A quality evaluation of chamomile and mint teas commonly consumed in Bosnia and Herzegovina.

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ABSTRACT

The quality of mint and chamomile tea brands available in the retail market in Bosnia and Herzegovina were assessed based on their total phenolic and flavonoids contents, total antioxidant capacity and levels of heavy metals (Cd, Pb, Cr, Fe and Cu). The results showed that both the mint and chamomile had high amounts of phenolic compounds and high antioxidant activity rates. However, the total phenolics and flavonoids were significantly higher in the mint than in the chamomile. The studied toxic heavy metals (Pb, Cd and Cr) were present at non-toxic levels in all the herbal tea samples, while the levels of Fe and Cu in some herbal teas slightly exceeded the World Health Organization's permissible limits. Accordingly, the results of this study indicate that the potential health risk of Cd, Cr and Pb exposure through the consumption of studied herbal teas appears to be very low with no significant health implications to consumers. Contrary, based on the samples examined in this study, the consumption of herbal teas could expose the consumer to the potential health risks associated with Cu and Fe. Therefore, further studies are needed to test this possibility.

KEY WORDS: Heavy metals, herbal, tea, phenolics, flavonoids, chamomile, mint, antioxidants

INTRODUCTION

The consumption of herbal teas has attracted attention due to their potential health promoting benefits, including neuroprotective, antimicrobial, antiproliferative, and anti-inflammatory effects. These health benefits are associated with the antioxidant activity of the biochemical and pharmacological compounds present in herbal teas, especially phenolic compounds (1).

Phenolic compounds, also denoted phenolics, are secondary plant metabolites characterized by the presence of one or more phenolic units (*i.e.*, an aromatic ring bearing one or more hydroxyls). Based on their chemical structures, phenolic compounds can be divided into different classes. They range from simple phenols with only one aromatic ring, bi-phenols and flavonoids, which contains 2 to 3 aromatic rings to polyphenols containing 12 to 16 aromatic rings. The number and arrangement of the hydroxyl groups in a particular aromatic ring leads to the variation in their antioxidant potential (2). Accordingly, there are several antioxidant mechanisms of phenolics including direct quenching of reactive oxygen species, inhibition of pro-oxidative enzymes and metal ion chelation ability. In addition, phenolic compounds can improve human

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health or help in the prevention of various diseases through mechanisms that differ from antioxidant action, such as cell signaling pathways and gene expression (3).

Unfortunately, aside the many biologically active substances such as phenolics, vitamins and carotenoids, herbal teas may also contain heavy metals such as Pb, Cd, Cr, Al, and Hg, which can be toxic to humans (4). High amounts of these metals in the human body can cause several health problems including kidney failure, neurological damage, loss of memory and skeletal muscle degenerative diseases (5). Knowing that the presence of toxic heavy metals in herbal teas may result in health risk to consumers, it is important to monitor heavy metal concentrations in both the herbs themselves and their infusions.

In Bosnia and Herzegovina, chamomile and mint are the main herbs used for brewing herbal teas. There has been concerns about the safety and quality of these herbs particularly since chamomile and mint are known to accumulate heavy metals in its above-ground parts when grown in contaminated soil (6-7). It is therefore essential to provide the public with factual information to ensure better understanding of the risks, as well as, benefits associated with the consumption of these herbal teas. Consequently, the objective of this study was to evaluate and compare the content of total heavy metals, total phenolics and flavonoids in 10 different samples of chamomile (*Matricaria chamomilla* L.) and mint (*Mentha piperita* L.) teas purchased at local herbal pharmacies (Sarajevo, Bosnia and Herzegovina). The health benefits and toxicity associated with the chemical composition of tested herbal teas were also analyzed.

MATERIALS AND METHODS

Reagents

Folin-Ciocalteu reagent, catechin, NaOH, $NaNO_2$, $AlCl_3$, 2,4,6-tris(1-pyridyl)-1,3,5-triazine (TPTZ), $FeSO_4 \times 7\ H_2O$, HCl, $FeCl_3$, HNO_3, $HClO_4$ (Sigma Aldrich, St. Louis, USA), gallic acid (Fluka Chemica, Switzerland), anhydrous sodium carbonate (Kemika, Zagreb, Croatia) and ethanol (Merck, Darmstadt, Germany) were used in this study. All chemicals were of analytical grade.

Sample collection and pretreatment

Five commercial brands of tea bags, that is five of chamomile tea (*Chamomillae flos*, *Matricaria chamomilla* L.) and five of mint tea (*Menthae piperitae folium*, *Mentha piperita* L.) were purchased from local herbal pharmacies in March 2021 (samples 1 to 10, Table 1). The brands were selected because they represent the best-selling chamomile and mint teas in Bosnia and Herzegovina. For each tea sample, the bags were opened separately, mixed and homogenized using an electric grinder (IKA® A-11 basic), and were then stored in paper bags in a dry and dark place until analysis.

Table 1 Description of investigated herbal teas

SAMPLE	SCIENTIFIC NAME	ORIGIN	BRAND NAME
1	*Mentha piperita* L.	Bosnia and Herzegovina	Seti
2	*Matricaria chamomilla* L.	Bosnia and Herzegovina	Seti
3	*Mentha piperita* L.	Bosnia and Herzegovina	Bonito
4	*Matricaria chamomilla* L.	Bosnia and Herzegovina	Bonito
5	*Mentha piperita* L.	Austria	Teekanne
6	*Matricaria chamomilla* L.	Austria	Teekanne
7	*Mentha piperita* L.	Bosnia and Herzegovina	Plantago
8	*Matricaria chamomilla* L.	Bosnia and Herzegovina	Plantago
9	*Mentha piperita* L.	Croatia	Franck
10	*Matricaria chamomilla* L.	Croatia	Franck

Extraction procedure

Herbal tea (1 g) was steeped in 30 ml of aqueous ethanol solution (water/ethanol 70/30 v/v) at room temperature for 24 hours. After the above steeping process, the samples were centrifuged at 3000 RPM for 10 minutes. They were then filtered (Millipore nylon filters, 0.45 μm) into a 50 ml volumetric flask and diluted to the mark with extraction medium. The obtained extracts were used for the quantification of total phenolics, total flavonoids and total antioxidant activity.

Determination of total phenolic content

Total phenolics were determined using the Folin-Ciocalteu method (8) as follows: The sample solution (0.1 ml) was mixed with distilled water (6 ml) and a Folin-Ciocalteu reagent (500 μl) was added (Folin-Ciocalteu reagent was diluted with distilled water in ratio 1:2 before use). After 3 minutes 20% Na_2CO_3 (1.5 ml) was added, and the total volume was adjusted to 10 ml with distilled water. The samples were heated at 40°C for 30 minutes in a water bath. After cooling to room temperature, the absorbency of the resulting solutions was measured at 765 nm against a reagent blank. Total phenolics quantification was based on a standard curve of gallic acid (0-500 mg/l), and the results were expressed as milligram gallic acid equivalent per gram dry matter of the sample (mg GAE/g DW).

Determination of total flavonoid content

The total flavonoids were determined using a Aluminium chloride colorimetric assay (9) as follows: The sample solution (1 ml) was mixed with distilled water (4 ml) and 5% $NaNO_2$ (0.3 ml) was added. After 5 minutes, 10% $AlCl_3$ (0.3 ml) was added. Then after 1 minute, 1M NaOH (2 ml) was added, and the total volume was adjusted to 10 ml with distilled water. The mixture was incubated at room temperature for 1 hour and, after which the absorbency was measured at 510 nm against a reagent blank. The total quantification of the flavonoids was based on a standard curve of catechin (0 -100 mg/l), and the results were expressed as milligram catechin equivalents per gram dry matter of sample (mg CE/g DW).

Ferric reducing antioxidant power (FRAP) assay

Total antioxidant capacity (TAC) was determined using the Ferric reducing antioxidant power (FRAP) assay (10) as follows: 240 μl distilled water, 80 μl extract and 2080 μl FRAP reagent were added into 10 ml flask and heated in water bath at 37°C for 5 minutes. The FRAP reagent was prepared immediately before use by mixing acetate buffer (300 mM, pH=3.6), 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM HCl and 20 mM $FeCl_3$ in a volume ratio of 10:1:1. The samples were incubated at 37°C for 15 minutes in a water bath, after which, the absorbency was measured at 595 nm against a reagent blank. Ferric reducing antioxidant power quantification was based on a standard curve of $FeSO_4 \times 7\ H_2O$ (0 - 2000 μM), and the results were expressed as μmol Fe^{2+} per gram dry matter of sample (μmol Fe^{2+}/g DW). Amersham ultrospec 2100 pro spectrophotometer (Biochrom, USA) was used for all spectrophotometric determinations.

Acid Digestion of the herbal teas

The extraction of the heavy metals from each sample was obtained by mixing 1 g of dry herbal tea with 14 ml of an acid mixture at the ratio of 2.5:1 (nitric acid: perchloric acid), which was then kept overnight in a fume hood. Thereafter, the mixture was heated to its boiling point on a hotplate under reflux for 30 minutes. After cooling to room temperature, the mixture was filtered through a Whatman No.42 filter paper into a 50 ml flask and diluted to the mark with deionized water (11). These samples were prepared in triplicate for each tea brand, and each of those were tested three times.

Analysis of heavy metals

The heavy metals quantification in the obtained extracts was performed using a Shimadzu AA-7000 atomic absorption spectrometer. Calibration curves for tested heavy metals were obtained by diluting stock solutions (1000 mg/l) of each element supplied from Merck, Germany. The concentrations of the heavy metals in the samples were determined using the calibration curve and regression equation.

Statistical analysis

All experimental measurements were carried out in triplicate and the results were expressed as mean values ± standard deviation. One-way analysis of variance (ANOVA) and least-significant-difference test (LSD) were performed to evaluate statistical significance between the means using Microsoft Excel software. Statistical significance was considered at $P < 0.05$. The Pearson correlation coefficients were calculated in order to identify the relationship between the phenolics/flavonoids and total antioxidant capacity. According to Dancey and Reidy (12) the Pearson correlation coefficient values from 0.1 to 0.3 indicate a weak, values from 0.4 to 0.6 indicate a moderate, and values from 0.7 to 1.0 indicate a strong linear relationship between two variables.

RESULTS

Total phenolics, total flavonoids and total antioxidant capacity in the herbal tea samples

Total phenolics, total flavonoids, total antioxidant capacity and the ratio of total flavonoids/total phenolics (TF/TC) in the herbal tea samples are presented in Table 2.

The TP, TF and FRAP values of different herbal tea samples were found to be in the range of 6.2-18.3 mg/g, 2.9-13.3 mg/g and 61.9-139.1 μmol Fe^{2+}/g DW, respectively. In this study, the mint tea samples showed significantly higher TP, TF and FRAP values than the chamomile tea samples, regardless of their origin. The highest levels of phenolic compounds was in a sample of mint originating from Austria (No 5), while the lowest content of these compounds was in chamomile tea samples originating from Bosnia and Herzegovina (Nos 2 and 8). A similar trend was observed for the flavonoid contents and total antioxidant capacity in all investigated samples. The present study showed no significant difference ($p \geq 0.05$) for TP, TF and FRAP in the chamomile tea samples. Furthermore, the chamomile tea samples showed lower ratios of flavonoids/phenolics compared with the mint samples.

The correlation of total phenolics and flavonoids with antioxidant capacity

The correlation of total antioxidant capacity with total phenolic and flavonoids in herbal tea samples is shown in Figure 1.

In the present study, there was a positive and highly significant relationship between the total antioxidant capacity and the total phenolics/flavonoids, indicating that phenolic compounds play a major role in the antioxidant activity in herbal tea.

Concentrations of heavy metals in the herbal tea samples

The concentrations of tested heavy metals in the herbal tea samples are listed in Table 3. The results are expressed as mass of heavy metal (mg) per kg of dry input sample.

Table 3 shows that the heavy metal concentrations varied among the herbal teas in the present study. In analyzed herbal tea samples, the metals that were present at the highest concentration were Fe and Cu, while the lowest concentrations were recorded for Pb. Presented data also showed that Fe, Cu, and Cr were detected in all of the analyzed herbal samples.

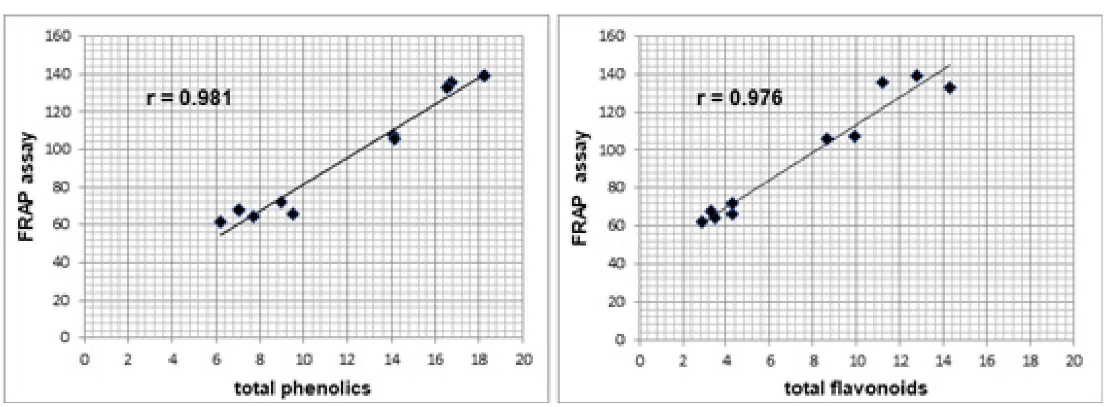

Figure 1 The relationship between antioxidant capacity (FRAP) and total phenolics/flavonoids in the herbal tea samples

Table 2 Total phenolics (TP), total flavonoids (TF) and total antioxidant capacity (FRAP) in the herbal tea samples

SAMPLE	TP (mg/g DW)	TF (mg/g DW)	FRAP (µmol Fe^{2+}/g DW)	(TF/TC ratio)
1 (mint)	14.1 ± 4.2^{b}	9.9 ± 2.9^{cd}	107.4 ± 23.3^{b}	0.70
2 (chamomile)	6.2 ± 1.5^{c}	2.9 ± 0.2^{e}	61.9 ± 19.9^{c}	0.47
3 (mint)	16.7 ± 6.4^{ab}	11.2 ± 3.7^{bc}	135.8 ± 25.0^{a}	0.67
4 (chamomile)	9.5 ± 5.7^{c}	4.2 ± 2.0^{e}	66.0 ± 22.2^{c}	0.44
5 (mint)	18.3 ± 3.4^{a}	12.8 ± 2.5^{ab}	139.1 ± 29.9^{a}	0.70
6 (chamomile)	7.7 ± 3.0^{c}	3.5 ± 1.0^{e}	64.4 ± 40.0^{c}	0.45
7 (mint)	16.6 ± 10.3^{ab}	13.3 ± 2.8^{a}	132.9 ± 47.4^{a}	0.80
8 (chamomile)	7.0 ± 2.1^{c}	3.3 ± 0.7^{e}	67.7 ± 18.3^{c}	0.47
9 (mint)	14.2 ± 2.8^{b}	8.7 ± 2.3^{d}	105.9 ± 22.4^{b}	0.61
10 (chamomile)	9.0 ± 1.9^{c}	4.3 ± 1.5^{e}	71.8 ± 14.2^{c}	0.48
$LSD_{0.05}$	3.61	1.68	20.83	-

Results are reported as mean ± standard deviation. Different letters denotes significant ($p < 0.05$) difference between the samples.

Table 3 Heavy metal concentrations in the tea samples

SAMPLE	HEAVY METAL CONCENTRATION IN THE HERBAL TEAS (mg/kg dry weight)				
	Cd	Pb	Cr	Fe	Cu
1 (mint)	n.d.	0.26 ± 0.007c	0.47 ± 0.027bcd	449.8 ± 50.6abcd	16.6 ± 1.1bc
2 (chamomile)	0.053 ± 0.01d	n.d.	0.66 ± 0.021a	468.1 ± 104.3abc	16.4 ± 2.2bcd
3 (mint)	n.d.	0.27 ± 0.11c	0.35 ± 0.023d	358.8 ± 100.1ef	16.8 ± 1.4ab
4 (chamomile)	0.194 ± 0.04b	0.17 ± 0.11d	0.32 ± 0.015d	90.8 ± 27.6i	12.6 ± 2.0h
5 (mint)	n.d.	0.09 ± 0.07e	0.31 ± 0.014d	261.7 ± 85.4gh	15.8 ± 2.6bcde
6 (chamomile)	n.d.	0.31 ± 0.25c	0.63 ± 0.020ab	477.3 ± 131.9ab	18.0 ± 1.5a
7 (mint)	n.d.	0.44 ± 0.17b	0.44 ± 0.016b	510.6 ± 168.2a	14.9 ± 2.1efg
8 (chamomile)	0.248 ± 0.11a	0.28 ± 0.14c	0.44 ± 0.031d	298.9 ± 196.7fg	15.1 ± 1.7defg
9 (mint)	n.d.*	0.56 ± 0.13a	0.47 ± 0.027bcd	227.6 ± 117.2gh	15.2 ± 1.4def
10 (chamomile)	0.124 ± 0.04c	0.26 ± 0.06c	0.63 ± 0.029abc	388.8 ± 166.4bcde	14.1 ± 1.7fg
$LSD_{0.05}$	0.045	0.05	0.17	93.91	1.35

Results are reported as mean ± standard deviation. Different letters denotes significant ($p < 0.05$) difference between samples.
* n.d. - (below detection limit)

However, Pb and Cd were detected in 90% and 50% of the analyzed samples, respectively. In this study the three toxic elements Pb, Cr and Cd were quantified with average values in herbal teas bellow the World Health Organization's (WHO) permissible limits of 10 mg/kg, 1.3 mg/kg and 0.3 mg/kg, respectively (13).

DISCUSSION

Herbal teas are widely used for the prevention and alleviation of a wide variety of diseases. They often contain highly bioactive compounds including phenolics and flavonoids. Numerous studies have demonstrated that phenolic compounds are responsible, at least in part, for the health benefits of herbal teas (14-16).

Table 2 shows that both the chamomile and mint tea samples contained a reasonable amount of phenolic and flavonoid compounds, however, the values of total phenolics and flavonoids in the mint teas were significantly higher than that in the chamomile teas. Similar to these results, the studies of Cleverdon *et* *al.* (17) and Castañeda-Saucedo *et al.* (18) found that mint tea possess higher amounts of phenolics and flavonoids compared to chamomile tea. In this study, the flavonoids to phenolics ratio for mint were much higher than those for chamomile, indicating that mint is rich in flavonoids.

Significant correlations between total antioxidant capacity measured by FRAP assays and total phenolics and flavonoids were found in the present study. The high correlations confirm the role of phenolic compounds as the main contributors to the antioxidant properties of chamomile and mint tea. Several studies have also shown that phenolic compounds, mostly flavonoids, contribute significantly to the total antioxidant capacity of herbal teas (19-20). Overall, this study suggests that chamomile and mint teas can be a good source of natural antioxidants, and this hypothesis has, in fact, been confirmed previously by several scientists (21-22).

In parallel with the increasing interest in the health benefits of herbal teas, there has been an increasing concern over the safety and toxicity of herbal teas. Indeed, there is often little information available about the evaluation of herbal teas with respect to contaminants, especially heavy metals, that could pose potential health problems. Consequently, the evaluation of the levels of heavy metal in herbal teas has recently attracted significant interest (23-24).

Among the herbal tea samples analyzed in this study, there is a significant difference in the concentration of all tested heavy metals. The results obtained in this study are not surprising, considering the fact that geochemical soil characteristics, agroclimatic conditions, type of plant species, storage processes and human activity can significantly affect the heavy metal levels in plants, including teas (25).

This study showed that the levels of all tested heavy metals differed significantly even within the herbal teas originated from the same plants. For example, the levels of Fe in mint varied from 90.8 to 477.3 mg/kg. The only exception was the Cd levels in the chamomile tea samples. Namely, the Cd concentrations in all tested tea samples of chamomile were below detection limits, suggesting that chamomile plants may possess different mechanisms to reduce Cd accumulation in their physiologically most active apical tissues i.e., leaves and flowers. This finding is in line with the results reported by Kováčik *et al.* (26).

The levels of the highly toxic heavy metals in the herbal tea samples analyzed here, i.e., Pb, Cd and Cr were within the WHO's permissible limits (Table 3). These results suggest that the potential health risks associated with exposure to toxic heavy metals via the consumption of the analyzed herbal teas should be negligible. Contrary, the levels of Fe and Cu in some herbal teas exceeded slightly the WHO's stipulated limits of 30 mg/kg for Cu and 450 mg/kg for Fe, indicating that these could be a source of iron and copper, particularly for vegetarians and vegans. A relatively high concentration of Fe and Cu in herbal teas could be attributed to the importance of these elements in plant metabolism and generally for physiological processes within the plant. For example, Fe serves as a component of many vital enzymes and assists in metabolic processes such as DNA synthesis, respiration and photosynthesis, while Cu participates in plant respiration and metabolism of carbohydrates and proteins (27). Hence, the plants developed different mechanisms to allow both Fe and Cu uptake and transport in plants.

From a health point of view, the high concentration of Fe and Cu in the analyzed herbal tea samples indicate that a potential health risk could exist through the consumption of these teas. Nonetheless, more detailed information such as the levels of heavy metals in the tea infusions, tea contribution to the daily intake of the heavy metals, as well as, heavy metal hazard quotient are required to completely assess the potential human health risk associated with heavy metals in herbal teas. The measurement of above-mentioned parameters in the herbal tea samples and their infusions was not carried out.

CONCLUSION

The results of this study indicate that the chamomile and mint are a great source of phenolic compounds that may play a role in improving heart health. The results of this study also suggest that the potential health risk of Cd, Cr and Pb exposure through a consumption of studied herbal teas is very low, i.e., few health implications to the consumers. A potential health risk caused by Cu and Fe in the studied herbal teas could exist through the consumption of the teas. Therefore, further studies are needed to test this possibility. The findings of this study also highlight the importance of quality control of herbal teas to ensure adequate protection of public health and promoting safety.

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