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Bioactive compounds in herbal infusions

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Abstract. Herbal teas are very popular and known as important source of biologically active compounds. Some of popular Latvian herbal teas: Calendula (*Calendula officinalis* L.), Matricaria chamomilla (*Matricaria chamomilla*), Lady's-mantle (*Alchemilla vulgaris* L.), Jasmine (*Jasminum officinale* L.), Yarrow (*Achillea millefolium* L.) and Linden flowers (*Tilia spp*.) were selected for analysis. This study was carried out with the aim to investigate the effect of extraction time (10, 15, 20 min) on the content of total flavonoids and total phenols as well as antioxidant activity of herbal tea extracts. The infusions were prepared by usual domestic preparation technique using ground air-dried plant materials and boiling deionized water (0.055 μS cm^{-1}) for extraction. Content of total flavonoids, total phenols and antioxidant activity was determined spectrophotometrically. Dry matter content was determined in lyophilized herbal infusions. The obtained results indicated that extraction time did not affected the content of biologically active compounds in the herbal infusions significantly ($P > 0.05$). The highest level of flavonoids was found in Jasmine and Lady's-mantle infusions (average 104.98 ± 9.21 mg quercetin equivalent 100 g^{-1} and 115.28 ± 5.25 QE mg 100 g^{-1} respectivelly), while the lowest was determined in Matricaria chamomilla extract – (average 70.10 ± 4.68 QE mg 100 g^{-1}). Lady's-mantle tea contained the largest amount of total phenols (average 4126.62 ± 26.24 mg gallic acid equivalents 100g^{-1}), the lowest – Calendula tea 1828.04 ± 10.37 mg GAE 100 g^{-1}). Data analysis showed a close linear positive correlation between the content of total flavonoids and total phenols in herbal infusions ($R^2 = 0.872$; $r = 0.934$) with the probability of 99%. In general, all samples tested in this study, demonstrated high level of antioxidant activity (from 75.04 to 91.54 mmol Trolox equivalents 100 g^{-1}). Results of the present experiments demonstrated that content of dry matter in analysed herbal teas was significantly different ($P < 0.05$).

Key words: herbal infusion, phenols, flavonoids, antioxidant activity.

INTRODUCTION

Herbal teas are known as beverages throughout the world. Herbs are mainly used in form of infusion of dried herbs in hot water. Leafy herbal teas are widely known to contain a variety of active phytochemicals with biological properties that promote human health and help reduce the risk of chronic diseases such as allergies, insomnia, headaches, anxiety, intestinal disorders, depression, and high blood pressure (Craig, 1999). Their beneficial effects could be partly attributed to polyphenolic compounds, which are known to possess antioxidant and antimicrobial properties (Miguel, 2010;

Cushnie & Lamb, 2011). There are known many herbal teas in Latvia. Most common are chamomilla, calendula, lady's-mantle, yarrow and linden flowers. Chamomilla contains several classes of biologically active compounds including essential oils, coumarins and several polyphenols, primarily the flavonoids (Avula et al., 2014).

Calendula accumulates large amounts of carotenoids in its flowers. Carotenoids are known as biologically active compounds with multiple applications in therapy. It is important to humans as precursors of vitamin A and retinoids. The plant of lady's-mantle is rich in tannins, flavonoids, salicylic acid, essential oil, bitter substances and phytosterols. It also contains vitamin C and numerous minerals. The most medicinally active part of the yarrow is the flowering tops. Yarrow contains flavonoids (apigenin, luteolin, quercitin) that increase saliva and stomach acid, helping to improve digestion. Yarrow may also relax smooth muscle in the intestine and uterus, which can relieve stomach and menstrual cramps (Lakshmi et al., 2011). Linden flowers contain sugar, tannin, mucilaginous matter, fatty substance, wax, yellow coloring matter, and a volatile oil, to which their fragrant odor is due. The main constituents of linden flower tea are flavonoids (quercetin glycosides, kaempferol glycosides, tiliroside), phenolic acids, essential oils, phytosterols, organic acids, tannins, mucilage, minerals, niacin, and vitamin C. These antioxidants help to prevent and repair DNA damage. Its pharmacological activity is in feverish colds, infectious diseases, and bronchitis, but it also has sedative and diuretic actions (Sroka & Bełz, 2009). Jasmine flower tea is very popular in Asia countries. Phytochemical analysis revealed that the jasmine flower contains antioxidants, coumarins, cardiac glycosides, essential oils, flavonoids, phenolics, saponins, and steroids (Kunhachan et al., 2012).

Antioxidants are molecules that neutralize harmful reactive oxygen species (ROS) by inhibiting oxidative chain reaction, preventing lipid peroxidation, reducing free radical concentration and chelating metal ions (Zhou & Yu, 2004). Reactive oxygen species, produced during aerobic metabolism, are essential mediators of important functions. Studies have demonstrated the involvement of ROS in a number of disorders including Alzheimer, atherosclerosis, diabetes, inflammation, and neurodegenerative and cardiovascular diseases. ROS also plays a key role in certain types of cancers and the ageing process (Salganik, 2001). Free radicals are reactive oxygen species produced in the body as by-products of cellular aerobic respiration and lead to oxidative stress (Yanai et al., 2008). Antioxidant activity is defined as an inhibition of the oxidation of lipids, proteins, DNA or other molecules that occurs by blocking thepropagation step in oxidative chain reactions (Huang et al., 2005).

Primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent the formation of free radicals through Fenton's reaction. (Chan et al., 2010).

The antioxidant ability of phenolic components occurs mainly through a redox mechanism and allows the components to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice Evans et al., 1997).

Flavonoids are phenolic substances isolated from a wide range of vascular plants, with over 8,000 individual compounds known. They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellants, and for light screening. Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, antiinflammatory, and vasodilating actions. However,

most interest has been devoted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals (Pietta, 2000).

Polyphenols are a group of compounds synthesized exclusively by plants, especially for the protection against UV-radiation and activity of pathogens. About 8,000 plant polyphenol compounds have been identified so far whereas only some hundred occur in edible plants. They are present in fruits, flowers, leafs, roots, and woody parts of plants, whereas external tissues include bigger amounts of these components (Manach et al., 2004).

Therefore the aim of current research was to evaluate the effect of extraction time (10, 15, 20 min) on the content of total flavonoids and total phenols as well as antioxidant activity of herbal tea extracts.

MATERIALS AND METHODS

Investigations were carried out at the Latvia University of Life Sciences and Technologies, Department of Chemistry.

Chemicals and spectral measurements

All the reagents used were with the analytical grade from Sigma Aldrich, Germany. JENWAY 630 Spectrophotometer was used for the absorbance measurements.

Plant materials

Plants of Calendula (*Calendula officinalis* L.), Matricaria chamomilla (*Matricaria chamomilla),* Lady's-mantle (*Alchemilla vulgaris* L.), Jasmine (*Jasminum officinale* L.), Yarrow (*Achillea millefolium* L.) and Linden flowers (*Tilia spp.)* were grown in Latvia, Jelgava region (the GPS-coordinates: 56° 39' 3.992" N 23° 43' 16.874" E), collected during the flowering period in May till July 2017 and were dried at room temperature in a dark place.

Preparation of herbal infusions

Infusions were prepared in triplicate from the selected plants by usual domestic preparation technique. For this purpose 0.5 ± 0.0001 g of finely ground air-dried plant material were extracted in 50 mL of boiling distilled water (0.055 μS cm^{-1}) and stirred gently on a magnetic stirrer at room temperature for 10, 15 or 20 min. Each extract was then filtered through paper filter (11 μm, Whatman Inc., Clifton, NJ, USA). The supernatant was used for all determinations.

Analytical methods

Dry matter

The tea infusions were lyophilized (lyophilizator ALPHA 1-2 Ld plus CHRIST, Germany, 72 hours, -56 °C, 0.027 mBar vacuum) to determine the yield of extracted compounds (dried weight) in each of them. The results were expressed as g freeze-dried infusion l00 g^{-1} tea sample.

Total phenolic compound content

The total phenolics of the tea infusions were analyzed spectrometrically according to the Folin-Ciocalteu (Singleton et al., 1999, Dewanto et al., 2002). Briefly, 2.5 mL of Folin–Ciocalteu reagent (diluted 10 times with distilled water) was added to 0.5 mL of

infusion. The mixture was then incubated for 3 min, after which 2 mL of sodium carbonate (Na_2CO_3) (7.5 g 100 g^{-1}) was added and well mixed. The control sample contained all the reaction reagents except the extract. After standing for 30 min at 20 ± 1 °C in dark place for color development, absorbance was measured at 760 nm using JENWAY 630 Spectrophotometer. Results were expressed as mg gallic acid equivalents (GAE) 100 g^{-1} dry-matter of herbal tea and analysis were carried out in triplicate for each herbal infusion.

Total flavonoid content

The content was quantified using aluminium chloride method (Xu & Chang, 2007) with some modifications. To 500 µL of tea infusion 2 mL of distilled H_2O was added, then mixed with 150 µL of 5% sodium nitrite $NaNO_2$. After 5 min, 150 µL of 10% aluminium chloride $AlCl_3$ solution was added. The mixture was allowed to stand for another 5 min, and then 1 mL of the 1M sodium hydroxide NaOH was added. The reaction solution was mixed well and incubated at 20 ± 1 °C in dark place for 15 min. The control sample contained all the reaction reagents except the extract. The absorbance was measured at 415 nm using JENWAY 630 Spectrophotometer. Results were expressed as milligram quercetin equivalent 100 g^{-1} in dry weight (mg QE 100 g^{-1} DW).

Antiradical activity

The antiradical activity of tea extracts was determined according to Afify (2012) with some modifications. Method is based on the radical scavenging ability in reacting with stable 2.2-diphenil-1-picrylhydrazyl (DPPH) free radical. 3.5 mL of DPPH solution (4 mg of DPPH reagent dissolved in 100 mL pure ethanol) was added to 0.5 mL sample extract. Solution was well mixed and stand in dark place at 20 ± 1 °C for 30 min. Absorbance was measured at 517 nm using JENWAY 630 Spectrophotometer. The antiradical activity was expressed as TROLOX (6-hydroxy-2.5.7.8-tertamethylchroman-2-carboxylic acid) equivalent antiradical activity (mmoL TE 100 g^{-1} DW).

Mathematical data processing

The results are the means and standard deviation for three replicates. Means were compared by analysis of variance (ANOVA) and correlation analysis. Significance was defined at $P < 0.05$. Statistical analysis was carried out by Microsoft Excel 2010 version software.

RESULTS AND DISCUSSION

Generally, the chemical properties of herbal tea depend on the applied temperature and time, as well as the extraction technique (Ong, 2004).

Based on the absorbance values after reaction with Folin–Ciocalteu reagent, results of the spectrophotometric analysis are given in Fig. 1. The total phenolics content (TPC) in the investigated herbal tea infusions ranged from 1,824.1 ± 9.08 to 4,350.3 ± 13.01 mg GAE 100 g^{-1} (Fig. 1). Lady's-mantle infusions were the most abundant in the total phenols (15 min extraction 4,350.3 ± 13.01 GAE 100 g^{-1} and 20 min extraction 4,226.4 ± 10.32 mg GAE 100 g^{-1}), while the lowest amounts were determined in Calendula tea infusions (in average 1,829.9 ± 9.65 mg GAE 100 g^{-1}). The

obtained results indicated that extraction time did not significantly affected the content of total phenols in the herbal tea infusions ($P > 0.05$).

Previous study Kaya et al. (2012) from Turkey proved the Lady's-mantle are well known as rich sources of polyphenolic compounds, flavonoids and phenolic acids.

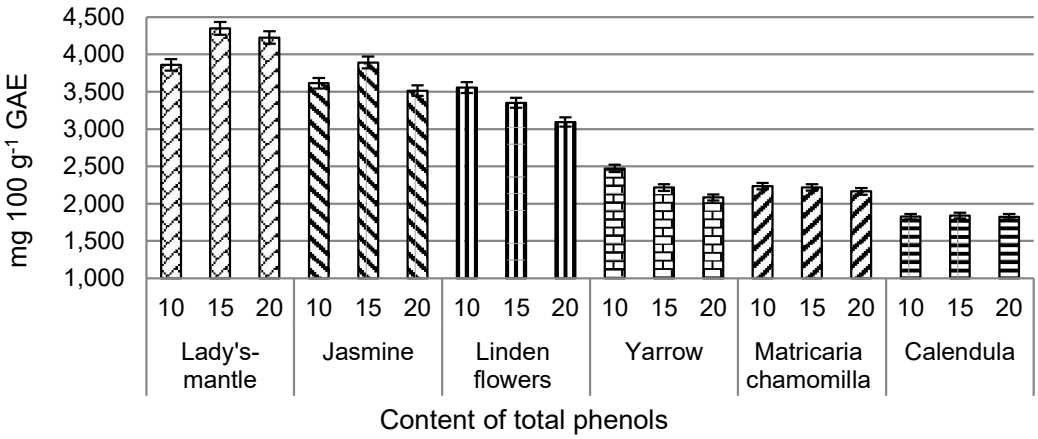

Figure 1. Content of total phenols in herbal tea infusions.

In addition, Rigane et al. (2013) from Tunisia reported that total phenolics content of Calendula herbal tea infusions was 109.27 mg GAE g^{-1} dry weight extracts. Scientist Toda (2011) from Japan reported that total polyphenol content in Arabian jasmine tea-water extract was 101.2 ± 17.8 mg GAE g^{-1}. In a study by Jungmin Oh (2013), it showed that the content of total phenols in green herb tea was 82.21 mg GAE g^{-1}, black herb tea was 82.86 mg GAE g^{-1}, and peppermint tea – 75.31 mg GAE g^{-1}. Accordingly to Formisano et al. (2015) total phenols content in Chamomile herbal tea was 2,689.2 ± 15 mg GAE 100 g^{-1} DW. Analyzing obtained experimental results we can conclude that our results confirm the results mentioned in scientific literature and that total phenols could be one of the main components responsible for antioxidant activities of these herbal teas.

Flavonoids are a large subgroup of secondary metabolites categorized as phenolic compounds, widely distributed throughout plants. Flavonoids protect plants against various biotic and abiotic stresses and exhibit adiverse spectrum of biological functions and play an important role in the interaction between the plant and their environment. Flavonoids absorbed the harmful UV radiation induced cellular damage (Amalesh et al., 2011). In herbal tea infusions the presence of flavonoids can explain the strong antioxidant activity of them. The elucidation of their structures is complex, as they can range from simple aromatic molecules to highly polymerized compounds and may involve synergism between compounds (Boroski et al., 2011).

The results of this study (Fig. 2.) point out that the highest level of flavonoids was found in Lady's-mantle and Jasmine infusions (average 115.28 ± 5.25 mg quercetin equivalent (QE) 100 g^{-1} and 104.98 ± 9.21 QE mg 100 g^{-1} respectivelly), while the lowest was determined in Matricaria chamomilla extract – (average 70.10 ± 4.68 QE mg 100 g^{-1}). The obtained results indicated that extraction time 10, 15 and 20 min did not significantly affected the content of flavonoids compounds in the herbal tea infusions ($P > 0.05$).

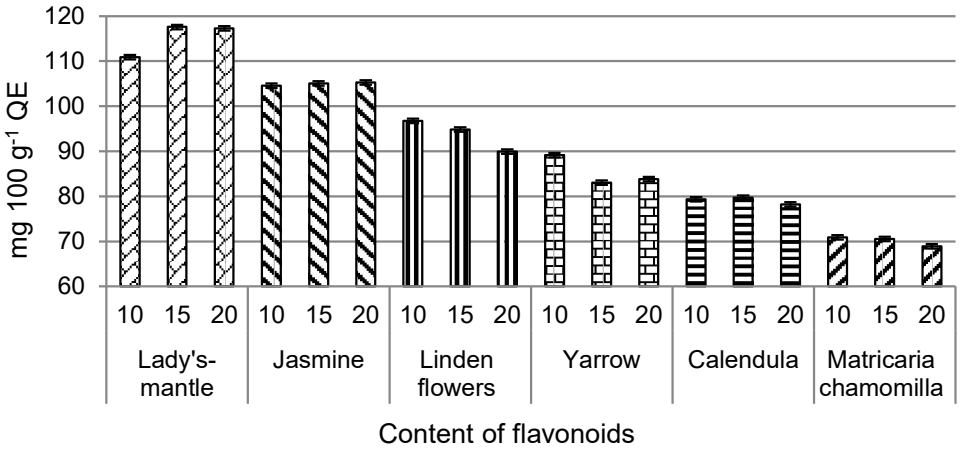

Figure 2. Content of flavonoids in herbal tea infusions.

In a study by Jungmin Oh from Korea (2013), it showed that total flavonoid levels in herb teas can ranged from 2.51 to 48.33 mg QE g^{-1}. In addition, Al-osaj (2016) from Iraq Nahrain University Baghdad, reported that total flavonoids in Lady's-mantle tea infusions was 1,831 quantity mg 100 g^{-1}. Scientist from Iraq reported that the Lady's-mantle is the richest source of flavonoids, containing catechin (250 mg 100 g^{-1}), epicatechin (524 mg 100 g^{-1}) and a significant amount of rutin (1,057 mg 100 g^{-1}) (Al-osaj, 2016). In addition, Rigane et al. (2013) from Tunisia reported that total flavonoids in Calendula herbal tea infusions ranged between 44.91 and 76.44 mg QE g^{-1} dry weight in leaf and flower extracts, respectively. Total flavonoids content in chamomile herbal tea was 710.7 ± 9 mg QE 100 g^{-1} DW to 530.9 ± 20 mg QE 100 g^{-1} DW (Formisano et al., 2015).

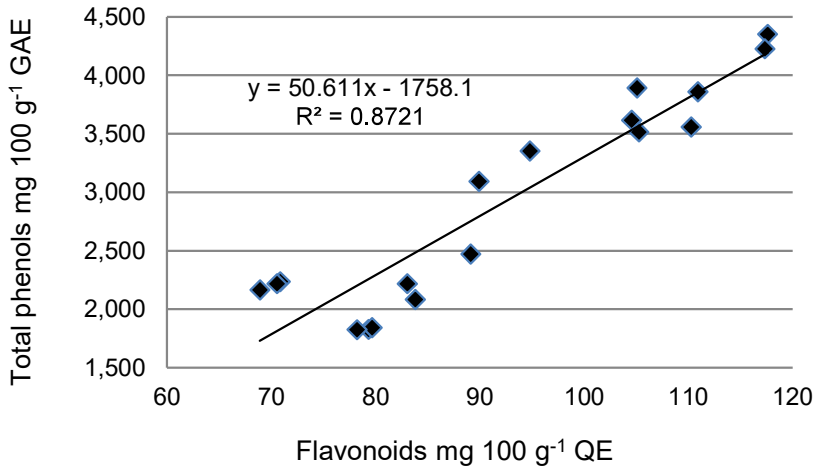

Figure 3. The correlation between total phenols and flavonoids content.

The correlation between total phenols content and flavonoids of the 6 herbal tea infusions is shown in Fig. 3. The result showed a positive linear correlation between total phenols and flavonoids content (R^2 = 0.8721).

The DPPH method is an efficient procedure commonly used to determine antioxidant activity (Scherer et al., 2009). The antioxidant capacity of the herbal tea infusions were studied through the evaluation of their free radical scavenging effect on the 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical. The results were expressed as μM Trolox ekv 100 g^{-1}.

The content of antioxidant activity, mmol Trolox eq. 100 g^{-1} is shown in Table 1. For the herbal tea infusions, the antioxidant activity varied from 75.04 ± 1.15 mmol Trolox eq. 100 g^{-1} to 91.54 ± 2.62 mmol Trolox eq. 100 g^{-1}. The highest antioxidant activity was observed for Linden flowers infusion: 91.19 ± 2.54 mmol Trolox eq. 100 g^{-1} with extraction time 10 min and 91.54 ± 2.62 mmol Trolox eq. 100 g^{-1} (extraction time 15 min), followed by Matricaria chamomilla 90.46 ± 2.66 mmol Trolox eq. 100 g^{-1} (extraction time 15 min).

Calendula had the lowest antioxidant activity – in average 80.08 ± 1.52 mmol Trolox eq. 100 g^{-1}. Extraction time did not have statistically significant influence ($P > 0.05$) on antioxidant activity.

Table 1. Antioxidant activity in herbal tea infusions

Herbal tea infusions	Antioxidant activity, mmol Trolox equivalents 100 g^{-1}		
	Extraction time		
	10 min	15 min	20 min
Calendula	75.04 ± 1.15	83.58 ± 2.02	81.60 ± 1.41
Matricaria chamomilla	89.03 ± 2.62	90.46 ± 2.66	89.13 ± 2.27
Jasmine	86.87 ± 1.64	87.54 ± 1.84	87.69 ± 2.06
Lady's-mantle	82.68 ± 1.35	87.05 ± 1.32	85.32 ± 1.33
Linden flowers	91.19 ± 2.54	91.54 ± 2.62	89.77 ± 2.36
Yarrow	89.50 ± 2.11	90.83 ± 2.21	86.30 ± 1.74

Antioxidant activity, mmol Trolox eq. 100 g^{-1} of the Herbal tea infusions value was expressed as the mean ± standard error (SD).

In addition, Rigane et al. (2013) from Tunisia reported that antioxidant activities of Calendula officinalis extracts was 0.35 ± 0.02 DPPH (IC_{50} mg mL^{-1}) or 28.37 ± 0.12 FRAP (mmol eq. of Trolox). In turn scientist Toda (2011) from Japan proved that antioxidant activity of Arabian Jasmin herb tea water extract was 144.0 ± 1.4 mmol L^{-1} PAO, but Chamomile herbal tea antioxidant activity was 238.2 ± 4 to 811.4 ± 1 mol TE 100 g^{-1} DW (Formisano et al., 2015).

The antioxidant capacities of samples may be influenced by lots of factors, such as cultivation, production, storage conditions and test systems, and cannot be fully described by one single method (Yashin et al., 2011). Most natural antioxidants are multifunctional. A reliable antioxidant evaluation protocol requires different antioxidant activity assessments to be performed to take into account various mechanisms of antioxidant action (Wong et al., 2006).

The obtained results indicated that extraction time did not significantly affected the content of dry matter in the herbal infusions ($P > 0.05$), but it depends on plants type. The highest content of dry matter was determined in Jasmine herbal tea extract (53.37 ± 0.27 g 100 g^{-1}), but the lowest in in Linden flowers and Yarrow herbal tea extracts, respectively 20.64 ± 0.10 g 100 g^{-1} and 20.57 ± 0.14 g 100 g^{-1}.

CONCLUSIONS

In summary, the present paper investigates the possibilities for obtaining herbal tea infusions with high values of bioactive compounds, using in Latvia well known and popular herbs. The obtained results indicated that extraction time (10, 15 or 20 min) did not affect the content of biologically active compounds, as well as antioxidant activity in the herbal infusions significantly, but content of tea`s dry matter was significantly different. The highest level of flavonoids was found in Jasmine and Lady's-mantle infusions, while the lowest (about two times) was determined in Matricaria chamomilla extract. Lady's-mantle tea also contained the highest amount of total phenols – in average 4126.62 ± 26.24 mg GAE $100g^{-1}$. Calendula and Matricaria chamomilla tea infusions are the poorest regarding these compounds. Data analysis showed a close linear positive correlation between the content of total flavonoids and total phenols in herbal infusions ($R^2 = 0.872$; $r = 0.934$). Due to higher content of bioactive compounds in tea infusions, all samples demonstrated high level of antioxidant activity (from 75.04 to 91.54 mmol Trolox equivalents 100 g^{-1}).

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