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Effects of brewing process on phenolic compounds and antioxidant activity of herbs

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Abstract Traditionally used medicinal plants contain a wide range of polyphenolic compounds that act as powerful antioxidants. The content of phenolic compounds in the infusions and decoctions of chamomile (*Matricaria chamomilla* L.) and St. John's wort (*Hypericum perforatum*), which are traditionally used medicinal herbs, was evaluated via liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. To obtain relevant antioxidant/reducing capacity of the prepared extracts, cupric reducing antioxidant capacity and Folin-Ciocalteu assay were applied. Rutin and apigenin were the major flavonoids in the aqueous extract of chamomile, whereas the predominant phenolic compounds of St. John's wort water extracts were rutin and catechin followed by chlorogenic acid. A longer time of infusion and decoction of St. John's wort herb significantly affected the rutin content. The increase of extraction time had very little impact on the antioxidant activities for chamomile but considerably higher impact on those for St. John's wort.

Keywords: medicinal plants, chamomile, St. John's wort, polyphenols, antioxidant capacity

Introduction

Commonly consumed medicinal plants contain different chemical compounds such as lipids, terpenoids, flavonoids, and phenolic acids. They exhibit several biological activities and offer numerous health benefits (1,2). Herbal teas, apart from their aromatic flavor, are popular owing to their antioxidant properties and therapeutic applications. It should be mentioned that the term herbal tea is commonly used for any beverage made from herbs, spices, or other plant material. These drinks are distinguished from true teas (black, green, and white) that are prepared from the leaves of the tea plant *Camellia sinensis*. Over the last decade, the global herbal tea market has exhibited robust growth due to the widespread acceptance of functional foods as well as the rise in consumer confidence to include herbs in preventive healthcare. Europe represents the largest market worldwide but the Asia-Pacific region is the fastest growing market at 8.1% compound annual growth rate (3).

The antioxidant abilities of herbal teas are mainly attributed to phenolic compounds, which can quench free radicals or inhibit the activity of free-radical-generating enzymes, can act as reducing agents or metal chelators (4,5). Herbal teas, as their chemical compositions vary depending on the plant used, exhibit a specific set of advantages. Chamomile tea exhibits antibacterial and antiseptic properties, calms the mind, and helps people relax (1). St. John's wort has been widely used as an anti-inflammatory, antidepressive, and healing agent (6).

Traditionally, herb brewing can be done as an infusion -where dried plant parts are steeped in boiling water- or as a decoction - where herbs are boiled in water. Decoction is the preferred method for brewing herbal tea from roots, bark, and berries. During these brewing processes, hydrophilic compounds are released; a majority of them, such as phenolic compounds, are water soluble. There are no data regarding the composition of herbal water extracts depending on the brewing condition. The effect of brewing temperature and time on the content of polyphenols, specifically flavonoids, was studied in green tea (7,8).

The aim of this study was to examine the content of phenolic compounds in herbal infusions and decoctions, the most consumed preparations of these herbs. Chamomile (*Matricaria chamomilla* L.) and St John's wort (*Hypericum perforatum*) were taken as the model herbs. The liquid chromatography-tandem mass spectrometry (LC-MS/MS) of the polyphenols group was conducted using a hybrid triple quadrupole-linear ion trap-mass spectrometer. The efficiency of extraction was examined using different brewing times. Additionally, the work was completed by investigating the antioxidant properties of the prepared extracts using the Folin-Ciocalteu (FC) assay and cupric reducing antioxidant capacity (CUPRAC) method.

Materials and Methods

Reagents The commercial standards of flavonoids and phenolic acids were from Sigma-Aldrich (St. Louis, MO, USA). The stock solutions

were prepared in acetonitrile. Acetonitrile was of HPLC grade from Merck (Darmstadt, Germany). Ultra pure water from Milli-Q system (Millipore, Billerica, MA, USA) was used for all experiments.

Determination of polyphenols by HPLC-MS The Shimadzu LC system and 3200 QTRAP Mass spectrometer (Applied Biosystem/ MDS SCIEX) were used for chromatographic analysis. The MS system equipped with electrospray ionization source was used in negative mode. Nitrogen was used as a curtain and as an auxiliary gas. Optimum conditions of selected reaction mode (SRM) for each compound were determined in the infusion mode. The continuous mass spectra were obtained by scanning mass-to-charge ratio (*m/z*) from 50 to 650 (9).

The information-dependent acquisition (IDA) method was programmed by combining SRM and precursor ion scans (PrecIS) as the survey scans and an enhanced product ion (EPI) and enhanced resolution (ER) scans as the dependent scans in the same injection. The survey scan contained 37 SRM transitions that correspond to the most intense selected (SRM) for each compound in the SRM method. SRM parameters used in the survey scan were previously optimized (10). Scanning of precursor ion scans of 269, 117, and 433 were applied to find unknown derivatives of apigenin. IDA parameters included the acquisition of ions whose peak height exceeded 5000 counts per second and exclusion for 30 s after three occurrences of the same ion. The EPI scan was performed with Q1 set at low resolution and the linear ion trap scanning from 50 to 650 amu at a rate of 4000 amu/s. The dynamic fill-time option was selected on the ion trap with a step size of 0.12 amu. The EPI scan at different collision energies (20 and 40 eV) was monitored for each SRM, PrecIS-IDA-EPI, ER cycle. The confirmation criteria applied to the target compounds in the samples were as follows: the presence of the characteristic SRM transitions at the correct retention time, a product ion scan, and enhanced mass spectrum of unknown compounds with the same substructure as standards. All experiments were performed in the negative ionization mode.

Compounds were separated on the SeQuant^{TM} ZIC-HILIC column (100×2.1 mm, 3.5 μm) from Merck at 30°C. 10 mM of ammonium acetate (pH 7) as eluent A and acetonitrile as eluent B were used. The gradient elution was as follows: 0-4 min 98% B, 6-7 min 90% B, 8-8.4 min 80% B, 8.4-12 min 50% B, and 13-20 min 98% B (10). The analytes were identified by comparing the retention time and *m/z* values obtained by MS and MS^2 with the mass spectra (11). Toluene was used as a void time marker.

The calibration curves in the SRM mode were generated by three repeated injections of standard solutions at five concentration levels were found to be in the range of 0.1-25 mg/L. A regression coefficient of 0.97 for luteolin and 0.99 for other compounds was obtained. The limits of detection (signal-to-noise ratio 3:1) were in the range of 0.1-0.25 mg/L. The relative standard deviations for the retention times of all peaks were lower than 3%. No recovery test was done as the sample preparation procedure contained only a filtration step.

Preparation of herb extracts Tea bags of chamomile (*M. chamomilla* L.) and St. John's wort (*H. perforatum*), manufactured by a leading Polish company Herbapol, were purchased from a local market. According to the information provided by the company, these herbs were collected in the 2015 season and air dried at room temperature.

Infusion was achieved by pouring 2 g (typical mass of herb in a tea bag) of dried plant in 50 mL of boiled water (~95°C) and was allowed to steep for an appropriate time interval. For decoction, 50 mL of cold water (~10°C) was added to 2 g of each herb; the liquid was heated under the cover over an appropriate amount of time. Each extract was then filtered and analyzed.

Evaluation of antioxidant activity of the extracts 1 mL of herb extract was mixed with 0.1 mL of FC reagent and 0.9 mL of water. After 5 min, 1 mL of sodium carbonate (7%, w/v) and 0.4 mL of water were added. The absorbance against a blank solution without an extract was measured at 765 nm after 30 min. The data were expressed as gallic acid equivalent in mg per g of dry matter.

The CUPRAC assay described by Apak *et al*. (12) was adopted. 1 mL of 10 mM $CuCl_2$ was mixed with 1 mL of 7.5 mM neocuproine alcoholic solution and 1 mL of 1 M acetate buffer (pH 7). Then, 0.5 mL of herb extract and 0.6 mL of water were added. The tube containing the sample and reagents was incubated in a water bath at 50°C for 20 min. The absorbance measurements were made at 450 nm. The results were expressed as trolox equivalent (mmol TR per g of dry matter).

Statistical analysis The results are expressed as the mean of triplicate determinations±SD. The statistical analysis was conducted using the software package STATISTICA 8.0 for Windows from Statsoft (Tulsa, OK, USA). A *p*-value of less than 0.05 was considered statistically different.

Results and Discussion

The content of phenolic compounds in the aqueous extracts of chamomile and St John's wort prepared as an infusion and a decoction was investigated to define the optimal extraction conditions for each type of herb. For the separation and determination of phenolic compounds in the prepared extracts, hydrophilic liquid interaction chromatography (HILIC) was used. The high organic content of the mobile phase in the HILIC mode enhances the ionization efficiency in the ion source of a mass spectrometer and simultaneously enhances the sensitivity of the detector (13).

In a preliminary study, we investigated the content of the selected compounds for each herb for a wide range of extraction time (5-30 min). Luteolin and hesperetin were chosen as the examples of compounds present in chamomile, while rutin and chlorogenic acid were studied in St. John's wort extracts. The results are presented in

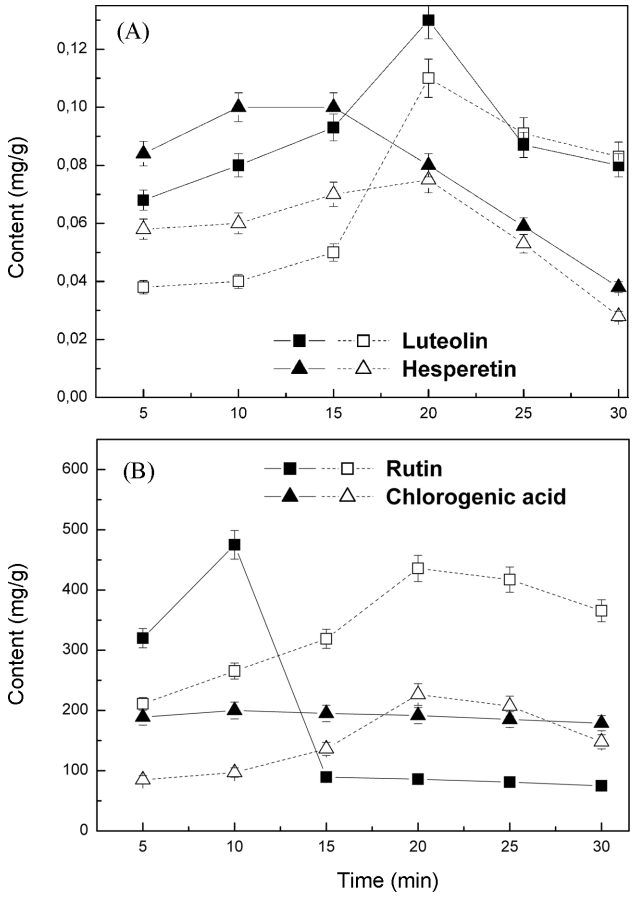

Fig. 1. The effect of extraction time on the content of selected phenolic compounds in the extracts of (A) chamomile and (B) St. John's wort for infusion (solid line) and decoction (dashed line) modes.

Fig. 1. The luteolin content in chamomile aqueous extracts increased up to 20 min of extraction time, which was faster than that in the infusion mode. Hesperetin content extracted from the same herb reached its maximum value in 15 and 20 min for infusion and decoction, respectively (Fig. 1A). After that time, similarly to luteolin, hesperetin content significantly decreased. Rutin was extracted from St. John's wort very quickly in the infusion mode (Fig. 1B), but the prolonged time of extraction caused its degradation. The content of this flavonoid was similar in decoction mode for 20 min (475±23.8 mg/L) in comparison to infusion conducted for 10 min (436 mg/L). The efficiency of chlorogenic acid extraction from St. John's wort did not depend on time.

The content of polyphenolic compounds determined in the aqueous extracts prepared in the infusion and decoction modes are given in Table 1 (for chamomile) and Table 2 (for St. John's wort). An example extracted ion chromatogram of determined compounds in chamomile extract after 10 min of infusion process is presented in Fig. 2. Rutin and apigenin were the major flavonoids present in the chamomile extract, whereas trace amounts of luteolin and hesperetin were found. The predominant phenolic compounds of St. John's wort water extracts were rutin and catechin, followed by chlorogenic

Table 1. The content of polyphenols in the aqueous extracts of chamomile

	Infusion time (min)		
	10	15	20
Luteolin	0.08±0.02$^{a1)}$	0.09±0.02^{a}	0.13±0.03^{a}
Rutin	4.21±0.25^{a}	3.60±0.21^{b}	3.22±0.26^{b}
Hesperidin	0.10±0.009^{a}	0.10±0.008^{a}	0.09±0.01^{a}
Hesperetin	0.10±0.006^{a}	0.10±0.005^{a}	0.08±0.004^{a}
Apigenin	2.60±0.19^{a}	2.59±0.13^{a}	2.57±0.15^{a}
p-HBA	0.62±0.051^{a}	0.56±0.049^{b}	0.79±0.054^{c}
Gallic acid	0.09±0.007^{a}	0.10±0.006^{a}	0.10±0.006^{a}
Caffeic acid	0.63±0.049^{a}	1.12±0.08^{b}	1.53±0.09^{c}
p-Coumaric acid	0.09±0.007^{a}	0.10±0.08^{a}	0.11±0.08^{a}
Chlorogenic acid	3.70±0.22^{a}	3.60±0.28^{a}	4.36±0.32^{b}
	Decoction time (min)		
	10	15	20
Luteolin	0.04±0.002^{a}	0.04±0.002^{a}	0.11±0.08^{b}
Rutin	0.26±0.011^{a}	0.35±0.002^{b}	0.89±0.060^{c}
Hesperidin	ND$^{2)}$	ND	ND
Hesperetin	0.06±0.003^{a}	0.07±0.002^{a}	0.08±0.005^{a}
Apigenin	2.24±0.10^{a}	2.30±0.13^{b}	2.29±0.11^{a}
p-HBA	0.28±0.014^{a}	0.27±0.012^{a}	0.49±0.022^{b}
Gallic acid	0.03±0.001^{a}	0.03±0.002^{a}	0.11±0.09^{b}
Caffeic acid	0.77±0.039^{a}	0.41±0.021^{b}	1.20±0.09^{c}
p-Coumaric acid	0.03±0.001^{a}	0.04±0.001^{a}	0.04±0.002^{a}
Chlorogenic acid	0.07±0.003^{a}	0.04±0.002^{b}	0.06±0.003^{a}

[1)]Values are expressed as mean (mg/L)±SD (n=3); In each row, different letters mean significant differences (p<0.05)
[2)]ND, not detected

acid. Catechin has been found to be the most powerful free-radical scavenger from among different classes of flavonoid compounds (14). This results from its chemical structure, mainly with the presence of the catechol moiety on ring B and the presence of a hydroxyl group activating the double bond on ring C. Chlorogenic acid, besides having well-known antioxidant activities, has been suggested as an inhibitor of inflammation and tumor promotion via deactivation of a range of pro-oxidative enzymes (15). Based on their antioxidant properties, quercetin and rutin exhibit the preventive effect of an oxaliplatin-induced painful peripheral neuropathy (16).

The derivatives of apigenin in chamomile extracts were identified using the hybrid triple quadrupole-linear ion trap-mass spectrometer. The IDA allows the use of specific survey scan modes to trigger sensitive and confirmatory product ion and MS spectrum scans acting as dependent scans (17,18). Chamomile extracts were screened for the presence of apigenin derivatives with IDA methods. Two ions of *m/z* 285 and *m/z* 473 were found in the extracts, according to PrecIS of 269. These ions could correspond to apigenin-4-methyl ether (acacetin, t_R =1.53 min) and apigenin-7-*O*-β-D-(6-*O*-acetylglucoside) (t_R =11.5 min), respectively. The product ion spectra of the corresponding precursor ions was recorded and presented in Fig. 3. The presence of these compounds was described in chamomile samples (19-21). Apigenin-7-*O*-β-D-glucoside was also identified at

Table 2. The content of polyphenols in the aqueous extracts of St. John's wort

	Infusion time (min)		
	10	15	20
Catechin	446.0±26.8[c]	381±22.9[b]	367±20.7[a]
Quercetrin	27.0±1.62[c]	25.8±1.55[b]	22.9±1.45[a]
Rutin	475.0±23.8[c]	89.3±5.35[b]	85.9±6.30[a]
Quercetin	1.12±0.08[b]	1.41±0.11[c]	0.47±0.026[a]
Hesperidin	4.70±0.28[b]	4.20±0.29[a]	4.02±0.26[a]
Gallic acid	3.01±0.42[b]	2.40±0.19[a]	2.60±0.28[a]
Caffeic acid	0.83±0.06[a]	1.52±0.11[b]	1.61±0.15[b]
p-Coumaric acid	0.23±0.07[a]	0.28±0.02[b]	0.58±0.07[c]
Chlorogenic acid	201±10.1[a]	195±9.75[a]	192±9.91[a]
	Decotion time (min)		
	10	15	20
Catechin	189±9.38[a]	228±11.2[b]	392±15.4[c]
Quercetrin	10.4±0.49[a]	11.4±0.56[b]	25.1±1.60[c]
Rutin	266±13.1[a]	319±15.6[b]	436±21.8[c]
Quercetin	0.38±0.02[a]	0.45±0.02[b]	1.56±0.12[c]
Hesperidin	3.10±0.14[a]	3.71±0.17[b]	6.10±0.39[c]
Gallic acid	2.30±0.11[a]	2.89±0.13[b]	3.24±0.49[c]
Caffeic acid	0.65±0.03[a]	1.03±0.05[b]	1.72±0.06[c]
p-Coumaric acid	0.33±0.02[a]	0.40±0.02[b]	0.59±0.03[c]
Chlorogenic acid	97.10±4.83[a]	136±6.80[b]	226±10.9[c]

[1)]Values are expressed as mean (mg/L)±SD (n=3); In each row, different letters mean significant differences (p<0.05)
[2)]ND, not detected

m/z 431 (17); however, this compound was not found in tested extracts of chamomile. Due to a lack of acacetin and apigenin-7-*O*-β-D-(6-*O*-acetylglucoside) standards, these compounds could not be quantified.

The extraction efficiency from a plant material by water depends on how these compounds are strongly bound to cell structural components such as cellulose, lignin or pectin. The optimal extraction conditions for a given plant depend on the presence of different active compounds (22). Generally, the rate of the extraction process and its efficiency increase by increasing temperature. This can be explained by cell wall disruption or by the breakdown of insoluble phenolics into soluble compounds. In our study, the infusion and decoction extraction of two herbs were conducted for the same interval times but at different temperature conditions. In decoction, the herb is placed in cold water and only then brought to a boil. For this reason, the content of studied phenolics is lower in decoction in comparison with the infusion mode. Similar results were reported for infusion and decoction of *M. recutita* L. herb (11).

When the time of extraction was increased, the content of some polyphenolic compounds changed. Brewing of chamomile in the infusion mode afforded the concentrations of apigenin, hesperidin, and luteolin at similar levels, while rutin content decreased. A longer time of infusion as well as decoction process significantly affected the content of rutin. Simultaneously, the content of its aglycones form, quercetin, increased; this was probably due to degradation of this glycoside. It should be noted that quercetrin (quercetin-3-*O*-rhamnoside) was more easily liberated by hot water during decoction than infusion and that its content only minutely decreased during the increased extraction time.

The possibility of degradation of these compounds (originated by temperature, time, or presence of oxidative enzymes) should be taken into consideration. Several studies reported instability of phenolics during thermal processing of different types of foods (23-28). Gonthier *et al*. (29) found that polyphenol oxidase was able to decrease the chlorogenic acid content in the model system. The stability of that compound could be improved by adding epigallocatechin gallate and ascorbic acid (30).

Additionally, experiments with the mixtures of standard phenolic solutions confirmed that some studied compounds are unstable under applied infusion and decoction processes. Their concentration

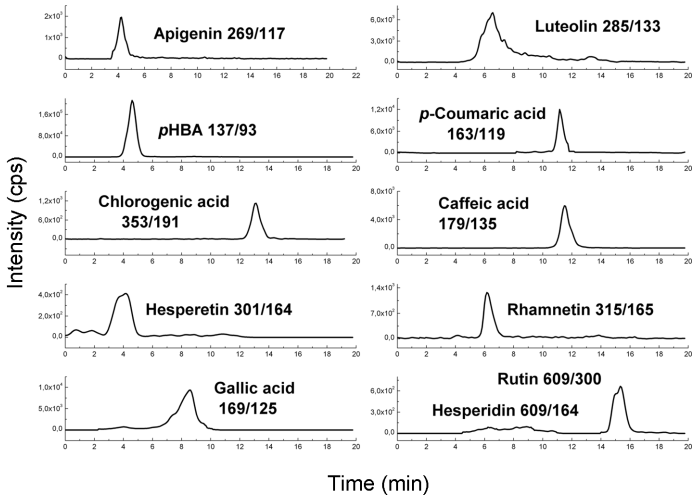

Fig. 2. Extracted ion chromatogram of determined phenolic compounds in chamomile extract after 10 min of decoction process. ZIC-HILIC column (100×2.1 mm, 3.5 μm). 10 mM ammonium acetate (pH 7) as eluent A and acetonitrile as eluent B were used under gradient mode.

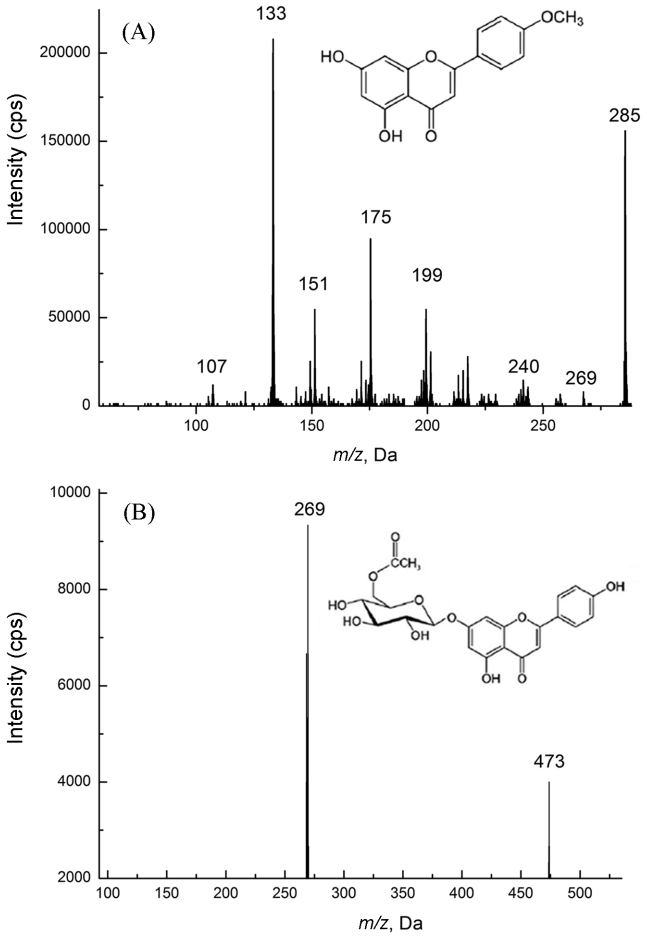

Fig. 3. The product ion spectra of: (A) apigenin-4'-methyleter; (B) apigenin-7-*O*-6''-*O*-acetylglucoside obtained in EPI mode.

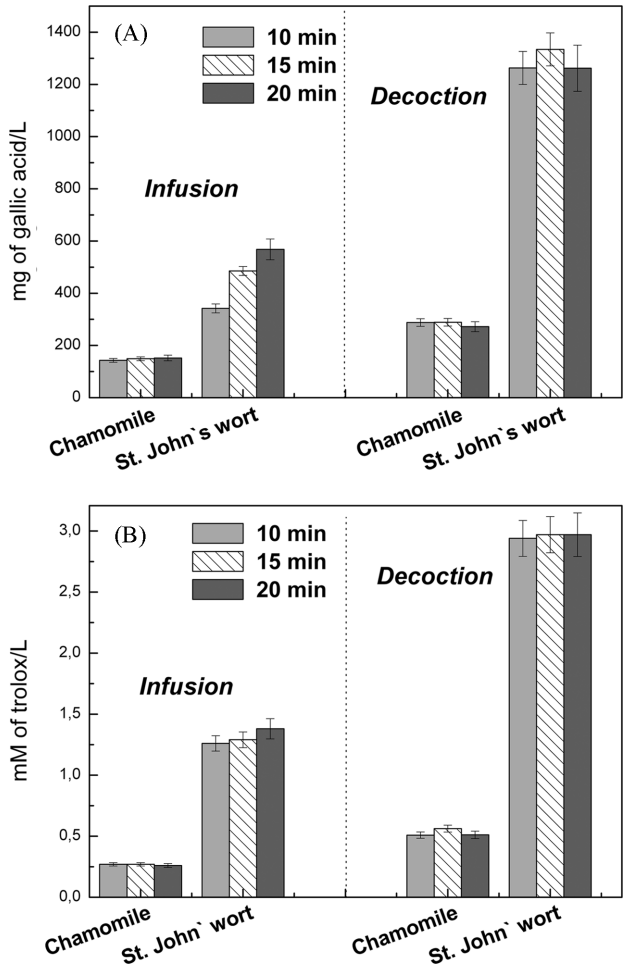

Fig. 4. The antioxidant activity of herb extracts using (A) FC method and (B) CUPRAC assay.

in the infusion extraction decreased in the following order: luteolin, myricetin, chlorogenic acid, *p*-coumaric acid>quercetin>hesperidin, *p*-HBA>gallic acid>catechin, caffeic acid>rutin>apigenin. For decoction extraction, the order was as follows: myricetin, quercetin, rutin, catechin, caffeic acid, chlorogenic acid, gallic acid>luteolin>*p*-coumaric acid>apigenin>hesperidin>*p*-HBA.

FC and CUPRAC assays were used to evaluate the antioxidant properties of prepared herb extracts. Both assays are based on a single electron transfer reaction, and it is assumed that the antioxidant activity is equal to the reducing capacity of the sample. Due to the chemical complexity of extracts, often a mixture of several compounds having different polarities and chemical behaviors, the approach with multiple assays in screening work is highly advisable (31). The results are presented in Fig. 4. Generally, St. John's wort extracts prepared by infusion and decoction exhibit higher antioxidant activity than chamomile extracts in both assays. This could be connected with the high concentration of catechin and chlorogenic acid (Table 2), which act as strong antioxidants (13,14). The increase in extraction time had very little impact on the values for chamomile but considerably higher for St. Johns wort.

Higher values for both herbs in FC and CUPRAC assays were obtained in the extracts prepared in the decoction mode (Fig. 4). During this process, probably more compounds with antioxidant (reductive) properties are released. Similar results were obtained for (*Melissa officinalis* L.) aqueous extracts (32). The FC assay, very often used for the determination of so-called total polyphenols, gives different responses for different phenolic compounds. However, the FC reagent could also oxidized several nonphenolic compounds to give higher results (32). CUPRAC assay is sensitive to thiol-type oxidants (11). Moreover, most phenolic compounds showed significant differences in their antioxidant activities according to the method used. Tabart *et al*. (33) found that only myricetin and gallocatechin (among 25 tested compounds) gave comparable activities in the various assays. The mechanisms involved in the assays used to evaluate antioxidant activity differ in their mechanisms and chemical conditions. Therefore, each herb preparation contains different compounds with specific capacities to participate in those mechanisms.

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Disclosure The authors declare no conflict of interest.

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