

## Lemon balm and sage herbal teas: Quantity and infusion time on the benefit of the content

Chás de sálvia e erva-cidreira: Quantidade e do tempo de infusão no benefício do conteúdo

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### ABSTRACT

Sage (*Salvia officinalis* L.) and lemon balm (*Melissa officinalis* L.) are important medicinal plants and their infusions (herbal teas) are the most common form of these plants consumed. In this study, herbal teas of both species cultivated and exported in Anatolia region were prepared using different infusion time (5 and 10 min) and sample amount (2 and 3 g). These herbal teas were analysed for their vitamin C, total phenolics, total flavonoids, total flavanols, free radical scavenging activities (1,1-diphenyl-2-picryl-hydrazyl and hydrogen peroxide), and also twelve minerals (K, Na, Mg, Ca, Fe, B, Ti, Sr, Ba, Li, Ag and Ga) were determined in each sage and lemon balm dry leaves as well as their herbal teas. Vitamin C in the herbal teas varied from 0.24 to 615.8 µg/mL. Total bioactive contents of the herbal teas ranged from 76.4 to 215.4 mg gallic acid equivalents (GAE)/cup for total phenolics, 16.2 to 69.9 mg quercetin (QE)/cup for total flavonoids, and 363.8 to 906.7 µg catechin equivalent (CE)/cup for total flavanol. The highest total bioactive contents and antioxidant activities were found in lemon balm herbal teas, and also in the herbal teas prepared with 3g sample amount and 10 min infusion time of both specie. As a result, the study also showed that the best sample amount in terms of the analysed minerals and vitamin C in herbal teas was 3 g for both species, but the best infusion time was 5 minutes for sage and 10 minutes for lemon balm.

**Index terms:** Antioxidant activity; minerals; vitamin C.

### RESUMO

A sálvia (*Salvia officinalis* L.) e a erva-cidreira (*Melissa officinalis* L.) são importantes plantas medicinais e suas infusões (chás de ervas) são a forma mais comum de consumo dessas plantas. Neste estudo, os chás de ervas de ambas as espécies cultivadas e exportadas na região da Anatólia foram preparados utilizando diferentes tempos de infusão (5 e 10 min) e quantidade de amostra (2 e 3 g). Estes chás de ervas foram analisados em termos de vitamina C, fenólicos totais, flavonóides totais, flavonóis totais, atividade sequestradora de radicais livres (1,1-difenil-2-picril-hidrazil e peróxido de hidrogênio) e doze minerais (K, Na, Mg, Ca, Fe, B, Ti, Sr, Ba, Li, Ag e Ga) foram determinados nas folhas secas de cada sálvia e erva-cidreira, bem como nos seus chás de ervas. A vitamina C nos chás de ervas variou de 0,24 a 615,8 µg/mL. O conteúdo bioativo total dos chás de ervas variou de 76,4 a 215,4 mg de equivalente de ácido gálico (GAE)/xícara para fenólicos totais, 16,2 a 69,9 mg de quercetina (QE)/xícara para flavonóides totais e 363,8 a 906,7 µg de equivalente de catequina (CE)/xícara para flavanol total. Os maiores conteúdos bioativos totais e atividades antioxidantes foram encontrados nos chás de erva-cidreira e também nos chás preparados com a quantidade de amostra de 3g e o tempo de infusão de 10 min de ambas as espécies. Como resultado, o estudo também mostrou que a melhor quantidade de amostra em termos de minerais e vitamina C analisados em chás de ervas foi de 3 g para ambas as espécies, mas o melhor tempo de infusão foi de 5 minutos para sálvia e 10 minutos para erva-cidreira.

**Termos para indexação:** Atividade antioxidante; minerais; vitamina C.

### INTRODUCTION

Herbal teas are widely consumed all around the world, drinks usually rich in antioxidants and other medicinal compounds which positive health effects (Atoui et al., 2005). Different herbal tea belonging to various plant families have been used as therapeutical, disease preventive or refreshment, in many forms of traditional medicine (Poswal et al., 2019). Since herbal teas are rich

in antioxidants, their consumption is becoming more and more preferred among health conscious people (Ivanova et al., 2005). They are usually consumed as infusions or oil extracts individually or as mixture of different materials (Costa et al., 2012; Yıldırım et al., 2017).

Sage (*Salvia officinalis* L.) belongs to a species of *Salvia* genus which is largest genera of Lamiaceae family. Some sage species are aromatic plants, native to Mediterranean region and consumed herbal teas in folk

medicine due to their medicinal properties since the ancient times. *Salvia officinalis* L. contains a significant amount of natural antioxidants, its medicinal effects can generally be associated with its high antioxidant content. Antioxidant capacity of the species has also strong antibacterial, anti-inflammatory, anticancer and antiproliferative properties (Hamidpour et al., 2014; Nutrizio et al., 2020). The previous studies also indicated that herbal teas of sage possess potential of antioxidant activity and this properties provide protection against oxidation-related disease, raises brain functions, prevent against dementia, depression, diabetes, obesity, even reduces sweating (Walch, 2011; Hamidpour et al., 2014).

Lemon balm (*Melissa officinalis* L) also belongs to Lamiaceae family. It is cultivated in many countries and native to the eastern Mediterranean and western Asia regions (Atashi et al., 2014). Because of essential oil of the species has strong antiviral, antibacterial, antidepressant and antispasmodic activities, it is used to treat sleep disorders and gastrointestinal disorders like abdominal pain or antidepressants (Sari; Ceylan, 2002; Usai; Atzei; Marchetti, 2016). Also, herbal tea of lemon balm was beneficial for many healths such as bronchial afflictions, gastric disorders, hypertension, nervous system disorders and insomnia (Skotti et al., 2014; Akbar, 2020). Most of the biological activities of these plants are associated with their high content of polyphenolic compounds, essential oils, vitamins and minerals. Most of the polyphenolic compounds consist of rosmarinic acid, quercetin, rutin, caffeic acid, chlorogenic acid and gallic acid in both sage and lemon balm. These compounds are related to impressive many health benefits, such as antidepressant, improved brain health and support memory, reduce blood sugar levels and cholesterol (Fonteles et al., 2016; Alagawany et al., 2017; Ghorbani; Esmaeilzadeh, 2017; Asadi et al., 2018; Khedher et al., 2018). These are especially due to high antioxidant and inflammatory activities of these compounds (Nunes et al., 2017; Shinjyo; Green, 2017). Antioxidant compounds help fortify your body's defences, reducing harmful effects of free radicals that are related to many diseases (Khansari et al., 2009).

Vitamin C is a water-soluble essential vitamin found high ratio in sage and lemon balm. Previous studies reported that Vitamin C slowed skin aging through stimulating collagen synthesis and also prevented various types of cancer (Verrax; Calderon, 2009; Chen et al., 2011), the oxidative stress (Harrison, 2012) and had strong antioxidant activity (Liu et al., 2019). Therefore, drinking herbal tea of Sage and lemon balms is of great importance for the human diet that contributed to increase its nutritional

and health benefits value. Moreover, drinking 240 mL of sage herbal tea twice daily significantly increased antioxidant defences and also had a positive effect on cholesterol on human health (Sá et al., 2009). Also, sage and lemon balm herb parts are rich sources of minerals like potassium, sodium, magnesium, calcium and iron. The minerals have many effects on human health. Potassium is an important mineral of cell and body fluids, which helps control heart rate and blood pressure (Nowak et al., 2019). Magnesium acts as a cofactor for many enzymes (Al Alawi; Majoni; Falhammar, 2018). In contrast, heavy metals such as Ag are toxic metals for human health. These heavy metal in plants often arise from contamination in cultivation conditions (soil and irrigation) or toxic emissions (Adamczyk-Szabela et al., 2017). Heavy metals in plants are taken by humans through the consumption of herbal tea. Therefore, the accumulation potential of heavy metal of plants is significant. Due to the fact that some regions and waters of Turkey and many countries are rich in toxic metals (Ag, Pb, Cd, As and etc.), it is important to analyze the toxic element accumulation of plants consumed by humans and animals (Sasmaz; Obek; Sasmaz, 2016; Yıldırım; Sasmaz, 2017; Marrugo-Negrete et al., 2019). There are many studies on the leaves of these species and their herbal teas (Arceusz et al., 2013; Herrera et al., 2018). There are no comprehensive reports about the variations in vitamin C, minerals, total flavonoid, total phenolic content and antioxidant activity of herbal teas made from sage and lemon balm prepared with different infusion time and sample amount. Therefore, the main of this study is to investigate effect of different sample amount and infusion time on the mineral compositions, antioxidant activities, total bioactive contents and vitamin C content of herbal teas of sage and lemon balm.

## MATERIAL AND METHODS

### Plant material

Cultivated and exported populations of the lemon balm (*Melissa officinalis* L) and sage (*Salvia officinalis* L.) species grown in Yalova province of Turkey were used to prepare herbal teas. The fresh leaves of the plants containing thirty individuals of each specie were harvested at the end of flowering in 2018, mixed and used to prepare herbal tea.

### Preparation of infusion herbal teas

The fresh leaves of both species were dried and powdered. As indicated in Figure 1, the infusions for

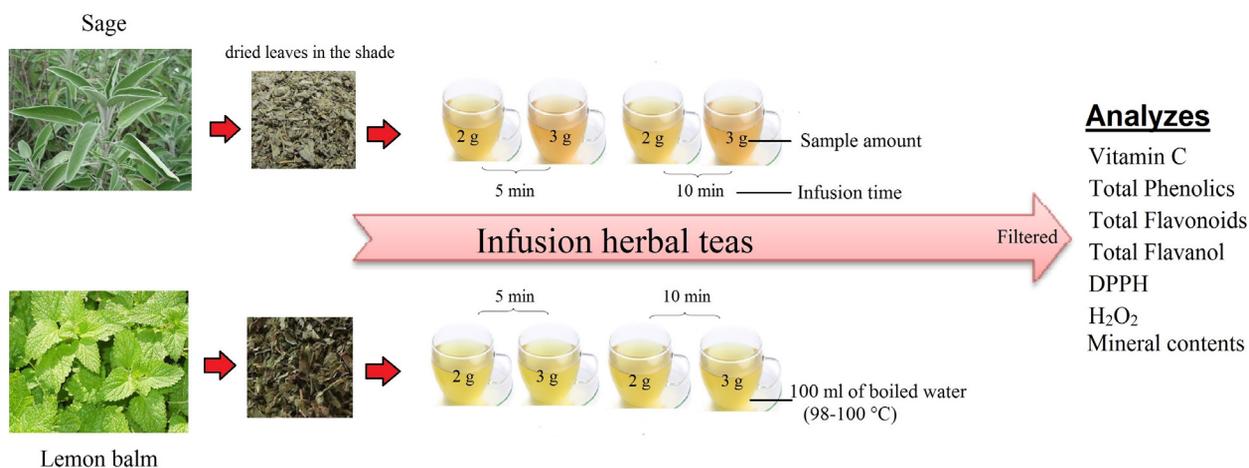
both species were prepared by adding two different amounts (2 and 3 g) in 100 mL of boiling water (98–100 °C) and mixtures of each amount were allowed to stay at room temperature for two different infusion times (5 min and 10 min) in order to determine the optimum infusing time (Gastaldi et al., 2018). Each experiment was conducted for 4 replicates. Each herbal tea samples obtained were filtered through Whatman filter papers and divided into two parts, kept at -20 °C before mineral analyses and at +4 °C until other analyses (bioactive and vitamin C contents and antioxidant activities).

### Analysis of vitamin C

Vitamin C contents of the herbal teas were analysed according to the methods Vieira, Teixeira and Silva (2000) and Yuan and Chen (1999) with slight modification, using Shimadzu LC-20AT method. The liquid chromatographic method used for the determination of L-ascorbic acid (AA) with Photo diode array detection (PDA) at 272 nm. Separations were carried out on a reverse phase column C18 (Inertsil ODS-4, Japan). The mobile phase employed was a mixture of distilled water and acetonitrile (9:1) at pH 2. Flow rate of the mobile phase was 0.8 mL/min and an injection volume of 10 µL was used in quantitative analysis. The temperature of analytical column was kept constant at 40°C. The calibration curve and quantitative evaluations were accomplished at 272 nm. The calibration curve was plotted in the concentration range of 25–1000 mg/L and based on a 6-point calibration. The vitamin C contents of herbal teas were calculated using calibration curve ( $y = 3052,8x + 1157,5$ ;  $R^2 = 0,9998$ ) of AA.

### Analysis of total phenolics, flavonoids and flavanol contents

The total phenolic contents in herbal teas of lemon balm and sage were measured using the Folin-Ciocalteu method (Singleton; Orthofer; Lamuela-Raventos, 1999). Each herbal tea (200 µL) was mixed with 0.2 mL Folin-Ciocalteu 0.6 mL of 20% sodium carbonate solution, and the total volume was adjusted to 10 mL with distilled water. After the mixtures were kept in the dark for 2 hours at room temperature, absorbance measurement was scored at 760 nm. The results were represented as mg gallic acid equivalents (GAE)/cup (150 mL) of herbal tea. The total flavonoid content of herbal teas was measured using the method developed by Arvouet-Grand et al. (1994) with minor modifications. Each herbal tea (200 µL) was mixed with 10% aluminum nitrate (100 µL) and 1 M potassium acetate (100 µL). The total volume was adjusted to 5 mL with 99% ethanol. After the mixtures were incubated in the dark for 40 min at room temperature, absorbance measurement was performed at 417 nm. The total flavonoid content of herbal teas was calculated as mg quercetin equivalent (QE)/ cup (150 mL) of herbal tea. The total flavanol contents of herbal teas were analyzed using the method developed by Quettier-Deleu et al. (2000) with slight modification. Each herbal tea (250 µL) was mixed with 5 mL of 0.1% DMACA (*p*-dimethylaminocinnamaldehyde) in methanolic:HCl (3:1) reagent. After the mixtures were incubated for 10 min at room temperature, absorbance measurement was performed at 417 nm. Total flavanol content of herbal teas was calculated as µg catechin equivalent (CE)/ cup (150 mL) of herbal tea.



**Figure 1:** Preparation of infusion herbal teas of sage and lemon balm.

### Analysis of antioxidan capacity

The effect of the herbal teas on DPPH radical was tested according to Gezer et al. (2006). Each herbal tea (200  $\mu$ L) was mixed with a 3.2 mL of a 0.004% methanol solution of DPPH. After the mixtures were incubated in the dark for 30 min at room temperature, absorbance measurement was performed at 517 nm. DPPH radical scavenging activity of herbal teas was expressed as equivalents of trolox (TE)/cup (150 mL) of herbal tea. The hydrogen peroxide scavenging activity of herbal teas was measured using the phosphate buffer (0.04 M, pH = 7.4) by Ruch, Cheng and Klaunig (1989) with minor modification. Each herbal tea (100  $\mu$ L) was mixed with 2.4 mL of buffer solution (0.1 M, pH = 7.4 phosphate buffer) and 1 mL of H<sub>2</sub>O<sub>2</sub> (40 mM) prepared phosphate buffer. Absorbance measures were read at 230 nm after 10 min incubation. The H<sub>2</sub>O<sub>2</sub> scavenging activity was expressed as equivalents of ascorbic acid (AAE)/cup (150 mL) of herbal tea.

### Analysis of mineral content

Method for digestion of the samples as reported by Turksoy et al. (2019) was used to solubilize leave of the sage and lemon balm with a few modifications. 200 mg of samples were weighed and added to microwave teflon tubes. After adding 5 mL suprapure HNO<sub>3</sub>, 2 mL H<sub>2</sub>O<sub>2</sub> and 3 mL ultrapure water to the samples, the caps were closed and digested with Microwave Digestion System (Milestone Stat D). The tubes cooled to the room temperature. On the other hand, their herbal tea samples were analysed directly (without digestion). The digested samples were diluted to 1/1 with distilled water. Appropriately diluted samples were measured by an ICP-MS (Thermo Scientific, USA). The internal standard (Hafnium, Hf) for the minerals was used, and all analyses were performed at least three times. The R<sup>2</sup> values of the calibration curves of the minerals are as follows: 0.9782 (Na), 0.9752 (Mg), 0.9967 (K), 0.9786 (Ca), 0.9770 (Fe), 0.9902 (Ba), 0.9902 (Ti), 0.9902 (Sr), 0.9902 for (Ga), and 0.9902 (Ag). Determination of the minerals in sage and lemon balm as well as their herbal teas and were analysed using ICP-MS with the following operating conditions: RF power, 1550 W; RF matching, 1.80 V; carrier gas, 0.97 L/min; spray chamber temperature, 2.7 °C.

### Statistical analysis

The results were expressed as mean values and standard deviations ( $\pm$ SD). The experiments were conducted as a using one-way analysis of variance (ANOVA). It was used Levene's test to check homogeneity

of variances before ANOVA tests. Differences between the means were compared by Duncan's multiple range tests using MSTAT-C computer programme. Data given in percentages were subjected to arcsine ( $\sqrt{X}$ ) transformation before statistical analysis. The Pearson correlation analysis was used to evaluate of the correlation between antioxidant activities and total phenolic contents. Post hoc inter-group comparisons of mineral concentrations were performed by the non-parametric Mann-Whitney U test for two independent samples.

## RESULTS AND DISCUSSION

### Vitamin C content

The vitamin C contents of the herbal teas of lemon balm and sage were given in Table 1. Amount of vitamin C ranged from 0.24 to 615.8  $\mu$ g/mL in both species. S3 (6.65  $\mu$ g/mL) and L4 (615.8  $\mu$ g/mL) treatments respectively showed the maximum vitamin C content for sage and lemon balm. Vitamin C content increased with rising up sample amount in sage, but decreased with increasing the infusion time. Similar to this study, Vieira, Teixeira and Silva (2000) reported that ascorbic acid increased the rate of degradation of ascorbic acid with the increase of temperature application, and even same results were observed with increasing application time. On the contrary, as the infusion time in lemon balm was extended, vitamin C content also increased. Up to date there has no any report about vitamin C content related to interactions between infusion time and amount of herbal teas of sage and lemon balm.

### Total phenolic content

Total phenolic contents in sage and lemon balm were given in mg GAE/cup (Table 1.). Although total phenolic contents in sage varied from 76.4 to 132.5 mg GAE/cup that of lemon balm fluctuated between 56.0 and 215.4 mg GAE/cup. S4 and L4 herbal tea treatments in both species were found the most effect infusion herbal teas for total phenolic content (132.5 and 215.4 mg GAE/cup content respectively). All herbal tea treatments of lemon balm exhibited to have higher total phenolic content than that of sage and there was found statistically significant difference ( $p < 0.01$ ) among the total phenolic contents of each species. Similarly, previous most studies stated that lemon balm had higher total phenolic content than sage (Atanassova; Georgieva; Ivancheva, 2011; Skotti et al., 2014; Jiménez-Zamora; Delgado-Andrade; Rufián-Henares, 2016). Tahirović et al. (2014) reported that total phenolic contents in infusion herbal tea (for 1 g and 10

min) of sage at different growing areas varied from 72.6 to 13.7 mg GAE/100 mL. Rusaczonok, Świdorski and Waszkiewicz-Robak (2010) reported that total phenolic content in herbal infusions of lemon balm fluctuated between 232 and 158 mg GAE/g dry matter. When the infusion herbal teas of both species were examined, total phenolic contents increased with increasing sample amount and the infusion times. Papoti et al. (2019) reported similar results in terms of phenolic content of lemon balm.

### Total flavonoid content

Total flavonoid contents in sage and lemon balm were given in Table 1. The highest total flavonoid content was found in L4 herbal tea treatment of lemon balm (69.9 mg QE/cup). On the other hand, S1 herbal tea treatment of sage gave the lowest total flavonoid content (16.2 mg QE/cup). All herbal tea treatments of lemon balm had the higher total flavonoid content than that of sage as the results regarding the phenolic content content. Total flavonoid content were statistically influenced ( $p < 0.01$ ) by infusion times for both species. The total flavonoid contents of the

herbal teas were positively correlated to total phenolic content ( $R^2=0.996$ ) with highly significant correlation. Atanassova, Georgieva and Ivancheva (2011) reported similar results for dry matter of sage and lemon balm.

### Total flavanol content

Total flavonoid contents of all herbal teas were given in Table 1 and varied from 363.8 to 906.7  $\mu\text{g CE/cup}$ . Total flavanol contents of all herbal tea treatments exhibited the difference according to their total phenolic and total flavonoid contents. The highest total flavanol contents were found in L4 treatment for lemon balm (906.7  $\mu\text{g CE/cup}$ ) and S4 herbal tea of sage (853.3  $\mu\text{g CE/cup}$ ). S4, L4, L3 and L2 herbal tea treatments were statistically in the same group ( $p < 0.01$ ). The total flavanol content of the herbal teas were positively correlated to total phenolic ( $R^2=0.838$ ) and total flavonoid content ( $R^2=0.882$ ) with highly significant correlation for both species. In general, L4 herbal tea of lemon balm was found to have higher total phenolic, flavonoid and flavanol contents than infusion herbal samples in this study and in previous studies (Gastaldi et al., 2018).

**Table 1:** Comparison of the effect of sample quantity and infusion times on vitamin C, total phenolic, total flavonoid, total flavanol contents and radical scavenging activities of the herbal teas.

| Sample amount                              | Treatments |            |             |             |
|--|------------|------------|-------------|-------------|
|  | 2 g        |            | 3 g         |             |
| Infusion time                              | 5 min      | 10 min     | 5 min       | 10 min      |
| Abbreviated name of infusion herbal teas   |            |            |             |             |
| Sage                                       | S1         | S2         | S3          | S4          |
| Vitamin C                                  | 1.64±0.20c | 0.24±0.02d | 6.65±0.50a  | 2.47±0.01b  |
| Total phenolics (mg GAE/cup <sup>1</sup> ) | 76.4±0.2d  | 86.6±0.1c  | 110.1±0.3b  | 132.5±0.3a  |
| Total flavonoids (mg QE/cup)               | 16.2±0.3d  | 20.3±0.2c  | 27.2±0.4b   | 35.4±0.5a   |
| Total flavanol ( $\mu\text{g CE/cup}$ )    | 363.8c     | 440.0c     | 668.6b      | 853.3a      |
| DPPH (mg TE/cup)                           | 18.9±0.1c  | 19.1±0.3c  | 26.0±0.5b   | 35.3±0.4a   |
| H <sub>2</sub> O <sub>2</sub> (mg AAE/cup) | 33.84c     | 36.33c     | 51.55b      | 64.21a      |
| Abbreviated name of infusion herbal teas   |            |            |             |             |
| Lemon balm                                 | L1         | L2         | L3          | L4          |
| Vitamin C                                  | 70.7±3.6d  | 395.2±5.3b | 96.7.4±2.1c | 615.8±14.0a |
| Total phenolics (mg GAE/cup)               | 156.0±0.3d | 159.8±0.4c | 198.6±0.4b  | 215.4±0.1a  |
| Total flavonoids (mg QE/cup)               | 44.8±0.1c  | 45.1±0.3c  | 58.0±0.4b   | 69.9±0.2a   |
| Total flavanol ( $\mu\text{g CE/cup}$ )    | 611.4b     | 769.5ab    | 819.0a      | 906.7a      |
| DPPH (mg TE/cup)                           | 39.5±0.1d  | 42.9±0.3c  | 53.9±0.2b   | 78.7±0.5a   |
| H <sub>2</sub> O <sub>2</sub> (mg AAE/cup) | 66.93c     | 69.84c     | 105.20b     | 119.44a     |

<sup>1</sup> cup=150 mL. Values are shown as mean ± SD. Different letters in the same line indicate statistically significant differences ( $P < 0.01$ ).

## Antioxidant Capacity

Sage and lemon balm have very power antioxidant activity because of rich phenolic components (especially rosmarinic acid) and vitamin C content (Nicolai et al., 2016; Liu et al., 2019). Determination of antioxidant activities of the herbal teas were performed with two common methods DPPH and  $H_2O_2$  radicals scavenging activities in the present study. Herbal teas of lemon balm exhibited a very stronger antioxidant activity than herbal teas of sage. Even, the tea sample (L1, 39.5 mg TE/cup for DPPH and 66.93 mg AAE/cup for  $H_2O_2$ ) exhibiting the lowest activity among the lemon balm tea samples showed higher activity than the tea sample (S4, 35.3 mg TE/cup for DPPH and 64.21 mg AAE/cup for  $H_2O_2$ ) exhibiting the highest activity among sage tea samples. Toydemir et al. (2015) reported same results on herbal teas of both species. According to the results shown in Table 1, L4 herbal tea of lemon balm revealed both the highest free radical scavenging activity in DPPH (78.7 mg TE/cup) and in the  $H_2O_2$  (119.44 mg AAE/cup). S1 herbal tea of sage was the lowest active against both DPPH radical (18.9 mg TE/cup) and  $H_2O_2$  (33.84 mg AAE/cup) radical. Furthermore, a strong positive correlation ( $R^2=0.974$ ) was detected between these two antioxidant tests.

There is usually a significant relationship between polyphenols and antioxidant activities of plants (Beretta et al., 2017; Liu et al., 2019). In this study, the total phenolic contents of the herbal teas were positively correlated to antioxidant activity measured by DPPH ( $R^2=0.956$ ) and  $H_2O_2$  ( $R^2=0.979$ ) with highly significant correlation. The correlation between total flavanol content and antioxidant activities were positive and high ( $R^2>0.800$ ) for both radicals. The strongest positive correlations were found between antioxidant activities and total flavonoid contents ( $R^2=0.976$  for DPPH and  $R^2=0.982$  for  $H_2O_2$ ). This may indicate that phenolic acids, especially flavonoid components are the key contributors to the antioxidant effects.

## Mineral content in leaves and herbal teas of sage and lemon balm

Samples of sage and lemon balm leaves and their herbal teas (average) were analysed for major and minor mineral contents. The major part of minerals in sage and lemon balm leaves consists of K, Ca, Mg, Na, Fe and other minor minerals, such as Sr, Ba, Ti, Ga and Ag.

The concentrations of major minerals in sage leave are 34171.2 K ppm, 10731.3 Ca ppm, 2935.9 Mg ppm, 430.0 Na ppm and 428.7 Fe ppm. Similar major minerals and level of concentrations were reported by Pytlakowska

et al. (2012), but the data were higher compared to other study. In sage leaves, Başgel and Erdemoğlu (2006) reported higher amount of Ca (23560 ppm) and lower concentration of Mg and Fe (2143 and 297.4 ppm). Tokaloğlu (2012) found the higher concentration of Fe (890 ppm). These differences can be attributed to different factors such as analysis methods, physical and chemical properties of the soil, location and etc. The concentrations of minor minerals in sage leave are 16896.6 Sr ppb, 9759.6 Ba ppb and 2106.6 Ti ppb. Unlike this work, Pytlakowska et al. (2012) reported the higher concentration for Ba (25.8-27.4 ppm) and Sr (34.9-62.2 ppm). Compared to literature data similar concentration for Sr is reported for sage leaves (17.5  $\mu\text{g/g}$ ) (Başgel; Erdemoğlu, 2006).

The concentrations of major minerals in lemon balm leave are 18718.4 K ppm, followed by 8456.3 Ca ppm, 2166.5 Mg ppm. They are lower compared to published amounts in leave of lemon balm (2354 K ppm, 401 Ca ppm, 366 Mg ppm) (Pytlakowska et al., 2012). The content of Fe (435.4 ppm) found the higher than Na (60.8 ppm). In lemon balm leaves, Sussa et al. (2016) reported similar result for Fe(4138-475 ppm) and Na (266-23 ppm). Tokaloğlu (2012) reported the higher concentration for Fe (592 ppm). The concentrations of minor minerals in lemon balm leave are 9099.6 Sr ppb, 2423.5Ba ppb and 1624.4 Ti ppb. Ag and Ga, toxic minerals for human health found the lowest level in leaves of sage and lemon balm (1.17, 1.15 for Ga and 0.75, 0.71 ppb for Ag).

The analyzed minerals had higher concentration in sage leaves than in lemon leaves except Fe. Chizzola (2012) reported that Fe consent in leaves of lemon balm was the higher than that of sage. On the contrary, the herbal tea of sage had lower concentration of analyzed minerals than herbal tea of lemon balm. Sage were found to accumulate the minerals better than lemon balm, but the concentrations of the minerals in herbal tea of lemon balm were higher than sage except Ba and Ag. The source of minerals in both specie can be related to their growth media such as soil and water, as well as their characteristic feature (Szymczycha-Madeja et al., 2012). Transfer rates of the minerals from leaves to infusion herbal tea for lemon balm were found higher than sage except Ag (Table 2). The highest transfer rate was found as 85.7% for Na in lemon balm. The difference between the concentrations of the minerals found in sage and lemon balm herbal teas was statistically 1% significant for K, Ca, Fe, Ti, Sr, and 5% significant for Mg. No difference for Na, Ba, Ag and Ga was found. In infusion herbal teas of leaves, there was a high transition of Ag and Ga (28.0-52.1 % for sage and (8.5-66.1 % for lemon balm).

**Table 2:** The concentrations of major (ppm) and minor (ppb) minerals in sage and lemon balm leaves and their herbal teas, and transfer rates of the minerals in herbal teas.

| Minerals                 |            | Na     | Mg      | K        | Ca       | Fe    | Ba      | Ti       | Sr       | Ga    | Ag    |
|--------------------------|------------|--------|---------|----------|----------|-------|---------|----------|----------|-------|-------|
|                          |            | ppm    |         |          |          |       |         | ppb      |          |       |       |
| Leaves                   | Sage       | 430.0* | 2935.9* | 34171.2* | 10731.3* | 428.7 | 9759.6* | 2106.6*  | 16896.6* | 1.17  | 0.75  |
|                          | Lemon balm | 60.8   | 2166.5  | 18718.4  | 8456.3   | 435.4 | 2423.5  | 1624.4   | 9099.6   | 1.15  | 0.71  |
|                          | <i>p</i>   | 0.050  | 0.050   | 0.050    | 0.050    | 0.827 | 0.050   | 0.050    | 0.050    | 0.827 | 0.127 |
| Herbal teas<br>(Average) | Sage       | 47.0   | 52.2    | 116.9    | 13.2     | 0.9   | 182.4   | 623.6    | 226.5    | 0.61  | 0.21  |
|                          | Lemon balm | 52.1   | 68.2*   | 260.1**  | 25.9**   | 1.8** | 154.8   | 1028.0** | 404.5**  | 0.76  | 0.06  |
|                          | <i>p</i>   | 0.488  | 0.028   | 0.000    | 0.000    | 0.000 | 0.326   | 0.000    | 0.000    | 0.149 | 0.355 |
| Transfer<br>rate (%)     | Sage       | 10.9   | 1.8     | 0.3      | 0.1      | 0.2   | 1.9     | 29.6     | 1.3      | 52.1  | 28.0  |
|                          | Lemon balm | 85.7   | 3.1     | 1.4      | 0.3      | 0.4   | 6.4     | 63.3     | 4.4      | 66.1  | 8.5   |

$p < 0.01$ . \*\*,  $p < 0.05$ . \*

### Mineral content in infusion herbal teas of sage and lemon balm

The mineral contents in the herbal teas prepared different sample amounts (2 g and 3 g) and infusion times (5 min and 10 min) of sage and lemon balm leaves were analysed, and presented in Table 3. Effects of sample amounts and infusion times on the concentration of minerals in infusion herbal teas varied between mineral types and plant species. In the present study, the 3 g application of sample amount affected significantly the concentration of minerals in herbal teas of sage and lemon balm, and had the higher than its 2 g application. Na and Ag contents was statistically significant ( $p < 0.01$ ) according to the analysis of the variance of the sample amounts of sage, and the 3 g of sample amount had the lower concentration of these minerals than 2 g. Similar result for Ag content was observed in lemon balm.

But, the applications of infusion time had no effects on mineral contents in herbal teas of both

species. In general, with the increasing infusion time, the concentrations of the minerals in herbal teas of sage decreased and the balm increased. Compared to literature data, Pytlakowska et al. (2012) recorded similar findings for sage. However, Ag content increased with increasing infusion times (5 and 10 min) in herbal teas of both species. Özcan et al. (2008) reported that the best among infusion times (10, 15 and 20 min) were 15 min for Ag content in herbal teas of some spices.

In addition to individual effects of sample amounts and infusion times, interactive effects between these parameters were demonstrated to affect concentration of minerals in infusion herbal teas of sage and lemon balm. In the present study with both species, these parameters were found significant interactive effects on concentration of minerals in their herbal teas. The interaction between 3 g of sample amount and 5 min of infusion time had more effective effect on concentration of minerals in herbal teas (S3 and LB3) of both species.

**Table 3:** The effect of sample amount and infusion time on concentrations of minerals in infusion herbal teas of sage and lemon balm.

| Minerals      | Sage   |        |        |        |       |         |        | Lemon balm |       |        |        |       |         |       |      |         |         |        |       |       |  |
|---------------|--------|--------|--------|--------|-------|---------|--------|------------|-------|--------|--------|-------|---------|-------|------|---------|---------|--------|-------|-------|--|
|               | Na     | Mg     | K      | Ca     | Fe    | Ba      | Ti     | Sr         | Ga    | Ag     | Na     | Mg    | K       | Ca    | Fe   | Ba      | Ti      | Sr     | Ga    | Ag    |  |
| Sample amount | ppm    |        |        |        |       |         |        | ppb        |       |        |        |       |         |       | ppb  |         |         |        |       |       |  |
| infusion time | ppm    |        |        |        |       |         |        | ppb        |       |        |        |       |         |       | ppb  |         |         |        |       |       |  |
| 2 g           | 56.3** | 43.1   | 104.8  | 10.9   | 0.8   | 158.6   | 527.6  | 187.4      | 0.54  | 0.35** | 49.2   | 61.9  | 221.3   | 23.4  | 1.6  | 139.6   | 963.1   | 353.7  | 0.58  | 0.07  |  |
| 3 g           | 37.7   | 61.2** | 129    | 15.4** | 1.1** | 206.3** | 719.5  | 265.6**    | 0.69  | 0.07   | 55.1** | 74.5  | 298.9** | 28.5  | 1.9  | 169.9** | 1092.8  | 455.3* | 0.94* | 0.06  |  |
| <i>p</i>      | 0.004  | 0.004  | 0.262  | 0.004  | 0.003 | 0.004   | 0.055  | 0.004      | 0.2   | 0.092  | 0.004  | 0.15  | 0.004   | 0.2   | 0.15 | 0.004   | 0.149   | 0.025  | 0.016 | 0.63  |  |
| 5 min         | 53.4   | 56     | 128.2  | 14.1   | 1.0   | 200.7   | 674.3  | 245.7      | 0.66  | 0.1    | 50.6   | 64.7  | 248.4   | 23.7  | 1.7  | 143.6   | 982     | 389.9  | 0.72  | 0.05  |  |
| 10 min        | 40.7   | 48.4   | 105.7  | 12.2   | 0.9   | 164.2   | 572.8  | 207.3      | 0.57  | 0.32   | 53.6   | 71.8  | 271.9   | 28.2  | 1.9  | 165.9   | 1073.9  | 419.2  | 0.8   | 0.07  |  |
| <i>p</i>      | 0.15   | 0.377  | 0.749  | 0.81   | 0.45  | 0.15    | 0.631  | 0.262      | 0.522 | 1.00   | 0.15   | 1.00  | 1.00    | 0.873 | 1.00 | 0.262   | 1.00    | 1.00   | 0.378 | 0.261 |  |
| 2 g           | 59.5*  | 43.2   | 101.7  | 10.8   | 0.8   | 161.3*  | 491.1  | 189.2      | 0.46  | 0.07   | 47.5   | 43.5  | 187.3   | 17.1  | 1.2  | 118.6   | 799.4   | 288.1  | 0.52  | 0.08  |  |
| 10 min        | 53.1   | 43.1   | 108.0* | 11.1   | 0.7   | 155.8   | 564.2  | 185.7      | 0.61  | 0.64*  | 50.8*  | 80.3* | 255.4*  | 29.7* | 1.9* | 160.6*  | 1126.8* | 419.4* | 0.64  | 0.06  |  |
| <i>p</i>      | 0.05   | 0.827  | 0.05   | 0.275  | 0.513 | 0.05    | 0.127  | 0.275      | 0.275 | 0.05   | 0.05   | 0.05  | 0.05    | 0.05  | 0.05 | 0.05    | 0.05    | 0.05   | 0.513 | 0.825 |  |
| 3 g           | 47.2*  | 68.8*  | 154.6* | 17.4*  | 1.2*  | 240.0*  | 857.5* | 302.2*     | 0.86* | 0.13*  | 53.7   | 85.8* | 309.5*  | 30.3* | 2.1* | 168.7   | 1164.7* | 491.6* | 0.92  | 0.03  |  |
| 10 min        | 28.3   | 53.7   | 103.4  | 13.4   | 1.0   | 172.5   | 581.5  | 229        | 0.53  | 0.01   | 56.4*  | 63.2  | 288.3   | 26.7  | 1.8  | 171.1   | 1020.9  | 419    | 0.96  | 0.09* |  |
| <i>p</i>      | 0.05   | 0.05   | 0.05   | 0.05   | 0.05  | 0.05    | 0.05   | 0.05       | 0.05  | 0.05   | 0.05   | 0.05  | 0.05    | 0.05  | 0.05 | 0.275   | 0.046   | 0.05   | 0.376 | 0.046 |  |

*p*<0.01. \*\*, *p*<0.05.\*

## CONCLUSIONS

Vitamin C, total phenolic, total flavonoid, total flavanol, antioxidant activities and mineral contents in infusion herbal teas of lemon balm and sage were analysed in the study. The vitamin C, total phenolic, total flavonoid, total flavanol contents and antioxidant activities increased with increasing infusion time and sample amount for each species. The best sample amount in terms of analysed these parameters in the herbal teas of both species was 3 g, and the best infusion time was 10 min (S4 and LB4 treatments). However, Vitamin C in herbal teas of sage showed difference, increased with increasing sample amount, whereas it decreased with increasing infusion time. The most efficient vitamin C content among herbal teas of sage was found in S3 (3 g and 5 min). The best mineral content for human health in infusion herbal teas was found 3g sample and 5 min infusion time of sage and lemon balm (S3 and LB3). Considering the parameters examined, it was concluded that the highest quality infusion tea is S3 for sage, LB3 and LB4 for balm. The evaluation of the data obtained in this study together with examining food quality parameters, toxicological properties and chemical components will illuminate the accurate consumption of these herbal teas.

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