

ANTIOXIDANT CHARACTERISTICS OF LATVIAN HERBAL TEA TYPES

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*The aim of the study was to characterise antioxidative properties and antiradical activity of the herbal tea collection in Latvia. High-pressure liquid chromatography and spectrophotometry methods were used to characterise antioxidant properties of herbal tea. Antiradical activity was determined spectrophotometrically. The antiradical scavenging activity was measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) reaction. The herbal tea antiradical scavenging activity was estimated as the broken down quantity of DPPH on 100 grams of the herbal tea. Individual polyphenols in the herbal tea were identified and determined by liquid chromatography. Antiradical scavenging activity of the herbal tea was found to be $104 \text{ mol } 100 \text{ g}^{-1} \text{ tea}$. The following polyphenols were identified chromatographically in herbal tea: gallic, caffeic, chlorogenic, ferulic acids, rutin, catechin, vanillin, and epicatechin. Altogether 12 different varieties of herbal tea samples were analyzed. The results showed that different types of herbal tea substantially differed in composition. Polyphenol content of herbal teas was in the range of 1 mg in 100 g of tea up to 8 g per 100 g of tea. In the case of virus-related disease, infections and weakness of the organism, it is recommended to use tea with high content of rutin, as in *Verbascum thapsiforme* Schr., *Alchemilla vulgaris* L., *Comarum palustre* L. herbal tea.*

Key words: *herbal tea, polyphenols, antiradical scavenging activity.*

INTRODUCTION

Herbal teas are widely used in traditional medicine. Antioxidant effect is one of the major descriptive characteristics of organic and medicinal activity.

In the body mitochondrial activity, during metabolism or as a result of environmental factors, such as pollution, radiation, smoking, pesticides, etc. exposure, leads to free radicals — atoms, molecules or ions with unpaired electrons — superoxide, hydrogen peroxide, singlet acid, hydroxyl radical. These are highly reactive, unstable and can lead to oxidative stress — a chain reaction that causes cell and tissue damage, contributing to aging and increased risk of developing various diseases, such as cancer, heart disease, cataracts, etc. (Sailaja *et al.*, 2011).

During oxidative stress, specific molecules actively react with proteins, lipids, and nucleic acid molecules, adding the missing electron, and thus creating other unstable molecules and inducing a chain reaction, such as via hydroxylated aromatic amino acids in protein molecules and oxidised-SH groups forming disulfide bridges — SS-bonds (Vīgants, 2008).

Unsaturated fatty acid-containing lipids with a large number of double bonds are readily oxidised. A variety of oxidation

products, such as peroxides, hydroperoxide, alkanols, aldehydes, carboxylic acids, etc., can inactivate the enzyme activity by binding to the active centres. Linoleic and arachidonic acid form malondialdehyde, as well as 4-hydroxynonenal, which is very toxic. Also cholesterol can be oxidised, resulting in total destruction of the cell membrane.

Nucleic acids participate in purine base guanine hydroxylation to form 8-hydroxy guanine (Vīgants, 2008).

Oxidative stress can reduce the body's antioxidant defense system and increase concentrations of oxygen and nitrogen in the active form (Vīgants, 2008).

Damage by free radicals can be reduced by antioxidant — molecules that react with them, eliminating the unpaired electron, or making the active radical less active, terminating the chain reaction.

The endogenous system in the body is composed of several antioxidant enzymes — superoxiddismutase, catalase, glutathionperoxidase, glutathionreductase etc.

Well-known exogenous (with dietary imbibed) antioxidants are vitamins C and E, lycopene, lutein, carotenoids, such as β -carotene, selenium, flavonoids, polyphenols, lignans,

coenzyme Q10, and others. Most of them are vegetable products (Sailaja *et al.*, 2011).

Polyphenols are chemical compounds with phenol rings. There is a big difference in the phenolic compounds regarding chemical structure, and they can be monomers or complex polymers.

Phenolic compounds of plants can be divided into two broad categories: 1) phenolic acids (oxometallic) and their derivatives, and 2) flavonoids (polyphenols). Phenolic acids and their derivatives, mainly esters, have more basic structures. Phenolic compounds constitute a wide group of representatives of caffeic acid, kumarin acid, vaniline, and gallic acid.

The flavonoid class of compounds has a more complex molecular structure, which is usually heterocyclic with attracted phenolic ring(s). Flavonoids include antocyanidines (water-soluble pigment, which are oxidised flavonoles), catechins, isoflavones and proanthocyanidin (Robarts *et al.*, 1999).

Polyphenols are the main antioxidant source from food. They are found in legumes, fruits and berries, and vegetables, red wine, chocolate, green tea, cereals and many herbal teas. Phenolic compounds constitute 50–70% of the weight of green tea, which is a key indicator of quality teas (Naghma and Hasan, 2007).

Plants consist of flavonoids, including catechins, proantocyanins, anthocyanins, flavones, flavonoles and their glycosides, which have antioxidant properties. The weight of these compounds can reach up to 30% of leaf dry matter (Naghma and Hasan, 2007).

The aim of the research was to describe the antioxidative characteristics of Latvian herbal teas by antiradical scavenging activity method and to determine the content of the main polyphenols.

MATERIALS AND METHODS

In this study, the antioxidative properties of Latvian herbal teas were estimated regarding polyphenol content and antiradical activity. Twelve varieties of herbal teas were analysed:

Origanum vulgaris L., *Hypericum perforatum* L., *Chelidonium majus* L., *Achillea millefolium* L., *Calluna vulgaris* L., *Artemisia vulgaris* L., *Plantago major* L., *Comarum palustre* L., *Alchemilla vulgaris* L., *Calendula officinalis* L., *Verbascum thapsiforme* Schr., *Rubus idaeus* L. (Rubine and Eniņa, 2004) (Table 1). All these plants are widely distributed in Latvia and are used every day in the form of herbal tea. They include a variety of polyphenols, which act as radical and nitrite neutralisers in the body, they may show antiviral effects (including HIV), and enzyme induction, such as in the cytochrome P-450 system and phase II conjugation systems. They are important in the human body, as they

Table 1

HERBAL TEA VARIETIES ANALYSED IN THE STUDY

No.	Tea sample	No.	Tea sample
1	Plantain (<i>Plantago major</i> L.)	7	Common origanum (<i>Origanum vulgaris</i> L.)
2	Marsh cinquefoil (<i>Comarum palustre</i> L.)	8	Common St. John's wort (<i>Hypericum perforatum</i> L.)
3	Lady's mantle (<i>Alchemilla vulgaris</i> L.)	9	Greater celandine (<i>Chelidonium majus</i> L.)
4	Calendula (<i>Calendula officinalis</i> L.)	10	Milfoil (<i>Achillea millefolium</i> L.)
5	Wool mullein (<i>Verbascum thapsiforme</i> Schr.)	11	Wild wormwood (<i>Artemisia vulgaris</i> L.)
6	Red raspberry (<i>Rubus idaeus</i> L.)	12	Heather (<i>Calluna vulgaris</i> L.)

aid to avoid polyunsaturated fatty acid autoxidation (Higdon and Frei, 2012).

The scavenging activity of tea samples for the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described (Meda *et al.*, 2005) with some modifications.

1 g of dry tea was extracted in 50 mL methanol. Extraction was carried out for 1 hour at 25 °C. The filtrate of the extract was used for further analysis. A volume (1.5 ml) of each tea extract solution was mixed with 3 ml DPPH solution in methanol (0.02 mg·ml⁻¹), with methanol serving as the blank sample. The mixtures were left for 15 min at room temperature and then the absorbance was measured at 517 nm.

As mentioned above, in the control analysis, methanol was used instead of tea extract. Based on the DPPH solution and sample solution concentration, light absorption was used to calculate the break down amount of DPPH (μmol per 100 g of tea), in relation to the control solution.

Chromatographic determination of individual polyphenols in tea samples was made by liquid chromatography. Eight polyphenols in teas were determined: rutin, gallic acid, epicatechin, catechin, ferulic acid, chlorogenic acid, vanillin and caffeic acid.

The analyses were carried out using a Shimadzu liquid chromatography LC-20AD, with a DAD (diode array detector: SPD M20A) using analytical C18 column chromatography. The eluting solvent was methanol (A, 20%), water (B, 78.4%) and acetic acid (1.6% C) in gradient mode: 17:50 minutes – 58.5% B concentration, C concentration of 1.2%; 35 minute – end of analysis.

The sample injection into the chromatograph, using an automatic sample injection system SIL – 20AC. Fluent flow rate 1.0 ml/min.

Determination of polyphenols was made by using multiple wavelengths: 253, 268, 278 and 298 nm. 253 nm wavelength was used to determine rutin, and 263 nm — gallic acid, 278 nm — catechin, caffeic acid and vanillin, and 298 nm — chlorogenic acid, epicatechin and ferulic acid.

RESULTS

A significantly higher concentration of rutin was observed in *Hypericum perforatum* and *Calluna vulgaris* tea (Fig. 1).

The highest concentration of gallic acid occurred in *Rubus idaeus* tea (Fig. 2). *Rubus idaeus* tea had a relatively higher concentration of catechin (Fig. 3).

Alchemilla vulgaris, *Calluna vulgaris* and *Hypericum perforatum* teas had higher concentrations of caffeic acid (Fig. 4).

Vanillin is known as a spice, and is used in a variety of foods. Vanillin is also found in tea. A relatively higher vanillin concentration was found in *Alchemilla vulgaris*, *Calendula officinalis* and *Artemisia vulgaris* tea (see Fig. 5).

A relatively higher concentration of chlorogenic acid was found in *Comarum palustre*, and *Achillea millefolium* tea (Fig. 6). High chlorogenic acid concentration was also found in *Alchemilla vulgaris* and *Hypericum perforatum* teas.

The epicatechin concentration in teas was relatively low, and in the range from $0.07 \text{ mg} \cdot 100^{-1} \text{ g}$ to $3.6 \text{ mg} \cdot 100^{-1} \text{ g}$ of tea. Higher epicatechin concentration was found in *Alchemilla vulgaris* and *Calendula officinalis* teas ($2.6 \text{ mg} \cdot 100^{-1} \text{ g}$ and $3.6 \text{ mg} \cdot 100^{-1} \text{ g}$).

Ferulic acid had relatively high concentrations in *Origanum vulgaris* tea (Fig. 7). Higher concentration of ferulic acid was also observed in *Alchemilla vulgaris*, *Verbascum thapsiforme* Schr. and *Achillea millefolium* tea.

In order to assess the total anti-oxidant tea value, they were assessed by total content of polyphenols in tea, as well as by

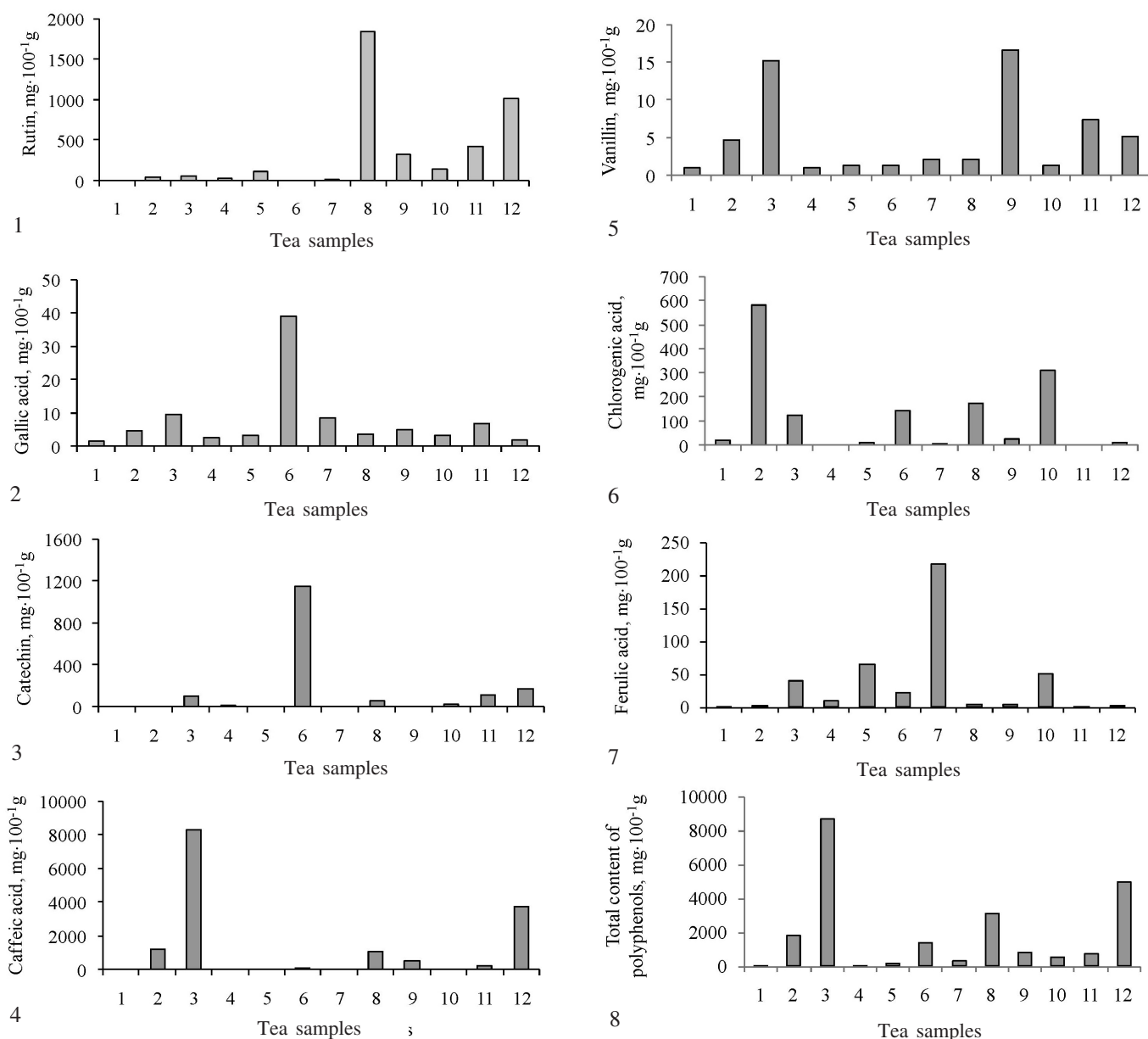


Fig. 1. Concentration of polyphenols in tea samples (numbers of tea samples identified in Table 1): 1) rutin; 2) gallic acid; 3) catechin; 4) caffeic acid; 5) vanillin; 6) chlorogenic acid; 7) ferulic acid; 8) total polyphenols.

certain antiradical activity (Fig. 8). The highest polyphenol concentration was found in *Alchemilla vulgaris* and *Calluna vulgaris* tea, and somewhat lower in *Comarum palustre*, *Rubus idaeus* and *Hypericum perforatum* tea.

The antiradical scavenging activity in the analysed tea ranged from 6 to 104 $\mu\text{mol}\cdot 100^{-1}$ g. The highest activity was in *Calluna vulgaris* tea (104 μmol break down DPPH reagent per 100 g of tea). In other teas, the antiradical activity was in the range of 21 to 38 $\mu\text{mol}\cdot 100^{-1}$ g.

DISCUSSION

It is known that rutin provides a more complete intake of vitamin C in the body. Therefore, it is used as a food supplement in combination with other substances. Rutin also has good anti-oxidant properties (Guardia *et al.*, 2001).

Catechins have antibacterial properties and it is able to destroy viruses and bacteria. Similarly, it is a good natural antioxidant (Higdon, *et al.*, 2003).

Caffeic acid has a spasmolytic and mitigating decreasing effect. Caffeic acid stimulates bile secretion. Chlorogenic acid has anti-bacterial, anti-inflammatory and antiviral properties. It also has antioxidant properties. Epicatechin is a powerful antioxidant. This is one of the rare flavonoids that in biologically active form is capable of reaching the brain (Mou-Tuan Huang *et al.*, 1991).

Ferulic acid is widely used in cosmetics as an antioxidant and as a protective measure against the adverse effects of UV rays. Ferulic acid inhibits skin aging (Graf, 1992).

High antiradical activity assessed by total polyphenols content was found only in heather tea. However, teas have many other polyphenols, which were not analyzed. Also, anti-oxidant activity can be attributed not only to polyphenols, but also to specific vitamins, amino acids, etc.

To conclude, our results demonstrate that increased antioxidant concentrations were found in *Alchemilla vulgaris*, *Calluna vulgaris*, *Comarum palustre*, *Rubus idaeus* and *Hypericum perforatum* teas.

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LATVIJĀ VĀKTO ZĀĻU TĒJU ANTIOKSIDATĪVO ĪPAŠĪBU RAKSTUROJUMS

Darbā ir pētītas dažādu Latvijā vāktu zāļu tēju antioksidatīvās īpašības, izmantojot augstspiediena šķidrums hromatogrāfijas un spektrofotometrijas metodes. Tika analizētas 12 tējas: parastā raudene (*Origanum vulgare* L.), divšķautņu asinszāle (*Hypericum perforatum* L.), lielā strutene (*Chelidonium majus* L.), parastais pelašķis (*Achillea millefolium* L.), sila virsis (*Calluna vulgaris* L.), parastā vibotne (*Artemisia vulgaris* L.), lielā ceļteka (*Plantago major* L.), purva vārnkāja (*Comarum palustre* L.), parastais rasaskrēsliņš (*Alchemilla vulgaris* L.), ārstniecības kļīņģerīte (*Calendula officinalis* L.), deviņvīru spēks (*Verbascum thapsiforme* Schr.), meža avene (*Rubus idaeus* L.). Tēju antiradikālā aktivitāte tika noteikta spektrofotometriski, izmantojot DPPH (2,2-difenil-1-pikrilhidrazils) reaģentu. Iegūtie antiradikālās aktivitātes rezultāti izteikti uz noārdītā DPPH reaģenta daudzumu 100 g tējas. Atsevišķi fenolu savienojumi — galluskābe, kafijskābe, hlorogēnskābe, ferulskābe, rutīns, katehīns, vanilīns un epikatehīns — identificēti, izmantojot šķidrums hromatogrāfiju. No iegūtajiem rezultātiem var secināt, ka palielināts antioksidantu saturs ir rasaskrēsliņu, viršu ziedu, asinszāļu, aveņu tējām. Dažādu infekciju, vīrusu saslimšanu un organisma vājuma gadījumā ieteicams lietot tējas, kas satur rutīnu, hlorogēnskābi un katehīnus. Tējas ar palielinātu ferulskābes saturu ieteicams lietot kosmētikā kā līdzekli pret ādas novecošanos un UV staru kaitīgo iedarbību.

In cases of virus diseases, infections and weakness it is recommended to use tea to increase rutin, chlorogenic acid and catechin intake.

Tea with increased ferulic acid content can be used to prevent against skin aging and UV destructive exposure.

The estimated total antioxidant content is not the only parameter that should be used in tea evaluation, and other polyphenol and antioxidant concentrations should be considered.

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