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Radical Scavenging and Anti-Lipoperoxidative Activities of *Smallanthus sonchifolius* Leaf Extracts

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Radical scavenging and anti-lipoperoxidative effects of two organic fractions and two aqueous extracts from the leaves of a neglected Andean crop−yacon (*Smallanthus sonchifolius* Poepp. & Endl., Asteraceae) were determined using various in vitro models. The extracts' total phenolic content was 10.7−24.6%. They exhibited DPPH (IC_{50} 16.14−33.39 μg/mL) and $HO^{\bullet}$ scavenging activities (4.49−6.51 mg/mL). The extracts did not scavenge phenylglyoxylic ketyl radicals, but they retarded their formation. In the xanthine/xanthine oxidase superoxide radical generating system, the extracts' activities were 26.10−37.67 superoxide dismutase equivalents/mg. As one of the extracts displayed xanthine oxidase inhibitory activity, the effect of the extracts on a nonenzymatically generated superoxide was determined (IC_{50} 7.36−21.01 μg/mL). The extracts inhibited *t*-butyl hydroperoxide-induced lipoperoxidation of microsomal and mitochondrial membranes (IC_{50} 22.15−465.3 μg/mL). These results make yacon leaves a good candidate for use as a food supplement in the prevention of chronic diseases involving oxidative stress.

KEYWORDS: Yacon; phenolic compounds; antioxidant activity; lipid peroxidation; protective effects

INTRODUCTION

Yacon (*Smallanthus sonchifolius*) is an Andean crop used for centuries by the native inhabitants of South America as a food and in traditional medicine. Yacon tubers, for the local population, are a regular dietary component, and the leaves are used for their hypoglycemic activity (*1*). The tubers contain β-oligofructans as the storage saccharides (*2*, *3*), and these are being at present recommended as prebiotics for improving mineral absorption and other beneficial effects (*4*−*6*). Because of their low caloric content, the tubers are a convenient dietary supplement for risk groups of the population (e.g., those with metabolic syndrome). However, until the 1980s, with the exception of Peru and Japan, practically no attention was devoted to this plant (*7*), which can be agriculturally cultivated in the European and northern American climate.

Phenolic compounds have been isolated from the tubers of yacon—mainly chlorogenic acid (*8*) and other caffeic acid derivatives (*9*, *10*)—and from the leaves of related *Smallanthus fruticosus* (centaureidin and sacuranetin) (*11*). We have already reported on the presence of large amounts of phenolic compounds in extracts from yacon leaves and tubers, mainly chlorogenic, protocatechuic, ferulic, rosmarinic, gallic, gentisic, and caffeic acids and their derivatives (*12*−*14*).

We have also provided evidence for the antioxidant activity of two extracts in relation to the content of phenolics. Moreover, we showed that these extracts exhibited cytoprotective effects against *tert*-butyl hydroperoxide induced oxidative damage to rat hepatocytes (*14*). More recently, we have presented the effects of four different extracts of yacon leaves on rat hepatocyte viability, on oxidative damage, and on glucose metabolism in these cells and their insulin-like effect on cytochrome P450 (CYP) isoformes 2B and 2E mRNA expression in the rat hepatoma cell line (Fao cells). Phenolic compounds were probably responsible for the observed activities of the extracts (*15*).

This study was undertaken to elucidate the antioxidant activity of four different extracts from *S. sonchifolius* leaves in different in vitro models of various degrees of complexity. The main objective was to contribute to the documentation of a new food supplement designed for the prevention of chronic diseases involving oxidative stress and disorders of glucose metabolism.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing 200−250 g were conditioned in standard boxes for 15 days before the experiments. They were fed a standard laboratory diet, provided with water ad libitum, and kept on a 12:12 h light−dark cycle.

Biological Material. Yacon (*S. sonchifolius* Poepp. & Endl., Asteraceae, Heliantheae, Melampodinae) plants, originally purchased from Ecuador, were grown at the Potato Research Institute in Havlíčkův Brod. Voucher specimens were deposited in our collection at the Institute of Medical Chemistry and Biochemistry, Olomouc, Czech Republic. The leaves were collected in October 2000 at harvest time of the tubers and dried at ambient temperature.

Extraction Procedure. Dried yacon leaves (20 g) were extracted as follows:

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(i) Organic fraction 1 (OF1): using a Soxhlet extractor with MeOH (3 × 8 h) and chlorophyll removal with petroleum ether, the aqueous layer was then acidified (0.01 M H_3PO_4) and extracted by ethyl acetate. Extract yield after evaporation of the solvent was 0.26 g.

(ii) Organic fraction 2 (OF2): the dried drug was extracted by cool percolation with methanol/water (3:7); the extract was then acidified (0.01 M H_3PO_4) and extracted by ethyl acetate. Extract yield after evaporation of the solvent was 0.25 g.

(iii) Decoction (DEC): the material was refluxed in water (20 mL) for 20 min and then left to cool at room temperature. Extract yield after freeze-drying was 3.30 g.

(iv) Tea infusion extract (INF): 1000 mL of boiling water was poured onto the leaves and then allowed to extract for 20 min while cooling. Extract yield after freeze-drying was 6.06 g.

All extracts were dried until a constant weight was achieved. The extraction procedure was repeated 3 times for each sample, and the reproducibility of the procedure was controlled by the measurement of total phenolic content in the samples.

Reagents. β-Nicotinamide adenine dinucleotide (NADH) sodium salt, 1,1-diphenyl-2-picrylhydrazyl (DPPH, 90%), dimethyl sulfoxide (DMSO) for cell cultures, nitro blue tetrazolium chloride (NBT), phenazine methosulfate (PMS), sodium dodecylsulfate (SDS), superoxide dismutase (SOD) E.C. 1.15.1.1 from bovine erythrocytes (30 000 U), *t*-butyl hydroperoxide (*t*BH, 70% in water), 2-thiobarbituric acid (TBA, 98%), trichloroacetic acid (TCA), tris(hydroxymethyl)aminomethane (Tris), xanthine (99%) and xanthine oxidase (XOD) E.C. 1.1.3.22 from buttermilk (5 U), *N*-*t*-butyl-α-phenylnitrone (PBN), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), and 30% H_2O_2 were purchased from Sigma-Aldrich Ltd., Czech Republic. $FeSO_4 \cdot 7\ H_2O$ and other chemicals and solvents were of analytical grade from Pliva-Lachema, Czech Republic.

Analyses. *Phenolic Content Analysis.* Total phenolics in all extracts were determined using the Folin–Ciocalteau reagent (*16*). A total of 25 μL of the tested fraction in distilled water was mixed with 500 μL of the reagent (previously diluted 10-fold with water) and maintained at room temperature for 5 min; 500 μL of sodium bicarbonate (75 g/L) was added to the mixture. After 90 min at 30 °C, the absorbance was measured at 725 nm. Results were expressed as gallic acid equivalents.

DPPH Scavenging. A total of 375 μL of a methanol solution of the tested sample (6.25, 12.5, 25, 50, 75, and 100 μg/mL) was mixed with 750 μL of a methanol DPPH solution (20 mg/L). After 30 min, the absorbance at 517 nm was measured, and IC_{50} values were obtained from the inhibition curves (*17*, *18*).

Hydroxyl Radical Scavenging. The Fenton reaction of H_2O_2 with $FeSO_4$ and spontaneous decomposition of hydrogen peroxide at 25 °C were used as sources of hydroxyl ($HO^\bullet$) radicals. The scavenging activity of the tested samples was determined using electron paramagnetic resonance (EPR) spectroscopy. Because $HO^\bullet$ radicals are very unstable and cannot be detected readily by the continuous wave EPR spectrometer, they were captured by a spin trap of DMPO, and the EPR spectra of this spin adduct were subsequently recorded. The reaction solution contained 5×10^{-4} M $FeSO_4$, 0.025 M DMPO, the tested substances (0.05–10 mg/mL), and 0.05 M H_2O_2 in a phosphate buffer (0.19 M Na_2HPO_4, pH = 7.4). The EPR spectra of these solutions were registered 40 min after the addition of H_2O_2, and the IC_{50} values were obtained from the dependences of scavenging activities on substance concentration.

Phenylglyoxylic Ketyl Radical Scavenging. The spontaneous decomposition of D,L-2,3-diphenyltartaric acid (DPTA) in propane-2-ol at 25 °C was used as a source of phenylglyoxylic ketyl radicals. Preparation and decomposition of DPTA is described in refs *19* and *20*. Phenylglyoxylic ketyl radicals rise during the first phase of this decomposition, and they are transformed into benzoyl radicals during the second phase. Since the lifetime of these radicals is relatively short, they were stabilized as spin adducts with the spin trap of PBN. The lifetime of such spin adducts is longer (some tens of minutes); therefore, they can be easily recorded by a continual wave EPR spectrometer. The EPR spectra of solutions containing 0.025 M DPTA, 0.05 M PBN (control), or the desired amounts of yacon extracts or caffeic acid in propan-2-ol were recorded.

Electron Paramagnetic Resonance Spectroscopy. All the EPR spectra were registered by a continual wave EPR spectrometer ERS 230 (ZWG Berlin, Germany), which operates in X-band (~9.3 GHz), at a modulation amplitude 0.1 mT and a microwave power 5 mW.

Superoxide Radical Scavenging Activity—Enzymatic Assay. Antiradical activity was determined spectrophotometrically by monitoring the effect of the tested substances on the reduction of NBT to the blue chromogen formazan by $O_2^{\bullet -}$. Superoxide radicals were generated by the xanthine/XOD system as described previously (*21*). Briefly, 0.1 mL of aqueous SOD standard solutions (5, 10, 25, 50, and 100 U/mL) or a sample solution (1 mg/mL) were separately added to a 1.0 mL mixture of 0.4 mM xanthine and 0.24 mM NBT in 0.1 M phosphate buffer (pH 7.8) containing 0.1 mM EDTA. Organic fractions were previously dissolved in DMSO (final concentration 0.1%). A total of 1.0 mL of XOD (0.05 U/mL), dissolved in the same phosphate buffer, was added, and the resulting mixture was incubated at 37 °C for 20 min. The reaction was terminated by adding 1.0 mL of 69 mM SDS solution, and the absorbance was measured at 560 nm (*22*). The superoxide scavenging activity was calculated as SOD equivalents (U/mg) from the SOD standard curve.

Effect on XOD Activity. The effect of the organic fraction OF1 on XOD activity was evaluated by measuring the formation of uric acid from xanthine in a double-beam spectrophotometer at room temperature. A total of 480 μL of XOD (0.592 U/mL) in a phosphate buffer (0.1 M) was mixed with 10 μL of the tested fraction in DMSO (final concentration 10, 25, 50, 75, 100, 125, and 150 μg/mL). The reaction was started by the addition of 500 μL of xanthine (400 μm) in the phosphate buffer, and the absorbance was recorded at 295 nm for 2 min.

Superoxide Radical Scavenging Activity—Nonenzymatic Assay. Superoxide radicals were generated by the NADH/PMS system following a described procedure (*23*). A total of 50 μL of the tested fraction (final concentration 0, 2.5, 5, 10, 12.5, 20, 40, and 60 μg/mL) was mixed with 40 μL of NADH (996 μm), 60 μL of NBT (250 μm), and 150 μL of PMS (5.4 μm). All the reagents were dissolved in a phosphate buffer (19 mM, pH 7.4). Organic fractions were previously dissolved in DMSO (final concentration 0.1%). The reaction mixture was incubated for 30 min at 37 °C, and the absorbance was measured at 560 nm. IC_{50} values were obtained from inhibition curves.

Protection Against tBH-Induced Lipoperoxidation. Rat liver microsomes and mitochondria were prepared by fractional centrifugation (*24*, *25*) of rat liver homogenate in 3 mM Tris buffer containing 250 mM sucrose and 0.1 mM EDTA (pH 7.4). The mitochondrial and microsomal fractions were characterized by their protein content (*26*). For inhibition of lipoperoxidation, a mixture containing 2 mg/mL mitochondrial or 1 mg/mL microsomal fraction, 5–120 μg/mL extracts or phenolic acids, and 1 mM *t*BH was incubated in a water bath at 37 °C for 60 min. The incubation was then terminated by adding 2 mL of a mixture of 26 mM TBA and 918 mM TCA; the samples were subsequently incubated at 90 °C for 30 min. After cooling, the samples were centrifuged (10 min, 1000*g*, 20 °C), and the absorbance of the supernatant was measured at 535 nm (*27*). IC_{50} values were obtained from inhibition curves.

Statistics. Data were analyzed with a one-way ANOVA using the StatView Statistical Package (SAS Institute, Inc.). Differences were considered statistically significant when p was <0.05 and p was <0.01. IC_{50} values were obtained using MS Excel 2000 with the extension Life Science Workbench (LSW) Data Analysis Toolbox (MDL Information Systems, Inc.).

RESULTS

Phenolic Content Analysis. The phenolic content in the extracts was 24.6 ± 0.6% for OF1, 22.1 ± 3.4 for OF2, 10.7 ± 0.4% for DEC, and 11.8 ± 0.4% for INF. The content in the two organic fractions was significantly different from both the aqueous extracts, while OF1 was not significantly different from OF2, and DEC did not differ from INF.

DPPH Radical Scavenging. The results of the DPPH radical scavenging test of the extracts and their main components are

Table 1. DPPH Radical Scavenging of Selected Phenolic Compounds and Extracts from *S. sonchifolius* Leaves[a]

substance	IC_{50} (μg/mL)	IC_{50} (μM)
chlorogenic acid	2.46 ± 0.17	6.94 ± 0.47
caffeic acid	0.86 ± 0.05	4.75 ± 0.25
rosmarinic acid	1.86 ± 0.05	5.15 ± 0.15
protocatechuic acid	1.37 ± 0.10	8.87 ± 0.64
ferulic acid	7.20 ± 0.55	37.10 ± 2.84
quercetin	2.00 ± 1.12	6.63 ± 3.70
OF1	16.14 ± 3.38	
OF2	24.34 ± 2.65	
INF	31.10 ± 1.44	
DEC	33.39 ± 0.82	

[a] Values are expressed as mean ± SD; $n = 10$.

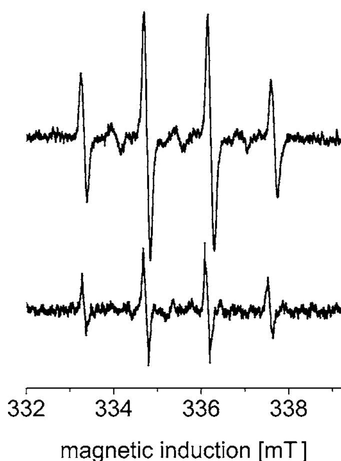


Figure 1. EPR spectra of the spin adduct DMPO with HO• without (upper line) or with 4 mg of OF1 (lower line) 40 min after the addition of H_2O_2.

shown in **Table 1** as concentrations of the tested substance that induced a 50% decrease of the reaction mixture absorbance at 517 nm (IC_{50}). The activity of phenolic acids and that of quercetin was higher that that of the tested extracts from yacon leaves, with the activity of aqueous extracts being almost half that of the organic fractions. No significant difference was found between OF1 and OF2 or between INF and DEC. The fraction OF1 was the most efficient among the extracts, as it was significantly different from the extracts INF and DEC ($p < 0.01$), and the fraction OF2 was significantly different only from DEC but not from INF ($p < 0.05$).

Hydroxyl Radical Scavenging. An EPR spectrum, which consisted of four lines with the intensity ratio of 1:2:2:1, was observed after the addition of H_2O_2 into the phosphate buffer containing $FeSO_4$ and DMPO (**Figure 1**). This EPR signal was in the range of free radicals with $g = 2.0038$, and distances among lines were 1.48 mT. This signal belongs to the spin adduct of DMPO and the HO• radical, with hyperfine splitting constants $A_N = A_H = 1.48$ mT (*28*). The signal intensity linearly increased up to 35 min, and later, until 120 min, it increased more slowly (about 3%). Therefore, the EPR spectra were recorded 40 min after the addition of H_2O_2. The intensity of the EPR spectra of the samples with studied substances decreased with an increasing amount of antioxidant (**Figure 1**). The IC_{50} values, calculated from the dependencies of the signal intensity upon the antioxidant concentration, were in the range of 4.5−6.5 mg/mL for yacon extracts, and caffeic acid was the most active of the substances tested with IC_{50} 0.90 ± 0.07 mg/mL (**Table 2**).

Phenylglyoxylic Ketyl Radical Scavenging. The spontaneous decomposition of D,L-2,3-diphenyltartaric acid was documented by the EPR spectra of spin adducts of PBN with their

Table 2. Hydroxyl Radical Scavenging Activity of Caffeic Acid and Extracts from *S. sonchifolius* Leaves[a]

substance	IC_{50} (mg/mL)
caffeic acid	0.90 ± 0.07
OF1	4.49 ± 0.45
INF	6.51 ± 0.22
DEC	5.37 ± 0.21

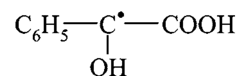
[a] Values are expressed as mean ± SD; $n = 5$.

Table 3. Superoxide Radical Scavenging Activity of Selected Phenolic Compounds and Extracts from *S. sonchifolius* Leaves in the Xanthine/Xanthine Oxidase Generating System[a]

substance	SOD equivalents (U/mg)
chlorogenic acid	56.13 ± 3.78
caffeic acid	410.37 ± 45.54
rosmarinic acid	516.76 ± 52.24
protocatechuic acid	82.91 ± 8.43
ferulic acid	5.32 ± 0.88
OF1	34.70 ± 5.76
OF2	30.30 ± 4.92
INF	26.10 ± 4.42
DEC	37.67 ± 7.83

[a] Values are expressed as mean ± SD, n=6.

radical intermediates. During the first phase (ca. 5−40 min), the PBN adduct with phenylglyoxylic ketyl radical was mainly registered (*19*, *20*).



The EPR spectrum of this spin adduct consisted of three doublets and one triplet as a result of the hyperfine interaction of the unpaired spin with one nuclear spin of nitrogen ^{14}N, $I = 1$ (triplet, $A_N = 1.4$ mT), and with one nuclear spin of hydrogen ^{1}H, $I = 1/2$ (doublets, $A_H = 0.24$ mT). The intensity of this signal reached a maximum at 35 min. After 15 min, besides the previously mentioned signal, a new signal could be observed. The latter was manifested first only as unequal peak intensities in doublets and as a shoulder on the first and last doublet. The intensity of the first signal began to decrease after 35 min, whereas the intensity of the new signal increased. The splitting constants of the new EPR signal were $A_N = 1.4$ mT and $A_H = 0.36$ mT. These EPR spectra were attributed to the spin adduct of PBN with benzoyl radical $C_6H_5C^{\bullet}{=}O$ (*19*, *20*). After 120 min, no EPR signals in the spectra could be registered. When the studied extracts from *S. sonchifolius* leaves at 5 mg/mL were added to the reaction mixture, no EPR signal was observed during the first 8 h. The signal of the PBN adduct with phenylglyoxylic ketyl radical appeared overnight and lasted several (5−6) days. Thus, the extracts displayed in this model first antioxidant and subsequently prooxidant activities.

Superoxide Radical Scavenging Activity. The scavenging ability of the tested substances was also checked on the xanthine/XOD superoxide radical generating system. Their scavenging activity was expressed as equivalents of SOD activity that induced the same effect (**Table 3**). SOD equivalents were acquired from the SOD calibration curve.

When this method is used, the effect of the extract on the XOD activity must be checked because an inhibitory effect of the enzyme would also lead to a decrease in the NBT reduction (*23*). In this regard, we evaluated the effect of the fraction OF1 on the XOD activity by the metabolic conversion of xanthine. This fraction presented XOD inhibitory activity ($IC_{50} = 84.11 \pm 7.18$ μg/mL). Inasmuch as this value did not differ ($p < 0.05$)

Table 4. Superoxide Radical Scavenging Activity of Selected Phenolic Compounds and Extracts from *S. sonchifolius* Leaves in the NADH/PMS Generating System[a]

substance	IC_{50} (μg/mL)
chlorogenic acid	3.43 ± 0.35 (9.69 ± 0.97 μM)
caffeic acid	1.57 ± 0.17 (8.70 ± 0.92 μM)
OF1	8.59 ± 2.93
OF2	7.36 ± 2.18
INF	7.75 ± 3.69
DEC	21.01 ± 5.75

[a] Values are expressed as mean ± SD; $n = 9$.

Table 5. Effect of Selected Phenolic Acids and Extracts from *S. sonchifolius* Leaves on *t*-Butyl Hydroperoxide-Induced Lipoperoxidation of Microsomal and Mitochondrial Membranes[a]

substance	microsomes	mitochondria
	IC_{50} (μg/mL)	
chlorogenic acid	64.06 ± 16.58 (180.8 ± 46.8 μM)	102.9 ± 16.4 (290.4 ± 46.3 μM)
caffeic acid	9.86 ± 1.92 (54.73 ± 10.66 μM)	37.43 ± 8.77 (207.8 ± 48.7 μM)
rosmarinic acid	7.63 ± 0.35 (21.18 ± 0.97 μM)	12.20 ± 1.09 (33.86 ± 3.03 μM)
protocatechuic acid	37.18 ± 5.80 (241.3 ± 37.6 μM)	48.45 ± 4.62 (314.6 ± 30.0 μM)
ferulic acid	100.4 ± 7.8 (517.0 ± 40.2 μM)	147.9 ± 14.4 (761.7 ± 74.2 μM)
OF1	23.79 ± 7.83	22.15 ± 9.33
OF2	32.83 ± 2.32	27.56 ± 9.87
INF	361.1 ± 24.6	212.8 ± 13.5
DEC	465.3 ± 13.9	404.1 ± 57.9

[a] Values are expressed as mean ± SD; $n = 10$.

from the IC_{50} for superoxide radical scavenging in this system (83.30 ± 5.60 μg/mL), it was not possible to show a clear-cut scavenging effect on $O_2^{\bullet-}$.

To clarify, we determined the effect of the substances on the superoxide radical generated by the NADH/PMS system. The tested substances were also active in this model with IC_{50} values in the range of 1.57 ± 0.17 and 21.01 ± 5.75 μg/mL (**Table 4**). Phenolic acids were again more active than the extracts. No differences were noted among the individual extracts with the exception of the extract DEC, which was significantly different from all other extracts. These results confirmed $O_2^{\bullet-}$ scavenging by the tested substances.

Protectivity Against *t*BH-Induced Lipoperoxidation. The antioxidant activity of the tested substances was confirmed on the model of lipid peroxidation of liver subcellular membranes. Inhibition of *t*BH-induced (final concentration 1 mM) lipoperoxidation of microsomal and mitochondrial membranes was measured in concentrations ranging from 5 to 200 μg/mL. All the substances tested inhibited peroxidation in the experimental models used; only the aqueous extracts did not reach 50% inhibition of lipoperoxidation, so they were tested up to 800 μg/mL. 100% inhibition was not reached with any of the tested compounds; IC_{50} values were in the range of 9.86−465.3 μg/mL (**Table 5**).

DISCUSSION

The correlation of total phenolic content with antioxidant activity of plant extracts has been demonstrated in several studies (*16*, *29*). The antioxidant activity of yacon leaf extracts could therefore be predicted on the basis of its content of phenolics.

DPPH radical scavenging was used for a screening of antioxidant activity. IC_{50} values of the tested phenolic compounds, with the exception of ferulic acid, were comparable in this model. A higher IC_{50} (lower antioxidant activity) value of the ferulic acid is in accordance with previously reported lowering of antioxidant activity of phenolic acids after methylation of the *o*-hydroxy group (*30*).

Table 6. DPPH Radical Scavenging by Plant Leaf Extracts[a]

plant	family	IC_{50} (μg/mL)
Ginkgo biloba (*25*)	Ginkgoaceae	40.72 ± 0.19
Vitex polygama (*25*)	Verbenaceae	21.94 ± 0.82
Anadenanthera peregrine (*25*)	Lamiaceae	11.56 ± 0.38
Lantana trifolia (*25*)	Moraceae	25.84 ± 0.67
Smallanthus sonchifolius	Asteraceae	
OF1		16.14 ± 3.38
OF2		24.34 ± 2.65
INF		31.10 ± 1.44
DEC		33.39 ± 0.82

[a] Values are expressed as mean ± SD.

The scavenging activity of the tested extracts from *S. sonchifolius* leaves can be compared with the activity of extracts of *Ginkgo biloba* and some Brazilian plant leaves (**Table 6**) (*31*). The activities of individual extracts did not differ markedly. Our results confirm the expected difference between organic fractions (OF1 and OF2) and aqueous extracts (INF a DEC), which can be determined by their lower content of phenolic compounds. In view of higher IC_{50} values of the extracts as compared to phenolic compounds, we assume the presence of additional substances that can lower the mean antioxidant activity of the extracts.

Although the DPPH radical scavenging test is broadly accepted for in vitro evaluation of natural substance antioxidant potential (*21*, *31*−*35*), this radical does not occur in vivo, and the experimental conditions involve the use of methanol. Therefore, we studied the effect of the tested substances on the hydroxyl radicals, generated by the Fenton reaction in a phosphate buffer. The IC_{50} value (0.902 mg/mL) for caffeic acid seems to be relatively high, but Ohsugi et al. (*36*) found that 0.05 mg/mL caffeic acid scavenged 83.8% of the $HO^{\bullet}$ radical in the Fenton reaction with $FeSO_4$. However, they used a concentration of H_2O_2 of only 0.035 mM. As is evident from **Table 2**, caffeic acid and the tested extracts scavenged $HO^{\bullet}$ radicals. The $HO^{\bullet}$ radical scavenging efficiency was approximately 1000 times lower than that on DPPH or superoxide radicals. To determine whether the scavenging of $HO^{\bullet}$ radicals was caused by the interaction of the studied substances with Fe^{2+}, the same experiments were carried out in water in the absence of $FeSO_4$ (i.e., the spontaneous decomposition of H_2O_2 was used as a source of $HO^{\bullet}$ radicals). The results of such experiments prove that caffeic acid and yacon extracts scavenge $HO^{\bullet}$ radicals, which are generated by spontaneous decomposition of H_2O_2.

Various ketyl radicals are formed in living organisms as reactive intermediates in certain enzymatic reactions (e.g., during the metabolism of xenobiotics by cytochrome P450 and during dehydration of both endogenous and exogenous substrates by certain dehydratases (*37*)). Thus, the scavenging of such radicals is of extreme biological importance. The method presented here determines antioxidant properties of the tested substances in propan-2-ol ($\epsilon = 18$) (*38*). This mimics the surroundings at the interface between the center interfacial ($\epsilon = 30$) and the hydrophobic parts of the biological membranes (*39*). In contrast to vitamin C or silymarin and its components (*40*), yacon extracts did not show clear scavenging activities in this system, but they delayed radical formation by several hours. This phenomenon might be important for the protection of living

cells from this kind of radical, if the delay is sufficient for elimination of the source molecules (xenobiotics) from the organism. Whether this ability is of biological relevance or not must be determined in in vivo experiments.

We also studied the effect of the tested substances on the superoxide radical, generated enzymatically in the xanthine/XOD system. SOD equivalents, obtained for individual phenolic compounds, better reveal the relation between antioxidant activity and chemical structure than DPPH scavenging. Superoxide scavenging confirmed the lowest activity of ferulic acid (5.32 ± 0.88 SOD equivalents/mg), and the $O_2^{\bullet-}$ scavenging activity increased in the following sequence: chlorogenic (56.13 ± 3.78) $<$ protocatechuic (82.91 ± 8.43) $\ll$ caffeic (410.37 ± 45.54) $<$ rosmarinic acid (516.76 ± 52.24 SOD equivalents/mg). A relatively high scavenging activity of protocatechuic acids was surprising. This acid (i.e., 3,4-dihydroxybenzoic) does not contain the sequence −CH═CH−COOH, which is responsible for the proton donating ability of the *o*-dihydroxy substitution for radical reduction (*30*). High antioxidant activity of caffeic and rosmarinic acids is due to the *o*-dihydroxy-substitution and −CH═CH−COOH sequence on the benzene ring in the *p*-position to one of the OH groups. The values measured for these acids are almost twice as high as those found in the literature (260 and 230 SOD equivalent/mg, respectively) (*21*).

SOD equivalents for yacon leaves were in the range of 26.10 ± 4.42 to 37.67 ± 7.83 without significant difference. The scavenging activity of the extracts corresponds to 50% of those for chlorogenic acid, which is considered to be an important antioxidant in food. IC_{50} was measured for the organic fraction OF1. Its value was 83.30 ± 5.60 μg/mL. This fraction also exhibited XOD inhibition with IC_{50} being 84.11 ± 7.18 μg/mL. The extracts and their main components were therefore tested for $O_2^{\bullet-}$ scavenging in a nonenzymatic assay.

In the NADH/PMS system, the IC_{50} values for yacon leaf extracts were in the range of 7.36 ± 2.18 μg/mL for the fraction OF2 to 21.01 ± 5.75 μg/mL for the aqueous extract DEC. The latter was the only one that was significantly less efficient; other extracts had comparable activity. Chlorogenic and caffeic acids were again more active than the leaf extracts (IC_{50} 3.43 ± 0.35 and 1.57 ± 0.17 μg/mL, respectively). If IC_{50} values are expressed in molar concentrations (9.69 ± 0.97 and 8.70 ± 0.92 μM, respectively), both acids had the same activity ($p < 0.01$).

For a more detailed evaluation of antioxidant activity of the tested substances in biological systems, we studied their protective effect on mitochondrial and microsomal membranes of rat hepatocyte. Both membranes are used as a model system for the evaluation of protectivity against lipoperoxidative damage. The hydrophobic−hydrophilic interface of the membranes is considered to be the key site of protective activity of phenolic compounds (e.g., silybin (*41*, *42*)). IC_{50} values obtained in the test of membrane damage by *t*BH for organic fractions and aqueous yacon leaf extracts were in the range of 22.15 ± 9.33 to 32.83 ± 2.32 μg/mL for fractions OF1 and OF2 and in the range of 212.8 ± 13.5 to 465.3 ± 13.9 μg/mL for the extracts INF and DEC. Organic fractions were comparably efficient as chlorogenic and caffeic acids but much more efficient than the aqueous extracts. For comparison, the IC_{50} value of an organic fraction from the *Prunella vulgaris* (Labiatae) aerial part extract was 39.2 ± 2.5 μg/mL. This medicinal plant is well-known for its protective effect in oxidative stress prevention (*43*).

The ability of phenolic acids (*44*) and flavonoids (*45*) to inhibit lipoperoxidation of model membranes or LDL lipoproteins is well-documented in the literature. The mechanism of membrane lipoperoxidation inhibition is a prevention of the chain reaction by interaction of the antioxidant with a free radical. Also, partition coefficients into the lipophilic region and the spacial orientation of the antioxidant on the membrane interface play a role in prevention of lipoperoxidative damage. The effectiveness of aqueous yacon leaf extracts in the model used is influenced by their high content of hydrophilic saccharides and glycosides. The hydrophilicity of these substances lowers their affinity to the lipid interface (*30*). This mechanism of the biological effect of antioxidants is also supported by the lower activity of an ester of the caffeic acid—the chlorogenic acid in comparison with the free caffeic acid, as reported here. The same effect has been described for ethyl esters of phenolic acids versus free acids using the model of inhibition of LDL oxidation (*44*).

Our results confirm the strong protective effect of the medicinal tea from yacon leaves used in Peruvian traditional medicine. These results are furthermore supported by previously found cytoprotective effects of the extracts on oxidative damage to rat hepatocyte primary cultures (*15*). A combination of radical scavenging, cytoprotective, hypoglycemic, and insulin-like (*15*) activity makes *S. sonchifolius* leaves a good candidate for use in the prevention of chronic diseases involving oxidative stress, particularly diabetes mellitus.

ABBREVIATIONS USED

CYP, cytochrome P450; DEC, decoction from *S. sonchifolius* leaves; DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide; DMSO, dimethyl sulfoxide; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DPTA, D,L-2,3-diphenyltartaric acid; EDTA, ethylenediamine tetraacetic acid; EPR, electron paramagnetic resonance; INF, infusion from *S. sonchifolius* leaves; NADH, β-nicotinamide adenine dinucleotide sodium salt; NBT, nitro blue tetrazolium chloride; OF1 and OF2, organic fractions 1 and 2 from *S. sonchifolius* leaves; PBN, *N*-*t*-butyl-α-phenylnitrone; PMS, phenazine methosulfate; SDS, sodium dodecyl (lauryl) sulfate; SOD, superoxide dismutase; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reacting substances; *t*BH, *t*-butyl hydroperoxide; TCA, trichloracetic acid; Tris, tris(hydroxymethyl)aminomethane; XOD, xanthine oxidase.

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