RESEARCH ARTICLE

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Total secondary metabolites and heavy metal profile of some medicinal plants frequently consumed as winter tea

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Abstract
In this study, sage (*Salvia officinalis* L.), linden (*Tilia platyphyllos* Scop.), and daisy (*Matricaria chamomilla* L.) herbs, which have rich phytochemical content and are frequently consumed in winter months for medicinal purposes, were obtained from three different herbalists located in Bingol and investigated in terms of their heavy metal contents, total flavonoid, total anthocyanin and total polyphenol contents. Some of the heavy metal (Cr, Fe, Co, Ni, Cu, Zn, As, Hg, Pb) contents of the collected herb samples were determined by ICP-MS. Total flavonoid, total anthocyanin and total polyphenol contents were determined by analyzing spectrophotometric methods. While the total phenolic content was found the most in sage (S1) ethylacetate and methanol extracts (760.1±2.0, 410.33±1.5 mg GAE/g), it was found the least in linden (L1, L3) hexane extracts (6.66±.1.3, 8.35±0.8 mg GAE/g). It was determined that total flavonoid content and total anthocyanin contents were higher in sage (S1) ethylacetate (294.31±2.1 mg CE/g and 291.31±2.1 mg MvGE/g), sage (S2) methanol (375.0±1.9 mg CE/g and 139.01*±2.0 mg MvGE/g) extracts, and less in linden (L1) and daisy (D1) hexane extracts respectively. It was determined that Cr and Cu metals were among the values suitable for consumption in terms of health in all samples except the chamomile (D1, D3) samples, and Zn metals were among the values suitable for consumption in all samples except the linden (L2) sample. Furthermore, the toxic metal Cd (0.05±0.00- 0.09±0.00 µg/g) in daisy (D1, D3) samples, as well as the other toxic metal Pb (4.50±0.01- 6.43±0.01 µg/g) in sage (S1,S2,S3) and linden (L1,L2,L3) samples, were found to be among the values suitable for consumption in terms of health. As a result, when the total anthocyanin, polyphenol and flavonoid contents were compared in all groups it was found that sage had the highest value in ethyl acetate extract. Additionally, Cu and Ni values of sage, and Fe and Pb values in both sage and linden were within safe limits in terms of health. They are of utmost importance in terms of supporting the daily mineral intake. However, As, Pb, and Hg were found to be toxic in daisy extract. This result can be shown as a result that the daisy will be a bioaccumulator. Therefore, consumption of daisy tea should be limited.
Keywords: Daisy, Heavy metal, Herbal Tea, Linden, Sage, Total anthocyanin, Total flavonoid, Total polyphenol

INTRODUCTION

Phytotherapy, which means treatment with herbs, is the use of chemical substances in herbs as medicines without subjecting them to an isolation process (Dündar 2001). Many herbs around us stand out as genuinely healthy physiological figures with their chemical programs and commonly used stakeholders of life with effects such as reducing stress, relieving anxiety, soothing the stomach, activating intestines or providing antiflatulent effects (Dündar and Aslan 2000). Benefiting from herbs for preserving body homeostasis or treating diseases is as old as the history of humanity. Thanks to these properties, herbs and phytochemicals are regarded as super ammunitions used in the body's defense and used against common risks, such as cancer, cardiovascular problems, hormonal disorders and diabetes, especially for their antioxidant effects (Evcimen and Aslan 2015). However, scientific reports that suggested that not every antioxidant substance could be used safely in under every circumstance motivated researchers to look for safer antioxidant herbs and herbal products (Dündar and Aslan 2000). Findings within this scope also update and change antioxidant herbal tea habits.

Herbal teas, which emerge as reliable fluid sources in winter periods when fluid intake and water cycle reduces, are also investigated by medicine, veterinary, biology and pharmacy disciplines. Thanks to being cheap, preferable and having positive effects, herbal teas have found a place in Turkish Pharmacopeia and the process of determining standards, licensing and determining indication fields have been going on. Many studies were conducted on using the teas made from herbs, such as classic black tea, green tea, white tea, jasmine, linden, sage, turmeric, ginger, melissa (lemon balm), parsley, daisy, rosemary, fennel, fennel-aniseed, stinging nettle, dill, thyme and rosehip as effective beverages in terms of physiological homeostasis and vitality and phytotherapeutic substances (Aslan 2018).

In color, smell and taste formation of medicinal herbs, phytochemicals are determinative as biologically active substances (Aslan, 2018). Considering the fact that there are more than ten thousand phytochemicals in the structures of herbs, they are impossible to investigate one by one (Balch and Balch 1997); (Craig 1997). Some of those that stand out in winter teas include catechins, isoflavones, indoles, carotenoids, anthocyanidins, ellagic acids and polyphenols, phenolic compounds, flavonoids, coumarins, catechins, anthocyanidins, carotenoids, lycopenes, phytates, indoles, sulfites, isothiocyanates, lignins, lactones, sponins, glucosinolates, phytosterols, phytoestrogens, saponins, terpenes, ellagic acids, quercetins.

In our study, each of the herbs investigated has medicinal effects. The scientific name for sage, which belongs to the lamiaceae family is Salvia officinalis. Sage (adaçayı in Turkish) is known to facilitate digestion and eliminate stomach gas due to its bitter taste. In addition, due to its soothing and disinfectant effects, it has been reported that it reduces sweating and saliva secretion, cleans the pores, is used as a mouthwash in rheumatic pain, tonsils, teeth, mouth and inflammation (Özer et al. 2001); (Aydıner 2006). The scientific name for linden, which belongs to the tiliaceae family is Tilia platyphyllos. Linden (ıhlamur in Turkish) is known to be used as a diaphoretic, chest emollient, sleep inducer and tranquilizer in addition to its use in strengthening cardiac muscle and nerves, activating and cleaning kidneys, treating epilepsy and migraines, and its properties such as antifebrile, fatigue eliminator and biligenic effects (Aydıner 2006); (Baytop 1999); (Şeker 2011). The scientific name for daisy, which belongs to the asteraceae family is Matricaria chamomilla. Daisy (papatya in Turkish) is known to be effective in increasing urine, whetting appetite, soothing nerves, reducing fever, eliminating diarrhea and gas, treating throat and tonsil pain, healing wounds, having biligenic effects and in the treatment of hemorrhoids, inflamed wounds, rheumatism, insomnia, flu, anemia, dizziness, eczema, waist and back pain (Aydıner 2006); (Koç 2002).

Heavy metals that are taken at high doses via the food chain also affect human health negatively (Öktüren and Sönmez 2006); (Okcu et al. 2009). For example, excess lead builds up in the bones and causes damages in the kidney, brain and nervous system functions while cadmium accumulation in the body causes serious problems that may lead to lung and prostate cancer (Kahvecioğlu et al. 2006). Herbs that are grown in regions polluted with heavy metals [such as roadsides and mineral deposits] may have high heavy metal contents (Pip 1991). Furthermore, the use of fertilizers with cadmium content and pesticides with organic mercury or lead increases the rate of herbs containing heavy metals (Arab et al. 1999).

Medicinal and aromatic herbs cover quite a large field in terms of herbs, their active ingredients and areas of consumption. These active ingredients have nutritional elements such as various vitamins, carotenes and calcium, while most of them do not have a nutritional element characteristic. These include flavonoids, polyphenols, monoterpenes, chlorophyll, dietary fiber, aliphatic sulfites, aromatic isothiocyanates, phytic acid (Stavric 1994); (Hollman et al. 1996). Flavonoids, which are the main sources of yellow, blue and red pigments in herbs, are polyphenolic compounds that are in a 2-phenyl-benzo-benzo-α-piron structure and found naturally in fruits, vegetables and beverages such as tea and wine (Herton et al. 1993); (Hollman et al. 1996). With the molecules that bind to this structure with benzene and heterocyclic rings, they get different names. These compounds in the flavonoid structure can be divided into groups, such as anthocyanins, flavonols, flavones, catechins, flavanones and isoflavonoids. Today, more than 4000 flavonoids that are naturally present in food are identified (Abacı et al. 2014)

The aim of this study is to investigate the total flavonoid, total anthocyanin and total polyphenol contents of the species sage (Salvia officinalis), linden (Tilia platyphyllos) and daisy (Matricaria chamomilla) that are sold as teas in herbalists and determine whether their heavy metal (Cr, Fe, Co, Ni, Cu, Zn, As, Cd, Hg, Pb) content are within the limits presented at World Health Organization (WHO) /

Food and Agriculture Organization (FAO) literature.

MATERIALS AND METHODS

Herbal Materials

Between the 10th and 11th months of 2019, three different plant species utilized for therapeutic purposes, such as antipyretic, sedative, muscle relaxant, and tranquilizer, were collected from three herbalists in Bingol. According to the Flora of Turkey and the Eastern Aegean Islands (Davis 1965-1985), plant species were recognized taxonomically by a faculty member of the Department of Biology, Faculty of Science, Bingöl University (Davis et al. 1988). Scientific names, family names, English names, Turkish names and used parts of the herbs that constituted the material of the study were presented in Table 1.

Table 1. Scientific, family, English and Turkish names of medicinal plant used different parts different parts

Scienctific name	*Family name*	*English name*	*Turkish name*	*Plant part used*
Salvia officinalis L.	*Lamiaceae*	Sage (S)	Adaçayı	Leafy and flowering branches
Tilia platyphyllos Scop.	*Tiliaceae*	Linden (L)	Ihlamur	Flower and buds
Matricaria chamomilla L.	*Asteraceae*	Daisy (D)	Papatya	F lowers

Preparing Samples for Heavy Metal Analysis

In the study, different parts of sage, linden and daisy herbs were used as materials. In the analyses, approximately 0.5 g was weighed from the ground samples and were placed in teflon containers of the microwave unit of CEM-Mars 6 240/50 (Corp. Matthews NC, USA) and 10 ml HNO_3 were added on top. The temperature conditions of the microwave unit were raised to 200 oC at the 25th minute. Then, this temperature was kept for 15 min and the temperature was lowered for a period of approximately 15 min. Therefore, it was enabled that the samples were turned into completely soluble states. Following the burning process, the 0.5 mL samples were cooled down to room temperature and they were completed by diluting with 1% (HNO_3-ultra pure water) on their volumes up to 15 mL.

Analysis Method

Heavy metal analyses of the medicinal herb samples were conducted with ICP-MS in three times of repetition. Heavy metal analyses of As, Cd, Cr, Cu, Fe, Co, Pb, Zn, Ni and Hg in these herbal samples were conducted according to ICP-MS method. The fundamental aspect of this method is that samples, which are turned into solutions by burning them via the microwave soluble method, are measured for concentrations of heavy metal contents by ICP-MS for certain standards. In table 2, the operating conditions of Perkin Elmer NexION 2000 model ICP-MS device used for the analysis were presented.

Table 2. ICP-MS (NexION) operating conditions

Parameter/Component	Description / Value
Nebulizer	MEINHARD® plus Glass Type C
Nebulizer şow	Optimized for < 2% oxides
Nebulizer gas şow rate	0,93 L/min
Spray Chamber	Glass cyclonic (bafşed), 2 °C
Injector	2.0 mm i.d.
Deşector voltage	−12 V
Analog stage voltage	−1750 V
RF Power	1600 W
Rinse time	45 second
Dwell time	50 ms
Aerosol Dilution	Set to 2.5x
Sample Delivery Rate	350 µL/min
Discriminator threshold	26
Alternating current (AC) rod offset	−4
Cones	Ni
Replicates	3

Analysis Methods for Bioactive Compounds

250 mg samples were obtained from dried herbs and they were solved in 5 mL hexane, methanol and ethyl acetate.

Determination of Total Polyphenolic Content

This process was conducted with Folin-Ciocalteu reactive method (Singleton et al. 1999). This method measures the required amount of the tested sample to inhibit the oxidation of the reactive (Vinson et al. 2005). Total phenolics were determined as mg gallic acid equivalents (GAE)/g of dried samples.

Determination of Total Flavonol Content

Total flavonols were determined wth the vanillin method according to Butler et al. (1982). Readings in spectrophotometer were conducted at 500 nm wavelength and total flavonols were determined as mg catechin equivalents (CE)/g of dried samples (Butler et al. 1982).

Determination of Total Anthocyanin Content

In plants types, anthocyanin analyses were conducted with the pH difference method according to Wrostad (Wrostad 1976). Readings in spectrophotometer were conducted at 520 and 700 nm wavelengths and at two different pH levels as 1.5 - 4.0. The values were determined as mg malvidin-3-glucoside equivalenst (MvGE) /g of dried samples.

Statistical Analysis

The data were calculated as the mean ± standard deviation of three replicates. IBM SPSS Statistics 28.0 software was used for statistical analysis. The Friedman test was used to analyze dependent variables. The Kruskal-Wallis test was used to analyze independent variables. The Mann-Whitney U test was used to determine the difference between groups with significant differences in the Kruskal-Wallis test.

RESULTS AND DISCUSSION

In this study, heavy metal contents of herbal samples were determined in mg/kg via the ICP-MS method. The limit of detection (LOD), limit of quantification (LOQ) and recovery (R, %) values of the calibration were presented in Table 3 while mean and standard deviation values of heavy metal contents of sage, linden and daisy herbs investigated in the study were presented in Table 4 and Table 5. There were statistically significant differences in the Cr, Fe, Co, Cu, Zn, As and Hg groups ($p<0.05$), but not in the Ni, Cd and Pb groups ($p> 0.05$). (Tables 4 and 5)

Abbreviations for plants in tables are as follows:

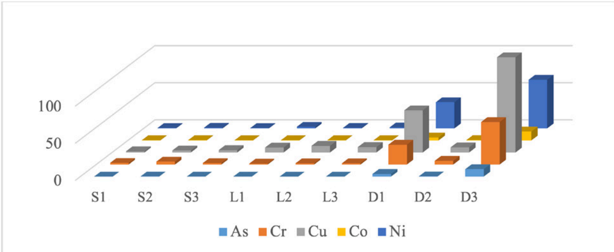
S1, S2, S3: Sage (*Salvia officinalis* L.) samples obtained respectively from the first, second and third herbalists

L1, L2, L3: Linden (*Tilia platyphyllos* Scop.)samples obtained respectively from the first, second and third herbalists

D1, D2, D3: Daisy (*Matricaria chamomilla* L.) samples obtained respectively from the first, second and third herbalists

When the heavy metal contents of the plants were examined, sage and linden were found to have heavy metal content below the toxic limits. However, it is shown in figure 1 that the daisy content is above the toxic limits (Figure 1).

Figure 1. Concentration values of the heavy metal contents of sage (S), linden (L) and daisy (D) herb samples



The solubility of phenolic compounds depends on the polarity of the solvent used, polymerization degree of phenolics and interaction of phenolics with other herbal compounds and formation of insoluble complexes. Therefore, there is no universal procedure to remove all of the phenolics or a branch of specific phenolic substances. Methanol, ethanol, acetone, water and combinations of these are frequently used for the extraction of phenolics. Free flavonoid aglycones are efficiently extracted by non-polar solvents such as methylene chloride, ethyl ether or ethyl acetate. Additionally, more polar glycosidic conjugate is solved in more polar solvents such as methanol and ethanol, thus, these organic solvents are generally used in Soxhlet extraction.

According to the statistical results, in the evaluation of

Table 3. Limit of detection (LOD), limit of quantification (LOQ) and recovery (R, %) values for the heavy metals in the study

Element	LOD (mg/L)	LOQ (mg/L)	R,%
Cr	0.017	0.056	97.380
Fe	0.223	0.746	98.743
Co	0.00032	0.00103	96.521
Ni	0.008	0.025	98.968
Cu	0.022	0.073	96.784
Zn	0.043	0.142	97.417
As	0.001	0.004	95.826
Cd	0.000165	0.00055	97.608
Hg	0.0016	0.0054	95.950
Pb	0.010	0.033	94.882

Table 4. Heavy metal contents (Cr, Fe, Co, Ni, Cu) of sage, linden and daisy herbs in the study

Herbal Material		Cr (µg/g)	Fe (µg/g)	Co (µg/g)	Ni (µg/g)	Cu (µg/g)
Herbalist 1	S1	2.18±0.01[a]	723.50±1.22[ab]	0.26±0.001[bc]	0.55±0.00	1.51±0.00[ab]
	L1	1.23±0.00[a]	649.37±0.69[ab]	0.26±0.00[bc]	2.06±0.01	6.62±0.01[ab]
	D1	26.69±0.03[a]	8783.62±11.48[ab]	3.9±0.04[bc]	35.26±0.04	56.67±0.07[ab]
Herbalist 2	S2	3.97±0.00[a]	1410.40±0.07[ab]	0.34±0.00[bc]	0.86±0.00	2.70±0.00[ab]
	L2	1.66±0.00[a]	684.57±0.63[ab]	0.27±0.00[bc]	0.74±0.00	8.88±0.01[ab]
	D2	5. 00±0.01[a]	1338.08±1.16[ab]	0.46±0.00[bc]	2.10±0.00	6.84±0.01[ab]
Herbalist 3	S3	1.97±0.00[a]	790.49±0.45[ab]	0.23±0.00[bc]	0.52±0.00	3.17±0.00[ab]
	L3	1.69±0.00[a]	1008.96±1.57[ab]	0.27±0.01[bc]	1.21±0.00	7.24±0.01[ab]
	D3	57.21±0.04[a]	22187.48±12.00[ab]	12.02±0.06[bc]	65.45±0.03	128.08±0.04[ab]
p value		0.044	0.019	0.033	p>0.05 (0.068)	0.028

Values were given as mean ± standard deviation of triplicate (n=3) determinations. Statistical analyzes were performed using the Kruskal-Wallis and post hoc Mann-Witney U test.

Table 5. Heavy metal contents (Zn, As, Cd, Hg, Pb) of sage, linden and daisy herbs in the study

Herbal Material		Zn (µg/g)	As (µg/g)	Cd (µg/g)	Hg (µg/g)	Pb (µg/g)
Herbalist 1	S1	27.87±0.04[ac]	0.11±0.00[bc]	Not detected	Not detected	6.43±0.01
	L1	69.09±0.10[ac]	0.05±0.00[bc]	Not detected	Not detected	5.92±0.00
	D1	90.36±0.14[ac]	3.50±0.01[bc]	0.09±0.00	Not detected	6.24±0.00
Herbalist 2	S2	92.76±0.05[ac]	0.25±0.00[bc]	Not detected	Not detected	6.07±0.00
	L2	25.57±0.02[ac]	0.04±0.00[bc]	Not detected	Not detected	4.70±0.01
	D2	32.45±0.03[ac]	0.33±0.00[bc]	0.09±0.00	Not detected	4.35±0.01
Herbalist 3	S3	56.83±0.03[ac]	0.11±0.00[bc]	Not detected	Not detected	4.50±0.01
	L3	96.38±0.17[ac]	0.07±0.00[bc]	Not detected	Not detected	4.53±0.00
	D3	1278.98±0.80[ac]	9.96±0.01[bc]	0.05±0.00	6.35±0.00[ab]	11.71±0.00
p value		0.037	0.031	p>0.05 (0.061)	0.003	p>0.05 (0.057)

Values were given as mean ± standard deviation of triplicate (n=3) determinations. Statistical analyzes were performed using the Kruskal-Wallis and post hoc Mann-Witney U test.

the extracts of the herbs obtained from three different herbalists, and by using three different solvents, it was determined that polyphenol content values obtained from ethyl acetate extracts of all of the herbs were significantly higher, except for sage 2 and linden 2 (Table 6).

In the evaluation of the total anthocyanin content of the herbs, in terms of sage extracts, it was determined that the anthocyanin content of ethyl acetate extract of sage obtained from the 1st herbalist and anthocyanin contents of methanol extracts of sage obtained from the 2nd and 3rd herbalists were higher. According to linden results, it was determined that ethyl acetate extracts of linden obtained from the 1st and 3rd herbalists and methanol extracts of linden obtained from the 2nd herbalist were higher in anthocyanin content. For daisy, it was determined that D1 ethyl acetate extract, D2 and D3 hexane extracts were higher in anthocyanin content (Table 7).

In the comparison of flavonoid contents of herbs obtained from different herbalists, it was determined that ethyl acetate extracts of sage, linden and daisy

Table 6. Total polyphenolic substance content of extracts

		Extracts (mg GAE/g)			
	Plants	Hexane	Methanol	Ethyl acetate	p
Herbalist 1	S1	300.33±1.5	410.33±1.5	760.1*±2.0	p<0.001
	L1	6.66±.1.3	31.74±1.2	58.24*±1.0	p<0.001
	D1	18.62±1.2	25.33±1.5	38.08*±2.0	p<0.001
Herbalist 2	S2	21.68±1.4	60.42*±1.7	36.38±0.99	p<0.001
	L2	26.34±1.5	145.29*±2.0	68.11±1.3	p<0.001
	D2	9.69±0.9	16.59±1.2	25.67*±1.5	p<0.001
Herbalist 3	S3	51.67±1.5	22.0±2.0	198.74*±2.0	p<0.001
	L3	8.35±0.8	13.31±0.9	28.41*±1.2	p<0.001
	D3	11.71±1.5	21.29±2.0	34.58*±1.2	p<0.001

*Shows the significant difference between the extracts of the same herb compared to other two extracts. Values were given as mean ± standard deviation of triplicate (n=3) determinations. Statistical analyzes were performed using the Friedman test.

Table 7. Total anthocyanin content of extracts

	Plants	Extracts (mg MvGE/g)			
		Hexane	Methanol	Ethyl acetate	p
Herbalist 1	S1	12.01±1.5	97.67±1.2	291.31*±2.1	p<0.001
	L1	7.65±0.9	38.37±1.1	66.1*±1.5	p<0.001
	D1	7.07±0.97	28.41±1.4	29.67±1.8	p<0.001
Herbalist 2	S2	56.29±1.0	139.01*±2.0	84.0±1.9	p<0.001
	L2	17.09±1.3	96.29*±1.8	52.64±1.5	p<0.001
	D2	14.74±1.1	11.71±0.9	13.82±1.5	p>0.1
Herbalist 3	S3	12.61±0.98	35.34±1.4*	27.69±1.2	p<0.001
	L3	10.13±1.0	15.67±1.5	76.04*±1.9	p<0.001
	D3	27.32±1.5*	15.13±1.5	23.52±1.7	p<0.05

*Shows the significant difference between the extracts of the same herb compared to other two extracts. Values were given as mean ± standard deviation of triplicate (n=3) determinations. Statistical analyzes were performed using the Friedman test.

Table 8. Total flavonoid content of extracts

	Plants	Extracts (mg CE/g)			
		Hexane	Methanol	Ethyl acetate	p
Herbalist 1	S1	108.67±1.5	124.31±1.2	294.31*±2.1	p<0.001
	L1	16.33±0.9	55.6±1.1	111.67*±1.5	p<0.001
	D1	12.03±0.97	48.67±1.4	55.0±1.8	p<0.001
Herbalist 2	S2	118.0±1.0	375.0*±1.9	192.0±2.	p<0.001
	L2	55.37±1.3	104.3*±1.5	97.52±1.8	p<0.01
	D2	28.71±1.5	20.35±1.1	23.36±0.9	p<0.05
Herbalist 3	S3	36.61±0.98	107.0±1.4	121.34*±1.2	p<0.001
	L3	18.64±1.5	41.78±1.9	156.31*	p<0.001
	D3	52.29*±1.7	29.59±1.5	43.0±1.5	p<0.05

*Shows the significant difference between the extracts of the same herb compared to other two extracts. Values were given as mean ± standard deviation of triplicate (n=3) determinations. Statistical analyzes were performed using the Friedman test.

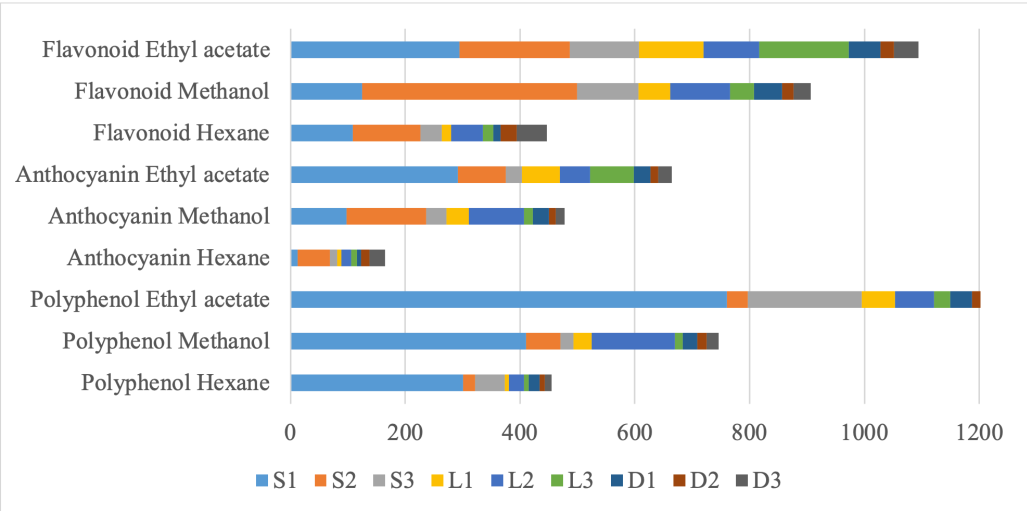


Figure 2. Bioactive components of different extracts of herbal tea samples

obtained from the 1st herbalist, methanol extracts of sage and linden and hexane extracts of daisy obtained from the 2nd herbalist, and ethyl acetate extracts of sage and linden and hexane extracts of daisy obtained from the 3rd herbalist were higher in flavonoid contents compared to other extracts (Table 8).

As seen in figure 2, considering the content of bioactive components, the best plant in terms of content is sage taken from the first herbalist. Solvents that form the best solvent medium for determining the polyphenolic, flavonoid and anthocyanin contents of different extracts of the same plants were determined as ethyl acetate, methanol and finally hexane, respectively (Figure 2).

In previous studies, it was reported that herbal substances were quite rich in micro and macro elements and teas obtained from these herbs were high in mineral contents (Sembratowicz and Rusinek-Prystupa 2014); (Stanojkovic-sebic et al. 2015); (Çolak et al. 2014). Therefore, including herbal teas in diets is of utmost importance in terms of supporting daily mineral intake. The minerals found in products result from the water and herbs used in its production. Leblebici et al. (2012) reported that Cr content of sage herb (7.30 ppm) was quite high (Leblebici et al. 2012). In our study, it was determined that daisy samples included more Cr content (Table 4). For medicinal herbs, the limit of containing Cr element is 2.0 ppm (20.000 µg/g) (WHO, 2007). For edible plants, this limit for Cu element was set at 3.0 ppm (FAO/WHO 1984). In our study, linden (1.23±0.00-1.69±0.00 µg/g) values were observed to be below Cr limit while sage (1.51±0.00, 2.70±0.00 µg/g) values were below the Cu limit. Additionally, in a study conducted in the Kisii region of Southwestern Kenya with medicinal herbs, Cr levels were determined as 2.035-.0567 ppm (Jabeen et al. 2010) while they were determined as 1.2-.29.49 ppm in the Haripur basin of Pakistan (Maobe et al. 2012).

According to Polat and Ogut (218), the Fe, As, Cd, Cr, Cu, Hg, Ni, Pb and Al metals' concentrations in six different medicinal plants *(Camellia sinensis, Tilia platyphyllos, Hypericum perforatum, Matricaria chamomilla, Salvia officinalis, Thymus vulgaris*) sold in herbal stores in Aydın, Denizli, Burdur and Isparta were determined via ICP-OES in samples sold unpackaged and as packaged tea for infusions. According to this study, more heavy metal residues were detected in the herbal tea samples sold unpackaged compared to the infused teas. At the end of the study, while the maximum amount of heavy metal in the samples sold unpackaged was Fe (302 mg/kg), Cd and Cr heavy metal residues were not identified in the samples made into infusions from packaged tea. The largest heavy metal residues were found in *Thymus vulgaris* samples sold unpackaged, and the smallest heavy metal residues were detected in the *Tilia platyphyllos* samples (Polat and Ogut 2018). Although Fe contents in types of plants are determined to be higher compared to other elements (415.65±0.13- 8783.62±11.48 µg/g), the limit of FAO/WHO for the value required in edible plants ranges below 20 ppm. Additionally, in this study, it was observed that most types of plants investigated in terms of Zn contents were quite high compared to the accepted limit values (27.4 ppm) (27.87±0.04-1278.98±0.80µg/g) (FAO/WHO 1984). While no reported toxic effect of Fe element was observed, excessive Fe, especially in children, was noted to form toxic effects as well as fatal effects of 60 mg/kg Fe intake (Kulhari et al. 2013). Small amounts of Co are a necessary element for the human body and especially, deficiency of Co may result in skin problems. Of the herbs investigated in the study, the highest amount of Co was determined in the types of daisies (3.9±0.04, 12.02±0.06 µg/g). No information for the determined limit was observed for Co element. Rajan et al. (2014) studied 4 different medicinal plants and they determined Co values as 0.284±0.099, 2.025±0.679, (0.059±0.001 ve 0.715±0.039 ppm (Rajan et al. 2014), which are quite below the values of our herbal samples. Co values we determined for sage and linden were observed to be similar to those found in the study conducted by Başgel and Erdemoğlu (2006).

High amounts of Cd can result in cancer, diarrhea, stomach problems and effects that can influence the central nervous system, and lead to death. World Health Organization's (WHO) acceptable limit is 0.3 ppm. This value for the Pb upper limit was set at 10 ppm. Accumulated lead in the body leads to acute and chronic poisoning and this can have negative effects on the kidneys, which may result in death (Heyes 1997). In our study, the amount of Hg, whose acceptable limit is 0.1 ppm, was not detected in all of the herbs while the amount of Cd could not be detected in all of the herbs, except for daisy (0.05±0.00-0.09±0.00 µg/g) (FAO/WHO 1984). Pb values for sage (4.50±0.01- 6.43±0.01 µg/g) and linden (4.53±0.00-5.92±0.00 µg/g) in our study were below the acceptable limit. Values for linden varied between 4.35 and 11.71 ppm. In a study conducted by Martín-Domingo et al. (2017) on herbal teas in Spain, it was reported that Cd content was 0.08 ppm and Pb content was 1.00 ppm (Martín-Domingo et al. 2017). These values are quite below the Cd and Pb contents of herbal samples in our study.

Natural antioxidant sources generally consist of herbal phenolics (Atoui et al. 2005). Antioxidant activities of phenolics are related to hydroxyl groups in their molecules (Ziakova and Brandsteterova 2003). The largest part of herbal phenolics comprises of flavonoids. This group includes more than 8000 known compounds (Pietta and Gardana 2003). Phenolic substances included in food compounds are of importance in terms of nutrition and health owing to their antioxidant properties. In a study conducted by İncedayı (2017), it was reported that linden plant and its beverage had total phenolic substance amounts of 7415.56±28.50 mg GAE/100g and 220.96±7.68 mg GAE/100mL, respectively (İncedayı 2017). Akış (2010) reported that the total phenolic substance content in the same plant was 5112 mg GAE/100g. Sinir et al. (2016) determined this value in carbonated erica beverage as 174.06±24.53 mg GAE/100mL and the results were deemed close to each other. The fact that the results were determined to be higher than the data obtained in this study (varies between 6.66 and 145.29 mg GAE/g) were linked to the fact that the linden concentrations that was extracted were higher (5% and 10%).

In a study that investigated the mean flavonoid contents in linden and mint samples depending on boiling, brewing and extraction processes, it was observed that boiling process for mint and linden samples and unprocessed linden samples were significant compared to the extraction process. It was also determined that the brewing process for processed mint, processed linden and unprocessed linden samples were more significant compared to the extraction process (Aksu 2010).

In studies conducted on types of sage (*Salvia spp.*), Miliauskas et al. (2004) determined that the flavonoid content of *Salvia officinalis* L. was 3.5 mg CE/g. In our study, the mean value was 6.70 CE/g, which is higher than that study. Miliauskas et al. (2004) investigated 12 different types of plants and determined the total phenolic content of *Salvia officinalis* L. as 22.6 mg GAE/g. Dinçer et al. (2013) determined the total phenol content of *Salvia tomentosa* type between 49.27 and 66.15 GAE/g, which indicated that their results were higher than those obtained in our study. In medicinal sage type (*Salvia officinalis* L.), Arıduru and Arabacı (2013) determined that the total phenol content, according to different solvents, was between 43.55 and 11.58 mg GAE/g.

As a result, antioxidant substances reduce the harmful effects of free radicals, which are known to play roles in the aging process and illnesses, by inhibiting reactions of free radicals, preventing the damages of oxidations via connecting oxygen and metals and preventing low-density lipoprotein (LDL) and lipoprotein oxidation. Antioxidant effects of herbal products, especially flavonoids, result from phenolic compounds such as cinnamic acid derivations and coumarins. Various studies demonstrated that phenolic compounds had antiallergic, anti-inflammatory, antidiabetic, antimicrobial, antipathogenic, antiviral and antithrombotic properties, and they had preventive effects in terms of diseases such as cardiovascular disease, cancer, osteoporosis, diabetes mellitus and neurodegenerative diseases. In the early 200s, more than 8000 phenolic compounds were identified and this number increases every day. Recently, several herbal phenolics were regarded as antioxidants and they are produced commercially. In this respect, it is deemed important to know the presence of these antioxidants, which provides preventive effects in diets, food and the levels they should be taken. Most of the phenolic compounds, whose efficiency on several subjects were scientifically proven, lack studies that investigate foreseen preventive and therapeutic properties of them, and multidisciplinary studies should be conducted on this subject.

Due to social accustomedness, linden, sage and daisy teas are frequently consumed during the winter months. There are many advantages to consuming them daily in terms of both antioxidant content and macro and micro element content. Especially sage is among the winter tea form that we should consciously use. In the comparison of total anthocyanin, polyphenol and flavonoid contents of the three herbs, it was determined that the highest content belonged to sage. This also has an important in terms of meeting daily mineral intake. Another winter tea that should be emphasized is linden tea. Every other herbal tea can be used as long as they meet the quality standards. However, because herbal teas, such as daisy, linden and sage teas, can show their effects after several uses, their use should be limited. Additionally, by determining the microelement concentrations of herbal samples, which are excessively consumed by purchasing from markets, the question of whether the mineral element levels of frequently used herbs are safe in terms of health.

CONCLUSIONS

As a result, It was determined that sage ethyl acetate extract had the highest value in terms of total anthocyanin, polyphenol, and flavonoid contents when compared to other plant samples. In addition, it was determined that the Cu and Ni values in sage and the Fe and Pb values in both sage and linden were within safe limits for health.

In conclusion, considering the winter periods, when fluid intake decreases and fluid excretion increases, and detoxification process based on sweating becomes less active, herbal teas, which passes into the extracellular fluid environment and excreted via sweating and urine, should be consumed more as long as they meet the minimum quality standards. They also have importance in terms of the development process of children and youth, and geriatric period health and vitality as well as in terms of homeostasis.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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