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*Article*

# Use of Liquid Chromatography with Electrochemical Detection for the Determination of Antioxidants in Less Common Fruits

**Zbynek Gazdik [1, 2, 3], Vojtech Reznicek [1], Vojtech Adam [2, 4], Ondrej Zitka [2], Tunde Jurikova [5], Boris Krska [6], Jan Matuskovic [7], Jan Plsek [1], Jan Saloun [8], Ales Horna [9] and Rene Kizek [2, *]**

[1] Department of Breeding and Propagation of Horticultural Plants, Faculty of Horticulture, Mendel University of Agriculture and Forestry, Valtická 337, CZ-691 44 Lednice, Czech Republic; E-mails: xgazdik@node.mendelu.cz (Z. G.), reznicek@node.mendelu.cz (V. R.)

[2] Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Zemedelska 1, CZ-613 00 Brno, Czech Republic; E-mail: ilabo@seznam.cz (V. A.)

[3] Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, , Faculty of Agronomy, Mendel University of Agriculture and Forestry, Zemedelska 1, CZ-613 00 Brno, Czech Republic

[4] Department of Animal Nutrition and Forage Production, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Zemedelska 1, CZ-613 00 Brno, Czech Republic

[5] Institute of Natural and Informatics' Sciences, Faculty of Central European Studies, Constantine the Philosopher University in Nitra, Nabrezie mladeze 91, SK-949 76 Nitra, Slovak Republic;

[6] Department of Fruit Growing, Faculty of Horticulture, Mendel University of Agriculture and Forestry, Valtická 337, CZ-691 44 Lednice, Czech Republic,

[7] Department of Fruit Production, Viticulture, and Enology, Horticulture and Landscape Engineering Faculty, University of Agriculture in Nitra, Trieda A. Hlinku 2, SK-949 76 Nitra, Slovak Republic

[8] Department of Applied Pharmacy, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackeho 1 - 3, CZ-612 42 Brno, Czech Republic

[9] Tomas Bata University, T.G. Masaryka 275, CZ-762 72 Zlin, Czech Republic

* Author to whom correspondence should be addressed; E-mail: kizek@sci.muni.cz; phone: +420-5-4513-3350; fax: +420-5-4521-2044.

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**Abstract:** Neurodegenerative disorders (NDD) have become the common global health burden over the last several decades. According to World Health Organization (WHO), a staggering 30 million people will be affected by Alzheimer's disease in Europe and the USA by 2050. Effective therapies in this complex field considering the multitude of symptoms associated with NDD indications, have not been found yet. Based on the results of NDD related studies, prevention appears to be the promise alternative. Antioxidative and anti-inflammatory properties are hypothesized for natural phenolics, a group of plant secondary products that may positively impact neurodegenerative diseases. In these studies, phenolic-rich extracts from less common fruit species: Blue honeysuckle (*Lonicera edulis*, Turcz. ex. Freyn), Saskatoon berry (*Amelanchier alnifolia* Nutt.), and Chinese hawthorn (*Crateagus pinnatifida* Bunge) were obtained and analyzed to detect neuroprotective substances content and establish a potential therapeutic value. High performance liquid chromatography with electrochemical detection was optimized and further applied on analysis of the extracts of less common fruit species. It was observed that Chinese hawthorn and Blue honeysuckle extracts are potent source of neuroprotective phenolic antioxidants. In accordance the results, it appears that the fruit or formulated products may have the potential for the prevention of neurodegenerative diseases.

**Keywords:** Phenolic compound; Electrochemical detection; Less common fruit species, liquid Chromatography; Neuroprotective food.

## Introduction

Recently, much interest has been generated in drug discovery and development from progress in the global propagation of neurodegenerative disorders (NDD). The number of people with neurological sequelae of nutritional disorders and neuropathies (352 million) and neurological sequelae secondary to injuries (170 million) also add substantially to the above burden. This trend, recorded over the several past decades [1], have led to acceleration in the search for therapeutic treatments, because none of the conventional strategies, nor any new pharmaceuticals (HST, CEI) have changed the complex problem with growing numbers of NDD-medicated patients.

The nervous system, including the brain, spinal cord, and peripheral nerves, is rich in both unsaturated fats (which are prone to oxidation) and iron [2]. The high lipid content of nervous tissue, coupled with its high metabolic (aerobic) activity, makes it particularly susceptible to oxidative damage [3]. The high level of brain iron can lead to oxidative stress via the iron-catalyzed reduce hydrogen peroxide to the highly reactive hydroxyl radical [4, 5]. It has been shown that reactive oxygen species (ROS) are the important mediators of cell signalling events such as inflammatory reactions (superoxides) and the maintenance of vascular tone (nitric oxide) [6-8]. Excessive production of ROS, outstripping endogenous antioxidant defence mechanisms, is referred to as oxidative stress [9]. Overproduction of ROS has been associated with the pathogenesis of variety of neurodegenerative disorders such as cognitive failures in Alzheimer's disease (AD) [10-15], retinal degeneration, ischemic dementia [6, 7, 9], Parkinson's disease (PD) [16], and amyotrophic lateral sclerosis (ALS,

"Lou Gehrig's disease") [17]. Although significant relationships between oxidative damage and neurodegenerative processes have been identified or are suspected, important pathways of relationship between β-amyloid production and exposure of hippocampal neurones to ROS attack has not been clarified yet [18-21], Reduction of oxidative stress and inflammation show the way of new therapeutic strategy for inhibiting the pathogenetical cascade associated with a multitude of symptoms such as brain atrophy, cerebrovascular hemodynamics, cognitive decline, inflammation-induced apoptosis, lipid metabolism dyshomeostasis, and amyloidal deposition and others. The situation has shown the necessity of precaution of the diseases. The importance of neuroprotective diet has been accepted by the public recently. Rather, current therapeutic strategies under investigation for AD include inhibitors of Ap production, compounds that prevent its oligomerization and fibrillization, anti-inflammatory drugs, inhibitors of cholesterol synthesis, antioxidants, neurorestorative factors and vaccines [22,23].

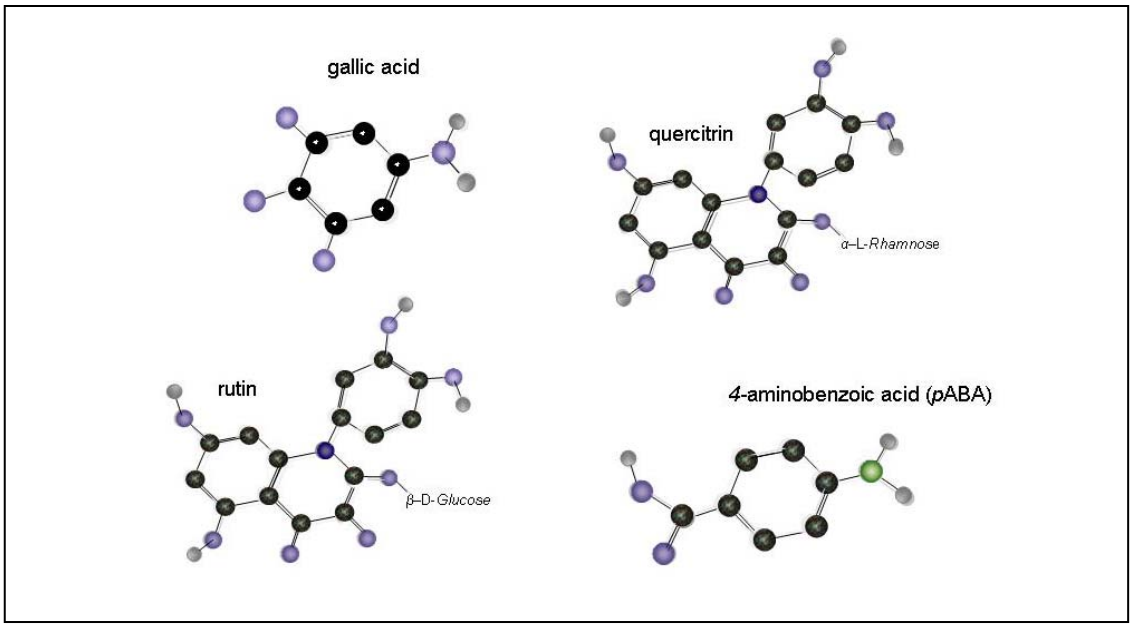
Free radicals are reactive organic or inorganic molecules with one or more unpaired electrons, commonly formed in the body as a result of metabolic processes which are normally eliminated by the antioxidant line of the body's defence systems [24]. Free radical scavengers are compounds that are capable of donating electrons or hydrogen atoms to inhibit a free radical reaction [2]. A number of *in vitro* models [25, 26], followed by *in vivo* studies [27-29], have shown that antioxidants, both endogenous and dietary, can protect neurocerebral system against oxidative stress [30, 31]. In addition, the research suggests that the phenolic compounds found mainly in fruits such as berries, may exert protection against NDD-related deficits in cognitive and motor function [20, 22, 23, 26].

Considerable epidemiological evidence suggests that regular consumption of fruit and vegetables decreases the risk of developing several neurodegenerative disorders. It has also been shown that the antioxidant activity varies with the types of phenolic compounds present in the fruit. Certain types of phenolic compounds show greater antioxidant activity than others [32, 33]. Less common fruit species or alternative fruit crops are characterized by resistance to both abiotic and biotic factors of an environment and valuable biochemical composition of fruits [34-38]. The lack of data in food composition leads to difficulties in quantifying the daily polyphenol intake. Methods to quantify the total polyphenol content of plant food products have been recently proposed [39-53]. It is extremely important to develop effective method to obtain novel opportunities for NDD prevention through the screening of every single target from a complex field of chemical diversity of plant substances. High performance liquid chromatographic analysis are most commonly employed to identify antioxidants in beverage samples using UV detection and to quantify them [54-57]. Significantly lower detection limits have been achieved by electrochemical detection [40, 58-61].

Gallic acid (3,4,5-trihydroxybenzoic acid, (GA, Figure 1) is a naturally occurring antioxidant that has shown a multitude of biological activities [62, 63], including neuroprotective ones. Analysis of cell apoptosis, intracellular GSH levels, production of ROS and the influx of $Ca^{2+}$ under oxidative stress had confirmed protective effects of gallic acid and it's derivatives in cell systems [64]. Over the several past decades, *p*-aminobenzoic acid (*p*ABA, Figure 1) has been frequently discussed as a compound that is an essential nutrient for microorganisms and some animals, but has not been shown to be essential for people. Recently (*p*ABA) has been well established a potent neutralizer of singlet molecular oxygen, a potent free radical which is a common by-product of normal metabolism [65-68]. More than 4,000 different polycyclic hydroxyphenols have been described to occur in food of plant origin [69]. Quercitrin (quercetin-3-*O*-a-L-rhamnoside, Figure 1) is the glycoside form derived from

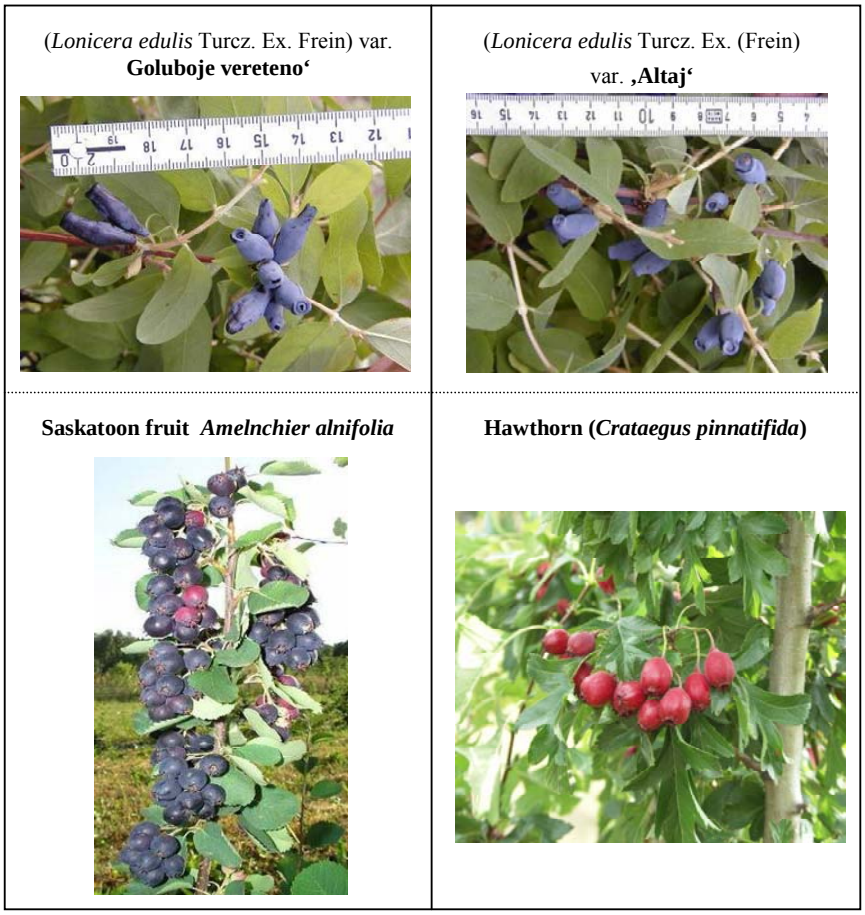
the pentahydroxyflavone quercetin that has been extensively studied over the past 30 years [70]. Quercetin is the flavonol that seems to be the most powerful flavonoid for protecting the body against reactive oxygen species [17,71,72]. Quercetin (Figure 1), through its catechol-*O*-methyltransferase (COMT) and monoamine oxidase (MAO) enzymes inhibiting properties, might potentiate the anticatabolic effect of L-dopa plus carbidopa treatment. Thus, quercetin can serve as an effective adjunct to L-dopa therapy in Parkinson disease [73]. Because plants contain many different classes and types of antioxidants, knowledge of total content of components to scavenge free radicals, would be useful for epidemiologic purpose [21,23,74-77]. In this regard, there is an urgent need to improve analytical methods to quantify various food flavonoids and their metabolites in biological fluids [78]. Liquid chromatography or electrophoresis with various types of detectors [40-42,59,79], or stationary electrochemical methods [40,58,60,61] are used for their determination.

**Figure 1.** Structure of rutin, quercitrin, gallic acid and 4-aminobenzoic acid.




This study was focused on the determination of neuroprotective plant phenols (rutin, quercitrin, gallic acid and 4-aminobenzoic acid, Figure 1) in fruits of three less examined plant species: Blue honeysuckle (*Lonicera edulis*, Turcz. ex. Freyn), Saskatoon berry (*Amelanchier alnifolia* Nutt.) and Chinese hawthorn (*Crateagus pinnatifida* Bunge) (Figure 2). The target analytes have been reported to possess a multitude of biological activities, including antiallergic, anti-inflammatory, antiviral, antiproliferative, and anticarcinogenic activities with a beneficial effects on mammalian metabolism [80-82], the antioxidant and anti-inflammatory activities in neurocerebral system contributes to their bioavailability and the dosage [78]. Keeping out the sequence of conditions involving the intestinal transport as well as structurally-specific mechanisms of target's absorption [83] and their mobility through the bloodstream, the ability to penetrate the hematoencephalic barrier plays the key role for neuroprotective effect [63,64]. Gallic acid, some *p*ABA derivates, rutin and quercitrin meet these requirements.

**Figure 2.** Analyzed fruit samples.




(A) (*Lonicera edulis* Turcz. Ex. Frein) var. "Goluboje vereteno". (B) (*Lonicera edulis* Turcz. Ex. Frein) var. "Altaj". (C) Saskatoon berry (*Amelanchier alnifolia* Nutt). (D) Hawthorn (*Crataegus pinnatifida*).

## Results and Discussion

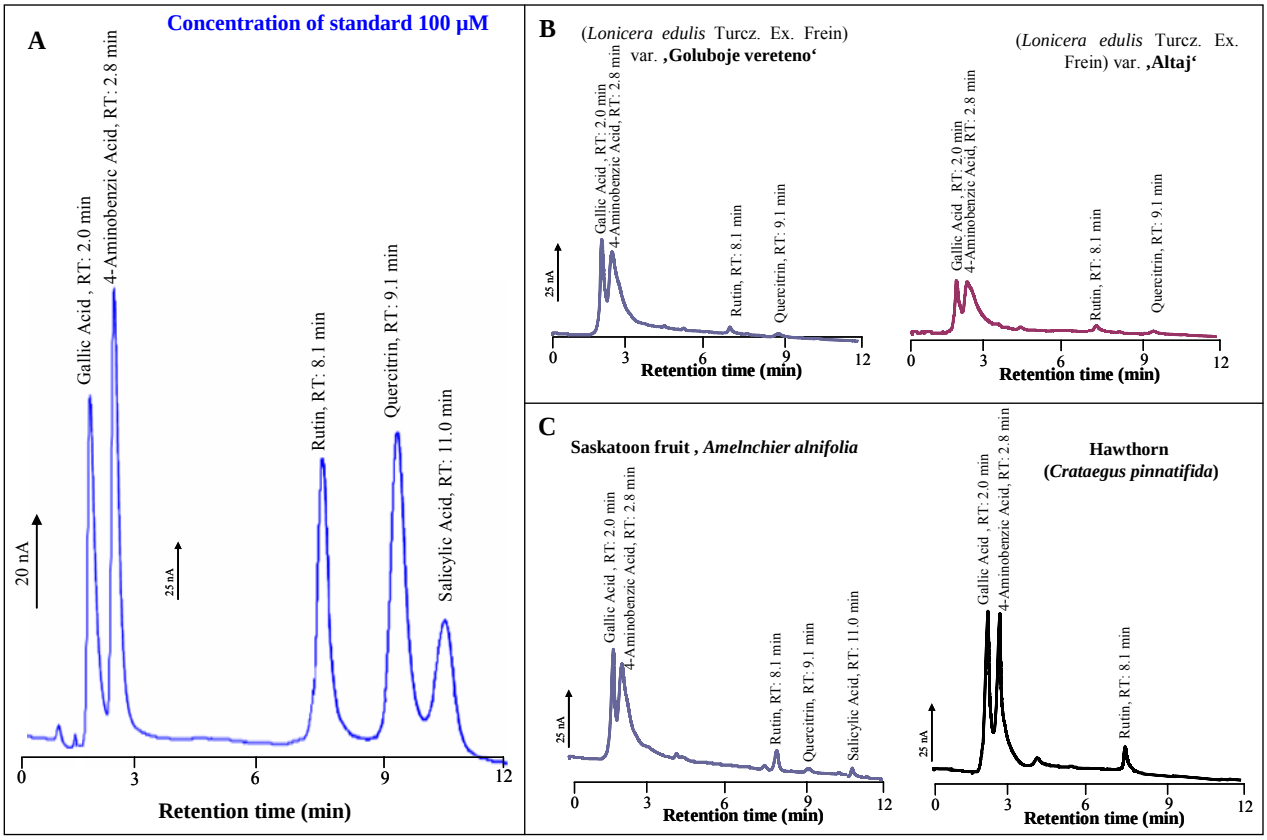
Antioxidants are known to take part in reducing reactions. Substances without the electrochemical activity are antioxidant activity less, on the other hand, the activity is expected from the sort of substances disposing low levels in half-undulate signal. Because there exists significant relation in between the substance antioxidant activity and the ability to provide electrochemical signal [32,78,84,85], the selective and sensitive electrochemical detection coupled with high performance liquid chromatography (HPLC-ED) presents an optimal analytical tool for their detection.

### *Identification and quantification of phenolic compounds in fruits by using HPLC-ED*

Primarily we focused on the study of basic electrochemical behaviour of compounds of interest (rutin, quercitrin, gallic acid and *4*-aminobenzoic acid – *p*ABA). Through the hydrodynamic voltammogram we characterized the oxidative signals and optimized frequency a pH of mobile phase for sensitive determination of phenolic antioxidants. We applied potentials from 100 mV to 950 mV (per 50 mV) on working glassy carbon electrode. We observed oxidative signals associated with

electrochemical conversion of hydroxyl groups of the target molecules [40,86]. The signals appeared already at 200 mV. To get sufficient sensitivity we choose 950 mV for the following experiments (Figure 3A).

**Figure 3.** (A) Typical HPLC-ED chromatogram of rutin, quercitrin, gallic acid and 4-aminobenzoic acid, and salicylic acid. (B, C) Typical HPLC-ED chromatograms of plant extracts.




*Content of phenolic compounds in less common fruits*

Separation of target molecules was carried out on a Restek Allure C18 column with reverse-phase elution. The solution of methanol - 60 % and (0.065 M) acetic acid - 40 % (*v*/*v*) was used as mobile phase. The conditions are given: column and detector temperature 55°C, flow rate of the mobile phase 1 mL/min, working electrode potential 950 mV. Under these conditions we analysed extracts from *Crataegus pinnatifida, Lonicera edulis and Amelanchier alnifolia* (Figure 3B, C). In the extract of Chinese Hawthorn (*Crataegus Sp.*) we determined that content of gallic acid was almost three times higher, compared to those of *p*ABA and rutin (Table 1), whereas quercitrin was not detected. *In vivo* quercitrin can be more important antioxidant and neuroprotective agent than quercetin because of its high bioavailability in the digestive tract [26].

**Table 1.** Levels of neuroprotective antioxidants*.

| **Fruit** | **Compound** | **Level of phenols** (mg/kg) |
|---|---|---|
| (*Crataegus pinnatifida* BUNGE) | gallic acid | 2,640 |
| | *4*-aminobenzoic acid | 970 |
| | rutin | 910 |
| | quercitrin | n.d. |
| (*Lonicera edulis,* Turcz. ex. Freyn) | gallic acid | 600 |
| | *4*-aminobenzoic acid | 170 |
| | rutin | 240 |
| | quercitrin | 220 |
| (*Amelanchier canadensis* L.) | gallic acid | 240 |
| | *4*-aminobenzoic acid | 20 |
| | rutin | 230 |
| | quercitrin | 230 |

*Twenty samples obtained from each fruit were analysed in triplicate; n.d.: not detected

A typical HPLC-ED chromatogram of the extract from *Crataegus pinnatifida* is shown in Figure 3C. Higher content of quercitrin was detected in *Lonicera edulis* and *Amelanchier alnifolia* fruits in tens of mg per 100 g of fresh weight. Quercitrin has been found in a wide range of berries such as whortleberry (158 mg/kg fresh weight), lingonberry (74 and 146 mg/kg), cranberry (83 and 121 mg/kg), chokeberry (89 mg/kg), sweet rowan (85 mg/kg), rowanberry (63 mg/kg), sea buckthorn berry (62 mg/kg) and crowberry (53 and 56 mg/kg) [54]. In addition, the highest content in *Lonicera edulis* and *Amelanchier alnifolia* was of gallic acid (Table 1). According to Pokorna and Matuskovic the level of the aglycone rutin obtained at several genotypes of *Lonicera* Sp. does not surpass a level of 300 mg/kg [87]. *para*-Aminobenzoic acid helps the body generate important nutrients such as vitamin K, folic acid and thiamine through stimulation the intestinal bacteria, enabling them to produce folic acid, assists in the formation of red blood cells which carry oxygen to sensitive brain tissue and to all parts of the body. The typical therapeutic dosage of *p*ABA is 300 to 400 mg daily [88]. From the point of view of total phenolic content, the fruits of *Crataegus pinnatifida* had an almost four times higher content of neuroprotective phenolics, compared to the other plant species we investigated. This can account for the considerable antioxidant activity of this plant species.

## Experimental

### *Chemicals*

Flavonoid standards for HPLC: gallic acid and 4-aminobenzoic acid (Sigma Aldrich Corp., USA), rutin trihydrate and quercitrin dihydrate (Roth GmbH, Karlstruhe, Germany) were used. Methanol (>99.9%; *v*/*v*), acetic acid, formic acid for HPLC and all other chemicals used were purchased from Sigma Aldrich.

*High performance liquid chromatography with electrochemical detection (HPLC-ED)*

The instrument used consisted of a solvent delivery pump (ESA Inc., Model 582, Chelmsford, MA, USA), a guard cell (ESA Inc., Model 5020), a Metachem Restek Allure reverse-phase chromatographic column (150.0 × 4.6 mm, 5 μm particle size, Canada), and an electrochemical detector. The electrochemical detector includes one low volume flow-through analytical cells (ESA Inc., Model 5040), which is consisted of glassy carbon working electrode, hydrogen-palladium electrode as reference electrode and auxiliary carbon electrode, and Coulochem III as a control module. The samples (5 μL) were injected using an autosampler (ESA Inc., Model 540 Microtiter HPLC). The data obtained were treated by CSW 32 software (Version 1.2.4, Data Apex, Czech Republic). Guard cell potential was set as 0 V. The glassy carbon electrode was polished mechanically with 0.1 μm alumina (ESA Inc.) and sonicated at room temperature for 5 min using a Sonorex Digital 10 P Sonicator (Bandelin, Berlin, Germany) at 40 W.

*Biological material*

The following fruit samples was analysed for a neuroprotective content evaluation: i) one genotype of Blue Honeysuckle (*Lonicera edulis*, Turcz. Ex. Freyn). The fruit was harvested in mid-May and stored at -18 ºC for seven months. ii) One variety of Saskatoon berry (*Amelanchier alnifolia* Nutt.). The fruit was harvested during July; after the harvest the fruit was stored at -18 ºC, for five months. iii) One variety of (*Crateagus pinnatifida* Bunge). The fruit was harvested in early October and stored at -18 ºC, for three months.

(*Lonicera edulis* Turcz. Ex. Frein) var. "Goluboje vereteno". This variety as a seedling obtained from free pollination has its origin in the "Sady Sibiri" orchards. The roundish weak branched shrubs are round shaped and shoot growth is fast. The level of frost resistance is high. The long berries with soft pulp are sweet, sour and lightly bitter. The yield is 2.5 kg.

(*Lonicera edulis* Turcz. Ex. Frein) var. "Altaj" is a hybrid variety obtained from breeding of *Lonicera kamtschatica* × *Lonicera turczaninowii.* This compact shrub exhibits dynamical growth and a high level of frost resistance. Hedge berries are cylindrically shaped, dark blue, with 1.05 g mean weight values. The pulp is juicy, sweet and sour, with an intense aroma. The harvest period comes very early; the shrub provides yield about 2.8 kg.

Saskatoon berry (*Amelnchier alnifolia*). *Amelanchier alnifolia* Nutt. is a tree or shrub providing good yields of purple, dark blue or black fruit so-called also Juneberries. There can be a multitude of levels in the fruit size, ranging on average from 0.6-1.6 mm. The thin-peeled fruit is very sweet and juicy, similar to commonly grown blueberries. Generally, *Amelanchier* fruit is known to be used by healers in folk medicine.

Hawthorn (*Crataegus pinnatifida*). Chinese Hawthorn belongs to *Rosacea* family, that suggests a high content of phenolic plants constituents. Fruit size is about 40 mm, drupes are red with 4-5 seeds in the yellow pulp. *Crataegus* fruit use to be very popular in folk medicine and it is frequently used nowadays by healers in eastern parts of Asia.

*Sample preparation*

The fruits of the analysed species were thawed at room temperature. Weighed fruits (approximately 6 g) were transferred to a mortar, and liquid nitrogen was added. The frozen samples were ground for 5 min. Then, 80% methanol (10 mL) was added to the mortar, and the sample was ground for 10 min. The homogenate was transferred to a test-tube and homogenised by shaking on a Vortex–2 Genie (Scientific Industries, New York, USA) at 4 °C for 30 min. The homogenate was centrifuged (14,000 g) for 30 min at 4 °C using a Universal 32 R centrifuge (Hettich-Zentrifugen GmbH, Tuttlingen, Germany). Before the analysis the supernatant was filtered through a membrane filter (0.45 μm Nylon filter disk, Millipore, Billerica, Mass., USA). Filtrate (150 μL) was diluted 1:400-1,080 with 80% methanol.

*Descriptive statistics*

Data were processed using Microsoft Excel® (USA). Results are expressed as mean ± standard deviation (S.D.), unless stated otherwise.

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## References and Notes

1. WHO. *The World Health Report 2002*. World Health Organization: Geneva, Switzerland, **2002**.
2. Halliwell, B. Reactive Oxygen Species and the Central-Nervous-System. *J. Neurochem.* **1992**, *59*, 1609-1623.
3. Dawson, V.L.; Dawson, T.M. Nitric oxide neurotoxicity. *J. Chem. Neuroanat.* **1996**, *10*, 179-190.
4. Aisen, P.S.; Davis, K.L. The search for disease-modifying treatment for Alzheimer's disease. *Neurology* **1997**, *48*, S35-S41.
5. Gerlach, M.; Benshachar, D.; Riederer, P.; Youdim, M.B.H. Altered Brain Metabolism of Iron as a Cause of Neurodegenerative Diseases. *J. Neurochem.* **1994**, *63*, 793-807.
6. Ashok, B.T.; Ali, R. The aging paradox: free radical theory of aging. *Exp. Gerontol.* **1999**, *34*, 293-303.
7. Hecht, S.S. Tobacco smoke carcinogens and lung cancer. *J. Natl. Cancer Inst.* **1999**, *91*, 1194-1210.
8. Rosenfeld, M.E. Inflammation, lipids, and free radicals: Lessons learned from the atherogenic process. *Sem. Reprod. Endocrin.* **1998**, *16*, 249-261.
9. Hayes, J.D.; McLellan, L.I. Glutathione and glutathione-dependent enzymes represent a Co-ordinately regulated defence against oxidative stress. *Free Radic. Res.* **1999**, *31*, 273-300.
10. Clausen, J. Demential Syndromes and the Lipid-Metabolism. *Acta Neurol. Scand.* **1984**, *70*, 345-355.

11. Harman, D. Aging - Prospects for Further Increases in the Functional Life-Span. *Age* **1994**, *17*, 119-146.
12. Hensley, K.; Butterfield, D.A.; Hall, N.; Cole, P.; Subramaniam, R.; Mark, R.; Mattson, M.P.; Markesbery, W.R.; Harris, M.E.; Aksenov, M.; Aksenova, M.; Wu, J.F.; Carney, J.M. Reactive oxygen species as causal agents in the neurotoxicity of the Alzheimer's disease-associated amyloid beta peptide. In *Pharmacological Intervention in Aging and Age-Associated Disorders*. New York Academy of Sciences: New York, USA, 1996; pp. 120-134.
13. Markesbery, W.R.; Carney, J.M. Oxidative alterations in Alzheimer's disease. *Brain Pathol.* **1999**, *9*, 133-146.
14. Smith, M.A.; Perry, G. Free radical damage, iron, and Alzheimer's disease. *J. Neurol. Sci.* **1995**, *134*, 92-94.
15. Wang, C.N.; Chi, C.W.; Lin, Y.L.; Chen, C.F.; Shiao, Y.J. The neuroprotective effects of phytoestrogens on amyloid beta protein-induced toxicity are mediated by abrogating the activation of caspase cascade in rat cortical neurons. *J. Biol. Chem.* **2001**, *276*, 5287-5295.
16. Ebadi, M.; Srinivasan, S.K.; Baxi, M.D. Oxidative stress and antioxidant therapy in Parkinson's disease. *Prog. Neurobiol.* **1996**, *48*, 1-19.
17. Olanow, C.W.; Arendash, G.W. Metals and Free-Radicals in Neurodegeneration. *Curr. Opin. Neurol.* **1994**, *7*, 548-558.
18. Cacabelos, R. Influence of pharmacogenetic factors on Alzheimer's disease therapeutics. *Neurodegener. Dis.* **2008**, *5*, 176-178.
19. Muthuswamy, A.; Tangpong, J.; Keller, J.N.; Markesbery, W.R.; Kiningham, K.K.; Murphy, M.P.; Flood, D.G.; St Clair, D.K. Beta-amyloid mediated nitration of MnSOD: Implication for oxidative stress in a APP NLh/NLh X PS-1P264L/PS-1P264L double knock-in mouse model of Alzheimer's disease. *Free Radic. Biol. Med.* **2005**, *39*, S62-S62.
20. Oliver, C.N.; Starkereed, P.E.; Stadtman, E.R.; Liu, G.J.; Carney, J.M.; Floyd, R.A. Oxidative Damage to Brain Proteins, Loss of Glutamine-Synthetase Activity, and Production of Free-Radicals During Ischemia Reperfusion-Induced Injury to Gerbil Brain. *Proc. Natl. Acad. Sci. U. S. A.* **1990**, *87*, 5144-5147.
21. Sakamoto, A.; Ohnishi, S.T.; Ohnishi, T.; Ogawa, R. Protective Effect of a New Antioxidant on the Rat-Brain Exposed to Ischemia-Reperfusion Injury - Inhibition of Free-Radical Formation and Lipid-Peroxidation. *Free Radic. Biol. Med.* **1991**, *11*, 385-391.
22. Emilien, G.; Maloteaux, J.M.; Beyreuther, K.; Masters, C.L. Alzheimer disease - Mouse models pave the way for therapeutic opportunities. *Arch. Neurol.* **2000**, *57*, 176-181.
23. Selkoe, D.J. Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature*. **1999**, *399*, A23-A31.
24. Noguchi, N.; Watanabe, A.; Shi, H.L. Diverse functions of antioxidants. *Free Radic. Res.* **2000**, *33*, 809-817.
25. Kerry, N.L.; Abbey, M. Red wine and fractionated phenolic compounds prepared from red wine inhibit low density lipoprotein oxidation in vitro. *Atherosclerosis* **1997**, *135*, 93-102.
26. Wagner, C.; Fachinetto, R.; Corte, C.L.D.; Brito, V.B.; Severo, D.; Dias, G.; Morel, A.F.; Nogueira, C.W.; Rocha, J.B.T. Quercitrin, a glycoside form of quercetin, prevents lipid peroxidation in vitro. *Brain Res.* **2006**, *1107*, 192-198.

27. Copp, R.P.; Wisniewski, T.; Hentati, F.; Larnaout, A.; Ben Hamida, M.; Kayden, H.J. Localization of alpha-tocopherol transfer protein in the brains of patients with ataxia with vitamin E deficiency and other oxidative stress related neurodegenerative disorders. *Brain Res.* **1999**, *822*, 80-87.
28. Tagami, M.; Yamagata, K.; Ikeda, K.; Nara, Y.; Fujino, H.; Kubota, A.; Numano, F.; Yamori, Y. Vitamin E prevents apoptosis in cortical neurons during hypoxia and oxygen reperfusion. *Lab. Invest.* **1998**, *78*, 1415-1429.
29. Yu, Z.F.; Bruce-Keller, A.J.; Goodman, Y.; Mattson, M.P. Uric acid protects neurons against excitotoxic and metabolic insults in cell culture, and against focal ischemic brain injury in vivo. *J. Neurosci. Res.* **1998**, *53*, 613-625.
30. Finch, C.E.; Cohen, D.M. Aging, metabolism, and Alzheimer disease: Review and hypotheses. *Exp. Neurol.* **1997**, *143*, 82-102.
31. Jama, J.W.; Launer, L.J.; Witteman, J.C.M.; denBreeijen, J.H.; Breteler, M.M.B.; Grobbee, D.E.; Hofman, A. Dietary antioxidants and cognitive function in a population-based sample of older persons - The Rotterdam study. *Am. J. Epidemiol.* **1996**, *144*, 275-280.
32. RiceEvans, C.A.; Miller, N.J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.* **1996**, *20*, 933-956.
33. Rosch, D.; Bergmann, M.; Knorr, D.; Kroh, L.W. Structure-antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice. *J. Agric. Food Chem.* **2003**, *51*, 4233-4239.
34. Sapers, G.M.; Jones, S.B.; Maher, G.T. Factors Affecting the Recovery of Juice and Anthocyanin from Cranberries. *J. Am. Soc. Hortic. Sci.* **1983**, *108*, 246-249.
35. Zatylny, A.M.; Ziehl, W.D.; St-Pierre, R.G. Physicochemical properties of fruit of chokecherry (Prunus virginiana L.), highbush cranberry (Viburnum trilobum Marsh.), and black currant (Ribes nigrum L.) cultivars grown in Saskatchewan. *Can. J. Plant Sci.* **2005**, *85*, 425-429.
36. Zatylny, A.M.; Ziehl, W.D.; St-Pierre, R.G. Physicochemical properties of fruit of 16 saskatoon (Amelanchier alnifolia Nutt.) cultivars. *Can. J. Plant Sci.* **2005**, *85*, 933-938.
37. Valentova, K.; Sersen, F.; Ulrichova, J. Radical scavenging and anti-lipoperoxidative activities of Smallanthus sonchifolius leaf extracts. *J. Agric. Food Chem.* **2005**, *53*, 5577-5582.
38. Psotova, J.; Kolar, M.; Sousek, J.; Svagera, Z.; Vicar, J.; Ulrichova, J. Biological activities of Prunella vulgaris extract. *Phytother. Res.* **2003**, *17*, 1082-1087.
39. George, S.; Brat, P.; Alter, P.; Amiot, M.J. Rapid determination of polyphenols and vitamin C in plant-derived products. *J. Agric. Food Chem.* **2005**, *53*, 1370-1373.
40. Adam, V.; Mikelova, R.; Hubalek, J.; Hanustiak, P.; Beklova, M.; Hodek, P.; Horna, A.; Trnkova, L.; Stiborova, M.; Zeman, L.; Kizek, R. Utilizing of square wave voltammetry to detect flavonoids in the presence of human urine. *Sensors* **2007**, *7*, 2402-2418.
41. Klejdus, B.; Vacek, J.; Adam, V.; Zehnalek, J.; Kizek, R.; Trnkova, L.; Kuban, V. Determination of isoflavones in soybean food and human urine using liquid chromatography with electrochemical detection. *J. Chromatogr. B.* **2004**, *806*, 101-111.
42. Mikelova, R.; Hodek, P.; Hanustiak, P.; Adam, V.; Krizkova, S.; Havel, L.; Stiborova, M.; Horna, A.; Beklova, M.; Trnkova, L.; Kizek, R. Determination of isoflavones using liquid chromatography with electrochemical detection. *Acta Chim. Slov.* **2007**, *54*, 92-97.

43. Klejdus, B.; Mikelova, R.; Petrlova, J.; Potesil, D.; Adam, V.; Stiborova, M.; Hodek, P.; Vacek, J.; Kizek, R.; Kuban, V. Determination of isoflavones in soy bits by fast column high-performance liquid chromatography coupled with UV-visible diode-array detection. *J. Chromatogr. A* **2005**, *1084*, 71-79.
44. Klejdus, B.; Mikelova, R.; Petrlova, J.; Potesil, D.; Adam, V.; Stiborova, M.; Hodek, P.; Vacek, J.; Kizek, R.; Kuban, V. Evaluation of isoflavone aglycon and glycoside distribution in soy plants and soybeans by fast column high-performance liquid chromatography coupled with a diode-array detector. *J. Agric. Food Chem.* **2005**, *53*, 5848-5852.
45. Klejdus, B.; Mikelova, R.; Adam, V.; Zehnalek, J.; Vacek, J.; Kizek, R.; Kuban, V. Liquid chromatographic-mass spectrometric determination of genistin and daidzin in soybean food samples after accelerated solvent extraction with modified content of extraction cell. *Anal. Chim. Acta*. **2004**, *517*, 1-11.
46. Vacek, J.; Klejdus, B.; Lojkova, L.; Kuban, V. Current trends in isolation, separation, determination and identification of isoflavones: A review. *J. Sep. Sci.* **2008**, *31*, 2054-2067.
47. Klejdus, B.; Vacek, J.; Lojkova, L.; Benesova, L.; Kuban, V. Ultrahigh-pressure liquid chromatography of isoflavones and phenolic acids on different stationary phases. *J. Chromatogr. A* **2008**, *1195*, 52-59.
48. Mimica-Dukic, N.; Simin, N.; Cvejic, J.; Jovin, E.; Orcic, D.; Bozin, B. Phenolic compounds in field horsetail (Equisetum arvense L.) as natural antioxidants. *Molecules* **2008**, *13*, 1455-1464.
49. De Marino, S.; Gala, F.; Zollo, F.; Vitalini, S.; Fico, G.; Visioli, F.; Iorizzi, M. Identification of minor secondary metabolites from the latex of Croton lechleri (Muell-Arg) and evaluation of their antioxidant activity. *Molecules* **2008**, *13*, 1219-1229.
50. Lamien-Meda, A.; Lamien, C.E.; Compaore, M.M.Y.; Meda, R.N.T.; Kiendrebeogo, M.; Zeba, B.; Millogo, J.F.; Nacoulma, O.G. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. *Molecules* **2008**, *13*, 581-594.
51. Simic, A.; Manojlovic, D.; Segan, D.; Todorovic, M. Electrochemical Behavior and antioxidant and prooxidant activity of natural phenolics. *Molecules* **2007**, *12*, 2327-2340.
52. Bendini, A.; Cerretani, L.; Carrasco-Pancorbo, A.; Gomez-Caravaca, A.M.; Segura-Carretero, A.; Fernandez-Gutierrez, A.; Lercker, G. Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. *Molecules* **2007**, *12*, 1679-1719.
53. Koblovska, R.; Mackova, Z.; Vitkova, M.; Kokoska, L.; Klejdus, B.; Lapcik, O. Isoflavones in the Rutaceae family: Twenty selected representatives of the genera Citrus, Fortunella, Poncirus, Ruta and Severinia. *Phytochem. Anal.* **2008**, *19*, 64-70.
54. Hakkinen, S.; Auriola, S. High-performance liquid chromatography with electrospray ionization mass spectrometry and diode array ultraviolet detection in the identification of flavonol aglycones and glycosides in berries. *J. Chromatogr. A* **1998**, *829*, 91-100.
55. Hong, V.; Wrolstad, R.E. Use of Hplc Separation Photodiode Array Detection for Characterization of Anthocyanins. *J. Agric. Food Chem.* **1990**, *38*, 708-715.
56. Stefova, M.; Kulevanova, S.; Stafilov, T. Assay of flavonols and quantification of quercetin in medicinal plants by HPLC with UV-diode array detection. *J. Liq. Chromatogr. Relat. Technol.* **2001**, *24*, 2283-2292.

57. Gitz, D.C.; Liu-Gitz, L.; McClure, J.W.; Huerta, A.J. Effects of a PAL inhibitor on phenolic accumulation and UV-B tolerance in Spirodela intermedia (Koch.). *J. Exp. Bot.* **2004**, *55*, 919-927.
58. Janeiro, P.; Corduneanu, O.; Brett, A.M.O. Chrysin and (+/-)-taxifolin electrochemical oxidation mechanisms. *Electroanalysis* **2005**, *17*, 1059-1064.
59. Klejdus, B.; Sterbova, D.; Stratil, P.; Kuban, V. Identification and characterization of isoflavones in plant material by HPLC-DAD-MS tandem. *Chem. Listy*. **2003**, *97*, 530-539.
60. Oliveira-Brett, A.M.; Diculescu, V.C. Electrochemical study of quercetin-DNA interactions: Part I. Analysis in incubated solutions. *Bioelectrochemistry* **2004**, *64*, 133-141.
61. Oliveira-Brett, A.M.; Diculescu, V.C. Electrochemical study of quercetin-DNA interactions - Part II. In situ sensing with DNA biosensors. *Bioelectrochemistry* **2004**, *64*, 143-150.
62. Isuzugawa, K.; Inoue, M.; Ogihara, Y. Catalase contents in cells determine sensitivity to the apoptosis inducer gallic acid. *Biol. Pharmacol. Bull.* **2001**, *24*, 1022-1026.
63. Lecanu, L.; Greeson, J.; Papadopoulos, V. Beta-amyloid and oxidative stress jointly induce neuronal death, amyloid deposits, gliosis, and memory impairment in the rat brain. *Pharmacology* **2006**, *76*, 19-33.
64. Zhao, B.; Lu, Z.; Nie, G.; Tang, H.; Belton, P. Structure-activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. *Free Radic. Res.* **2006**, *40*, S134-S134.
65. Nash, J.F. Human safety and efficacy of ultraviolet filters and sunscreen products. *Dermatol. Clin.* **2006**, *24*, 35-51.
66. Sayre, R.M.; Dowdy, J.C.; Gerwig, A.J.; Shields, W.J.; Lloyd, R.V. Unexpected photolysis of the sunscreen octinoxate in the presence of the sunscreen avobenzone. *Photochem. Photobiol.* **2005**, *81*, 452-456.
67. Kluczyk, A.; Popek, T.; Kiyota, T.; de Macedo, P.; Stefanowicz, P.; Lazar, C.; Konishi, Y. Drug evolution: p-aminobenzoic acid as a building block. *Curr. Med. Chem.* **2002**, *9*, 1871-1892.
68. Hu, M.L.; Chen, Y.K.; Chen, L.C.; Sano, M. Para-Aminobenzoic Acid Scavenges Reactive Oxygen Species and Protects DNA against Uv and Free-Radical Damage. *J. Nutr. Biochem.* **1995**, *6*, 504-508.
69. Beecher, G.R. Overview of dietary flavonoids: Nomenclature, occurrence and intake. *J. Nutr.* **2003**, *133*, 3248S-3254S.
70. Patterson, C.; Madamanchi, N.R.; Runge, M.S. The oxidative paradox - Another piece in the puzzle. *Circ. Res.* **2000**, *87*, 1074-1076.
71. Grace, P.A. Ischemia - Reperfusion Injury. *Br. J. Surg.* **1994**, *81*, 637-647.
72. Halliwell, B. How to characterize an antioxidant: An update. In *Free Radicals and Oxidative Stress: Environment, Drugs and Food Additives*. Portland Press Ltd: London, UK, **1995**; pp. 73-101.
73. Singh, A.; Naidu, P.S.; Kulkarni, S.K. Quercetin potentiates L-dopa reversal of drug-induced catalepsy in rats: Possible COMT/MAO inhibition. *Pharmacology* **2003**, *68*, 81-88.
74. Primiano, T.; Yu, R.; Kong, A.N.T. Signal transduction events elicited by natural products that function as cancer chemopreventive agents. *Pharm. Biol.* **2001**, *39*, 83-107.

75. Ren, W.Y.; Qiao, Z.H.; Wang, H.W.; Zhu, L.; Zhang, L. Flavonoids: Promising anticancer agents. *Med. Res. Rev.* **2003**, *23*, 519-534.
76. Yao, L.H.; Jiang, Y.M.; Shi, J.; Tomas-Barberan, F.A.; Datta, N.; Singanusong, R.; Chen, S.S. Flavonoids in food and their health benefits. *Plant Food Hum. Nutr.* **2004**, *59*, 113-122.
77. Zand, R.S.R.; Jenkins, D.J.A.; Diamandis, E.P. Flavonoids and steroid hormone-dependent cancers. *J. Chromatogr. B* **2002**, *777*, 219-232.
78. Holden, J.M.; Bhagwat, S.A.; Haytowitz, D.B.; Gebhardt, S.E.; Dwyer, J.T.; Peterson, J.; Beecher, G.R.; Eldridge, A.L.; Balentine, D. Development of a database of critically evaluated flavonoids data: application of USDA's data quality evaluation system. *J. Food Compos. Anal.* **2005**, *18*, 829-844.
79. Suntornsuk, L. Capillary electrophoresis of phytochemical substances. *J. Pharm. Biomed. Anal.* **2002**, *27*, 679-698.
80. Collins, A.R. Assays for oxidative stress and antioxidant status: applications to research into the biological effectiveness of polyphenols. *Am. J. Clin. Nutr.* **2005**, *81*, 261S-267S.
81. Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **2005**, *81*, 230S-242S.
82. Zheng, W.; Wang, S.Y. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* **2001**, *49*, 5165-5170.
83. Hamilton, R.D.; Foss, A.J.; Leach, L. Establishment of a human in vitro model of the outer blood-retinal barrier. *J. Anat.* **2007**, *211*, 707-716.
84. Fuhrman, B.; Aviram, M. Preservation of paraoxenase activity by wine flavonoids - Possible role in protection of LDL from lipid peroxidation. In *Alcohol and Wine in Health and Disease*. New York Academy Sciences: New York, USA, **2002**; pp. 321-324.
85. Fatouhi, L.; Ganjavi, M.; Nematollahi, D. Electrochemical study of iodide in the presence of phenol and o-cresol: Application to the catalytic determination of phenol and o-cresol. *Sensors* **2004**, *4*, 170-180.
86. Zitka, O.; Huska, D.; Krizkova, S.; Adam, V.; Chavis, G.J.; Trnkova, L.; Horna, A.; Hubalek, J.; Kizek, R. An investigation of glutathione-platinum(II) interactions by means of the flow injection analysis using glassy carbon electrode. *Sensors* **2007**, *7*, 1256-1270.
87. Pokorna-Jurikova, T.; Matuskovic, J. The study of irrigation influence on nutritional value of Lonicera kamtschatica - cultivar Gerda 25 and Lonicera edulis berries under the Nitra conditions during 2001-2003. *Hortic. Sci.* **2007**, *34*, 11-16.
88. Kantor, G.R.; Ratz, J.L. Liver Toxicity from Potassium Para-Aminobenzoate. *J. Am. Acad. Dermatol.* **1985**, *13*, 671-672.

*Sample Availability:* Samples of the compounds isolated from less common fruits are available from the authors.