrogen tank. The twin trough chamber was pre-conditioned with the vapor of the developing solvent system of chloroform: ethyl acetate: formic acid (5:4:1, *v:v:v*) for 20 minutes. Subsequently, it was developed up to 7 cm in the Camag Automatic Developing Chamber, with relative humidity fixed at 33% using magnesium chloride hexahydrate solution. After the development process, plates were automatically dried for 5 minutes and scanned in absorption/reflectance mode at 330 nm wavelength by Camag TLC Scanner 3. Rosmarinic acid contents were established through peak area via polynomial regression ($y=378.304+202.602x-0.007x^2$, R^2=0.99910).

Statistical Analysis

Statistical differences among the samples were analyzed using Minitab 17 software. The statistical significance of the results was determined through a one-way analysis of variance (ANOVA), and the significance levels were indicated using the Tukey post hoc test with a significance level of $p \leq 0.05$. The experiments were conducted in triplicate, and the results are presented as means ± standard error.

RESULTS and DISCUSSION

Anatomical Analysis

The stomatal structures of the samples were examined using chloral hydrate solution under a light microscope at 40x magnification (Figure 3). Among the samples, diacritic stomata surrounded by wavy epidermal cells were observed in six samples (S1, S2, S9, S12, S14, and S15), while anomocytic stomata with striated epidermal cells were present in seven samples (S3, S4, S6, S7, and S8). Additionally, paracytic stomata were identified in two other samples (S11 and S5). Furthermore, a few diacytic stomata were observed alongside anomocytic stomata in the S10 and S13 samples.

Among the observed stomatal types, diacritic stomata were found to have a width ranging from 15.17 to 17.54 µm, a height between 20.10 and 24.63 µm, and an outline area of 256.64 to 357.02 µm². Additionally, paracytic stomata exhibited an average width of 20.40 to 21.95 µm, a height of 23.80 to 25.49 µm, and an outline area of 396.58 to 442.81 µm². Anomocytic stomata, on the other hand, displayed an average width of 19.77 to 22.74 µm, a height ranging from 26.13 to 32.73 µm, and an outline area of 433.30 to 534.60 µm² (Table 2).

Table 2. The results regarding stomatal width, height, and outline in the tea samples. (*: Multiple stoma types were identified in samples S10 and S13, and only the measurements of the anomocytic stomata type were utilized)

Çizelge 2. Çay örneklerinde stoma genişliği, yüksekliği ve ana hatlarına ilişkin sonuçlar. (: S10 ve S13 örneklerinde birden fazla stoma tipi belirlenmiş olup sadece anomositik stoma tipinin ölçüleri kullanıldı)*

	Type of stomata	Epidermal cells	Stomatal width (µm)	Stomatal height (µm)	Outline (µm²)
S1	Diacritic	Sinuous	15.60±0.06	22.72±0.82	270.64±22.78
S2	Diacytic	Sinuous	15.68±0.77	20.10±1.32	256.64±32.25
S3	Anomocytic	Irregular and striated	19.77±0.29	28.66±0.81	433.30±4.69
S4	Anomocytic	Irregular and striated	22.74±0.14	26.13±1.65	456.19±12.93
S5	Paracytic	Polygonal	21.90±1.01	25.49±0.12	442.81±6.82
S6	Anomocytic	Irregular and striated	21.78±0.18	32.73±1.11	532.84±11.22
S7	Anomocytic	Irregular and striated	21.99±0.63	31.28±0.84	534.60±2.28
S8	Anomocytic	Irregular and striated	21.53±1.94	30.55±0.71	468.66±46.20
S9	Diacytic	Sinuous	17.08±0.14	23.88±2.14	315.67±7.43
S10*	Anomocytic	Irregular and striated	21.60±1.07	32.37±1.39	529.12±36.91
S11	Paracytic	Polygonal	20.40±2.02	23.80±1.39	396.58±55.25
S12	Diacytic	Sinuous	17.54±0.35	24.25±1.56	335.09±27.59
S13*	Anomocytic	Irregular and striated	22.19±0.94	26.86±1.02	467.34±8.38
S14	Diacytic	Sinuous	16.83±1.93	24.63±0.90	357.02±28.75
S15	Diacytic	Sinuous	15.17±1.04	24.08±1.47	280.03±10.98

Total Phenolic, Flavonoid, Anthocyanin and Antioxidant Contents

S1 and S2 samples showed the highest results in all analyses except for the total flavonoid experiment conducted without $NaNO_2$ in the medium, while S5 and S11 samples yielded the lowest results.

The total phenolic content of tea extracts varied between 305.08-117.80 mg gallic acid equivalent per gr extract. The S2 sample possessed the highest content with 305.08±2.32 mg gr^{-1} of hydroalcoholic extract (Table 3).

In the total flavonoid experiment, the two widely applied methods in other studies were used. In the absence of sodium nitrite, aluminum chloride, and sodium acetate reagent, the reaction occurs only with flavonols and luteolin in the flavonoid subclasses. However, in the presence of sodium nitrite, the reaction is more specific for rutin, luteolin, and catechins (Pękal & Pyrzynska, 2014; Shraim et al., 2021). The results showed that the S3 sample had the highest total flavonoid amount with 48.83±0.89 mg gr^{-1} of extract in the reaction without $NaNO_2$. When $NaNO_2$ was added to the reaction, the S2 sample exhibited the highest results with 260.17±2.96 mg gr^{-1} compared to other extracts (Table 3).

According to the thin-layer chromatography test, anthocyanin subclass compounds were identified on the plate in S15 (Figure 3). In the total monomeric anthocyanin content experiment; 2.03±0.21 mg cyanidin 3-glucoside equivalent total anthocyanin per g of extract was detected in the structure of tea extract coded as S15.

In the antioxidant experiments, it was defined that the S1 and S2 samples owned a higher antioxidant content than the other extracts. In the FRAP, ABTS, DPPH, and CUPRAC assays, the S1 sample yielded the following results; 410.91±9.99, 356.37±5.61, 300.96±5.70, 534.28±11.99 mg total antioxidant content per gram of extract, respectively. The S2 sample possessed 460.15±13.41 mg, 375.81±5.07 mg, 309.60±4.76 mg, and 643.05±11.58 mg of total antioxidant content per gram of extract in the same assays (Table 3).

High-Performance Thin Layer Chromatography

Rosmarinic acid and caffeic acid, which are found in high amounts in *M. officinalis* along with verbascoside, the main compound of lemon verbena, were visually examined (Figure 4). Rosmarinic acid was detected in 10 samples (S2, S3, S4, S7, S9, S10, S12, S13, S14, and S15), verbascoside in 7 samples (S3, S4, S6, S7, S8, S10, and S13), and caffeic acid in 4 samples (S1, S2, S9, and S12) based on chromatograms.

Anthocyanins are substances that can be observed in white light without any derivatization on a chromatogram plate. They exhibit maximum absorbance between wavelengths of 500-550 nm (Saha et al., 2020). Accordingly, the red substances separated in white light in S15 were identified as anthocyanin (Figure 5). Furthermore, in the chromatographic bands of S5 and S11 samples, completely different substances were observed.

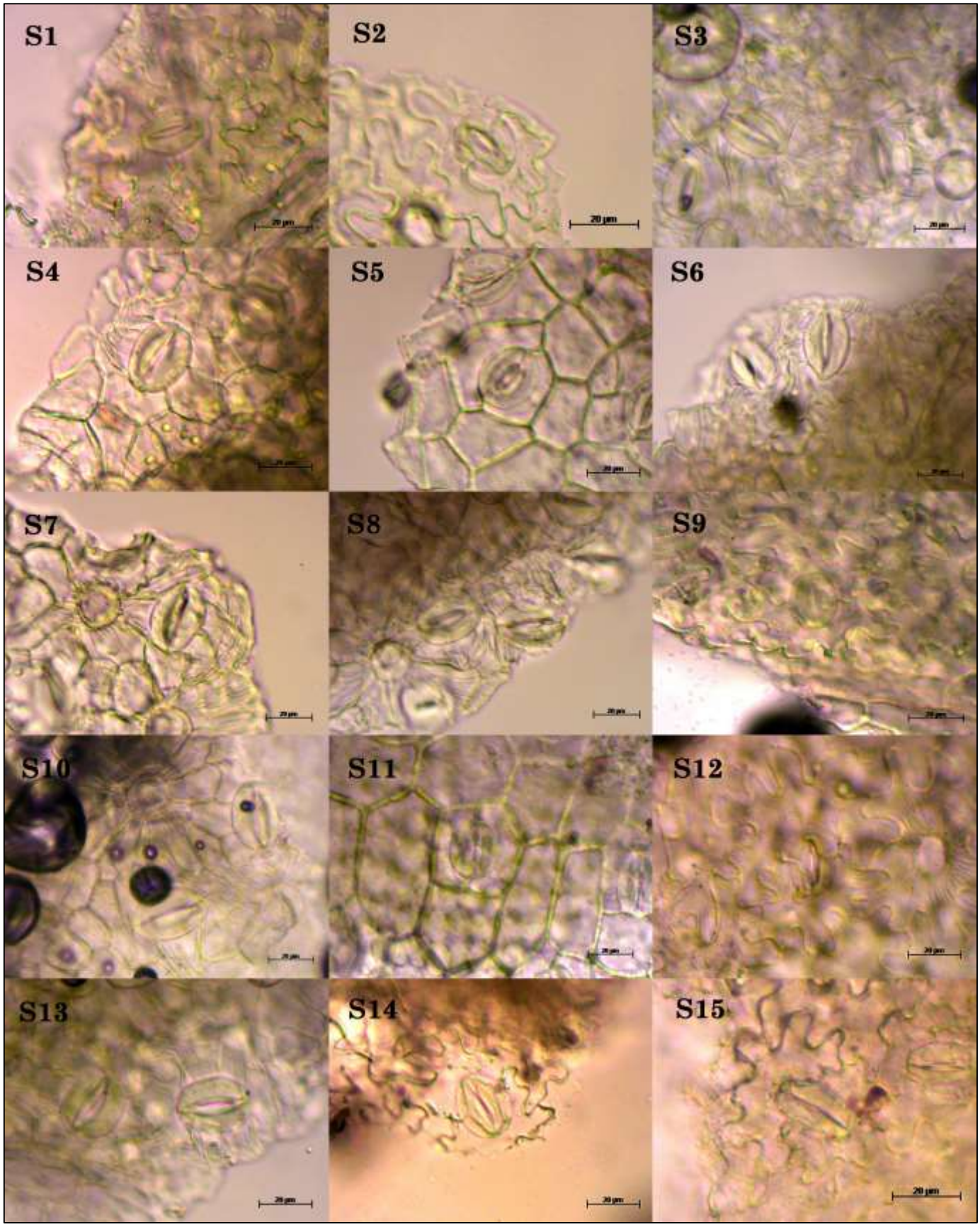

Figure 3. Major stomata types of tea samples with 40x magnification
Şekil 3. 40x büyütmede çay örneklerinin ana stomaları

Table 3. Total phenolic, flavonoid, antioxidant, and rosmarinic acid contents in *Melissa officinalis* L. tea extracts[6]. (N.D.: not detected)
Çizelge 3. Melissa officinalis L. çay örneklerindeki toplam fenolik, flavonoid, antioksidan ve rosmarinik asit miktarları (N.D.: tespit edilmedi)

	Total phenolic content[1]	Total flavonoid content[2, 3]		Total antioxidant content[4]				Rosmarinic acid content[5]
		$AlCl_3$	$NaNO_2$	FRAP	ABTS	DPPH	CUPRAC	
S1	268.53±5.45^{B}	27.86±0.33^{EF}	228.75±0.14^{B}	410.91±9.99^{B}	356.37±5.61^{A}	300.96±5.70^{A}	534.28±11.99^{B}	60.08±1.87^{A}
S2	305.08±2.32^{A}	32.49±1.02^{CD}	260.17±2.96^{A}	460.15±13.41^{A}	375.81±5.07^{A}	309.60±4.76^{A}	643.05±11.58^{A}	52.45±0.61^{B}
S3	148.94±1.13^{E}	48.83±0.89^{A}	135.60±4.68^{E}	164.34±1.75^{F}	188.86±3.00^{F}	190.35±6.39^{DEF}	261.45±2.93^{E}	1.10±0.11^{FG}
S4	121.43±3.76^{F}	27.81±1.41^{EF}	117.29±1.56^{FG}	125.11±2.68^{HI}	176.11±3.50^{FG}	158.83±4.30^{GH}	196.14±6.76^{G}	N.D.
S5	104.64±1.60^{F}	44.42±0.82^{B}	82.78±1.22^{H}	82.05±0.57^{J}	158.62±1.36^{G}	135.27±2.46^{H}	169.04±4.89^{G}	N.D.
S6	182.95±2.22^{D}	41.46±0.47^{B}	154.56±5.25^{D}	203±7.70^{E}	236.86±7.87^{C}	228.95±8.90^{C}	318.64±6.10^{D}	N.D.
S7	147.89±7.74^{E}	34.16±0.73^{CD}	134.68±5.66^{E}	156.92±2.22^{FGH}	204.18±2.99^{DEF}	197.88±6.10^{DE}	242.56±8.78^{EF}	1.80±0.18^{FG}
S8	158.36±2.58^{E}	34.50±0.53^{CD}	140.33±1.62^{E}	150.90±2.75^{FGH}	202±6.29^{DEF}	190.37±2.25^{DEF}	243.18±2.25^{EF}	N.D.
S9	215.46±1.27^{C}	30.54±0.53^{DEF}	156.25±1.29^{D}	277.12±3.00^{D}	305.77±6.78^{B}	259.44±2.16^{B}	420.84±10.91^{C}	35.73±1.46^{C}
S10	150.96±2.65^{E}	31.67±0.47^{CDEF}	127.46±0.11^{EF}	163.06±2.63^{F}	199.39±2.52^{DEF}	188.84±0.87^{DEF}	261.35±4.07^{E}	3.65±0.34^{F}
S11	118.42±3.88^{F}	34.78±0.82^{C}	45.34±0.33^{J}	98.47±2.19^{IJ}	227.16±1.59^{CD}	170.71±4.70^{FG}	173.06±12.33^{G}	N.D.
S12	253.02±2.40^{B}	27.71±0.34^{F}	194.17±1.61^{C}	333.28±13.77^{C}	312.35±13.34^{B}	273.90±5.06^{B}	528.58±10.84^{B}	57.68±0.72^{A}
S13	143.95±1.22^{E}	31.91±0.45^{CDE}	109.76±2.01^{G}	145.19±5.75^{FGH}	183.54±2.01^{FG}	177.54±2.96^{EFG}	240.34±6.99^{EF}	3.63±0.18^{F}
S14	117.80±0.76^{F}	22.27±1.50^{G}	62.06±1.09^{I}	128.56±3.73^{GHI}	197.90±2.65^{EF}	166.81±1.65^{FG}	205.15±0.51^{FG}	7.25±0.17^{E}
S15	159.25±2.19^{E}	35.37±0.54^{C}	113.44±2.05^{FG}	162.49±5.62^{FG}	220.63±4.20^{CDE}	212.02±9.15^{CD}	271.23±11.41^{E}	13.41±0.43^{D}

[1] mg gallic acid equivalent total phenolic content in gr extract
[2] $AlCl_3$ procedure, mg quercetin equivalent total flavonoid content in gr extract
[3] $NaNO_2$ procedure, mg catechin equivalent total flavonoid content in gr extract
[4] mg Trolox equivalent total antioxidant content in gr extract
[5] mg rosmarinic acid per gr extract
[6] Different letters in the same column indicate significantly different values at $P \leq 0.05$

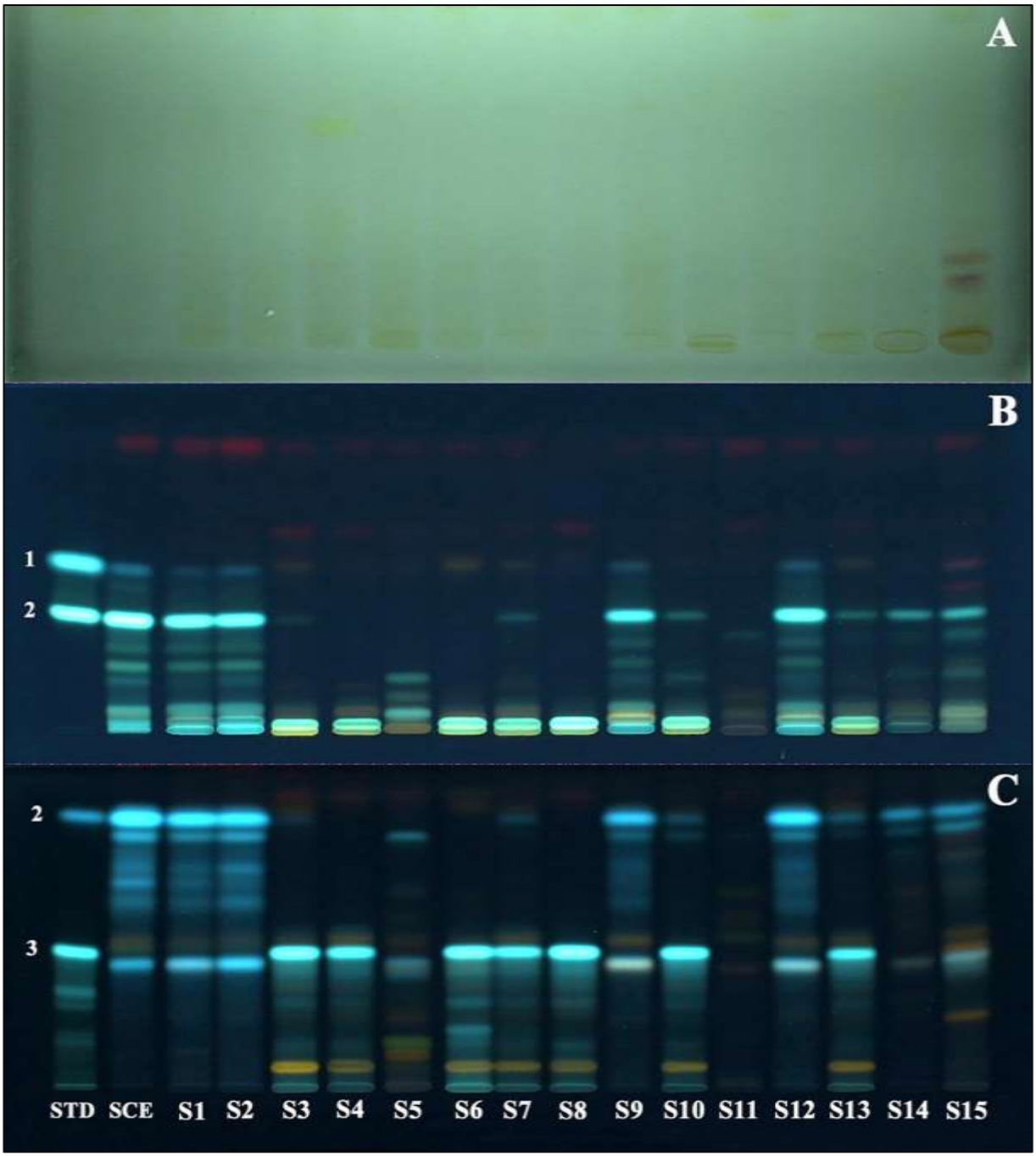

Figure 4. HPTLC chromatograms of tea extracts. STD: standards. 1: caffeic acid, 2: rosmarinic acid and 3: verbascoside. SCE: standardized commercial *M. officinalis* extract. (A) at white light, mobile phase: ethyl acetate–formic acid–acetic acid–water, (100:11:11:26, v:v:v:v), (B) at 366 nm and derivatized with NP/PEG, mobile phase: chloroform–ethyl acetate–formic acid (5:4:1, v:v:v:v), (C) at 366 nm and derivatized with NP/PEG, mobile phase: ethyl acetate–water–formic acid–acetic acid (15:2:1:1, v:v:v:v).

Şekil 4. Çay örneklerinin YPİTK kromatogramları. STD: referanslar. 1: kafeik asit, 2: rosmarinik asit ve 3: verbaskozit. SCR: standardize ticari M. officinalis ekstresi. (A) beyaz ışıkda, hareketli faz: etil asetat-formik asit- asetik asit-su, (100:11:11:26, v:v:v:v), (B) 366 nm'de ve NP/PEG ile türevlendirilmiş, hareketli faz: kloroform-etil asetat-formik asit (5:4:1, v:v:v), (C) 366 nm'de ve NP/PEG ile türevlendirilmiş, hareketli faz: etil asetat-su-formik asit-asetik asit (15:2:1:1, v:v:v:v).

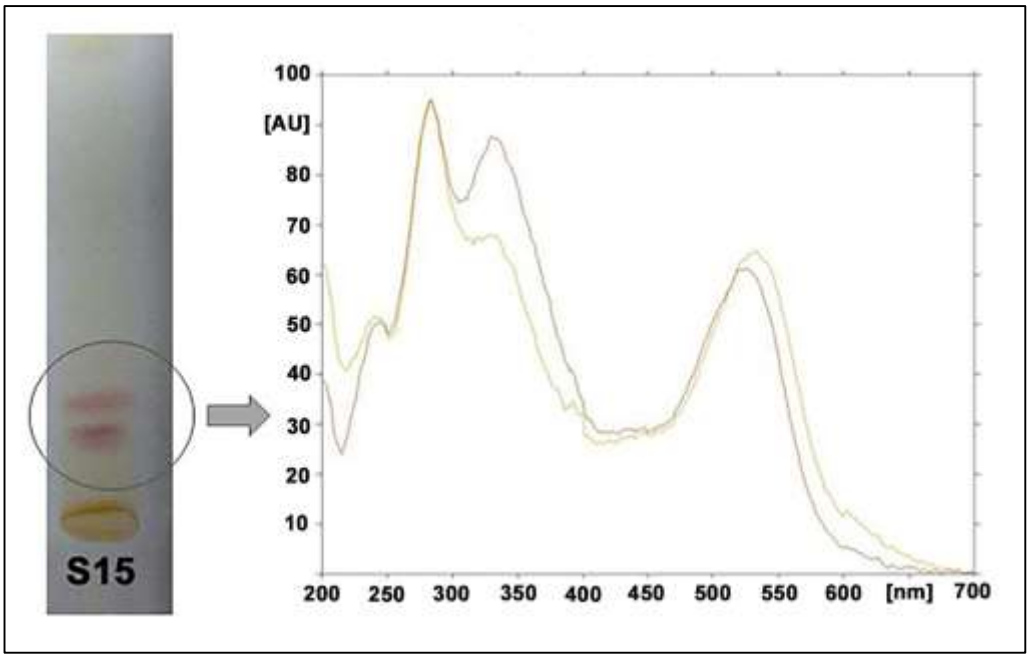

Figure 5. Overlaid spectra of the red-colored spots in S15 between 200 and 700 nm wavelength
Şekil 5. S15'teki 200 ve 700 nm dalga boyu arasındaki kırmızı renkli noktaların üst üste bindirilmiş spektrumları

Quantitative Rosmarinic Acid Content by HPTLC

According to the European Pharmacopeia, *M. officinalis* leaf extract must contain at least 2% rosmarinic acid (Anonymous, 2023). In the analysis, rosmarinic acid was found in 11 of the 15 samples and defined only 4 tea extracts (S1: 6%, S2: 5.2%, S9: 3.6%, and S12: 5.8%) possessed rosmarinic acid in their chemical structure above 2% (Table 2). While the S1 sample was the extract with the highest content of rosmarinic acid with 60.08±1.87 mg gr^{-1} extract in its chemical composition (Figure 6), it was observed there was no rosmarinic acid in the structure of the 5 tea extracts (S4, S5, S6, S8, and S11).

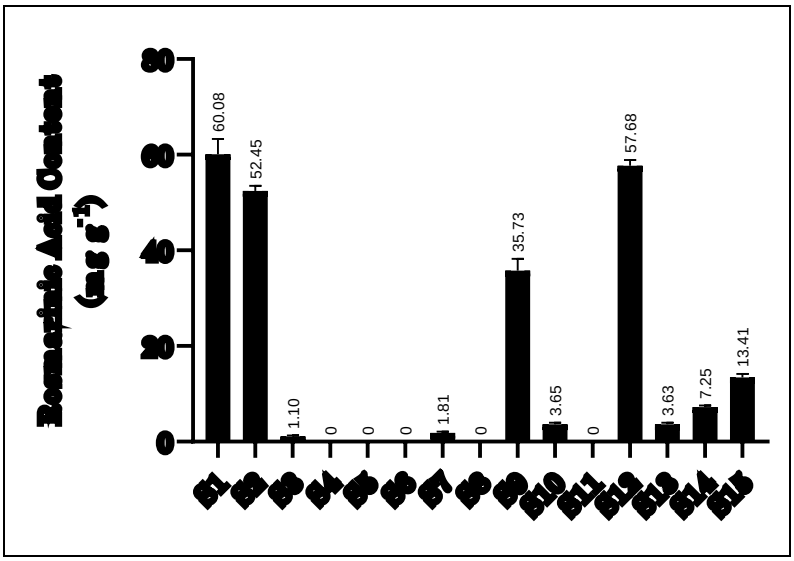

Figure 6. Rosmarinic acid content in samples
Şekil 6. Örneklerdeki rosmarinik asit miktarları

From an anatomical perspective, three types of stomata were identified in the examined samples: diacritic, anomocytic, and paracytic. The stomatal structure of the *M. officinalis* leaf, which belongs to the Lamiaceae family, is diacritic (Anonymous, 2023), and this stomatal structure was observed in six samples (S1, S2, S9, S12, S14 and S15). Additionally, it was found that the sizes of these three stoma types varied, with diacritic stomata being smaller than the others in width, height, and outline (Table 2).

The most abundant substance in the chemical composition of *M. officinalis* is rosmarinic acid

(Shakeri et al., 2016), and over 2% of rosmarinic acid was detected in the structure of 4 samples (S1, S2, S9, and S12) of these 6 samples. In some studies on lemon balm, the ratio of rosmarinic acid in the chemical composition of the hydroalcoholic extracts of the lemon balm leaf varies between 3-8% (Dastmalchi et al., 2008; Kim et al., 2010; Shekarchi et al., 2012; Calleja et al., 2017; Kittler et al., 2018; Kittler et al., 2018).

In addition to chlorogenic acid, caffeic acid, another major substance found in the structure of *M. officinalis*, was also detected in the chromatogram of S1, S2, S9, and S12 samples. Hydroxycinnamic acids are substances with antioxidant properties (Ockun et al., 2022), and Papoti et al. (2019) stated that a higher hydroxycinnamic acid content increased the total phenol and antioxidant content of the *M. officinalis* extract obtained using different extraction methods. Therefore, these four examples have higher total phenol and antioxidant content compared to other samples. However, these samples demonstrated low results in the absence of $NaNO_2$ in terms of total flavonoid content due to the lower amount of flavonols in their structures compared to other samples. Due to the high content of phenolic acid in the structure of *M. officinalis*, these samples reached the highest total flavonoid content in the presence of $NaNO_2$. Although the presence of $NaNO_2$ increases the selectivity in flavonoids, phenolic acids also exhibit the same absorbance with flavonoids at 510 nm wavelength (Pękal & Pyrzynska, 2014).

Anomocytic stomata were observed in 7 samples (S3, S4, S6, S7, S8, S10, and S13), and verbascoside (acetonide), the main compound found in *Aloysia* (Bahramsoltani et al., 2018; Costa de Melo et al., 2019; Tammar et al., 2021), was found in the thin layer chromatography chromatogram of these samples. However, in the phytochemical studies, verbascoside was not identified in the chemical composition of *M. officinalis* (Shakeri et al., 2016). Interestingly, diabetic stomata, resembling the epidermal surface of *M. officinalis*, were also found in 2 of these 7 samples (S10 and S13). This suggests that these two specimens may have been adulterated and sold as *M. officinalis*.

S5 and S11 samples were considered to be completely different plants from *M. officinalis* and *Aloysia* species. They exhibited paracytic stomata type, and the substances separated in their chromatograms were distinct from those in other samples. Additionally, their phenolic and antioxidant contents were lower compared to the other samples.

The low rosmarinic acid, total phenolic, and antioxidant content in the S14 sample may be attributed to unfavorable storage conditions for this product.

Despite the showing of 1.34% rosmarinic acid in the phytochemical composition of the S15 sample and its anatomical diacytic stomata structure, the presence of anthocyanin in its structure is an indication that this sample was likely adulterated.

CONCLUSION

As a result of this study, appropriate stomatal structure, rosmarinic acid ratio above 2%, and presence of caffeic acid in its composition were determined in only 4 samples (S1, S2, S9, and S12). While substituting *Aloysia* for *M. officinalis* as a herb in the markets poses a problem, much more significant issues were observed in crushed-type herbal teas. These issues include selling different plants instead of lemon balm, adulteration, and low rosmarinic acid content. For these reasons, it is recommended to frequently conduct quality controls, especially in markets selling medicinal herbs and teas.

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