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IN VITRO ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF THE INFLORESCENCES, LEAVES AND FRUITS OF *SORBUS TORMINALIS* (L.) CRANTZ

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Abstract: The antioxidant potential of 70% methanolic extracts from the inflorescences, leaves and fruits of *Sorbus torminalis* (L.) Crantz was evaluated using three *in vitro* test systems: the DPPH (2,2-diphenyl-1-picrylhydrazyl) and the ABTS [2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid)] free radical scavenging assays, and the AAPH [2,2'-azobis-(2-amidinopropane) dihydrochloride]-induced linoleic acid (LA) peroxidation test. The results were compared with the activity of the extracts obtained from the model antioxidant *Sorbus* species (*Sorbus aucuparia* L.), and also with the activity of phenolic standards such as quercetin, Trolox [(±)-6-hydroxy-2,2,7,8-tetramethylchroman-2-carboxylic acid], BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene) and TBHQ (*tert*-butylhydroquinone). The radical scavenging capacities of the *S. torminalis* extracts towards the DPPH radical were in the range of 62.0-244.1 micromolar Trolox equivalents/g d.w. of plant material. They were significantly ($p < 0.05$) correlated with the results of the ABTS test ($r = 0.8535$), and with the chain-breaking activities determined in the LA-peroxidation test ($r = 0.9831$). In comparison with the synthetic standards, the free radical scavenging capacity of the *Sorbus* extracts was remarkably higher than their chain-breaking activity. Both kinds of antioxidant effects of the extracts were significantly ($R^2 > 0.8097$, $p < 0.05$) influenced by the total phenolic content (TPC) as determined by the Folin-Ciocalteu method. The plant tissues derived from *S. torminalis* exhibited lower antioxidant potentials than those of *S. aucuparia* by a factor of 1.5-3.2, partially due to the lower TPC levels (multiplicity factors of 1.2-1.9). After the original antioxidant capacities of the extracts were recalculated according to the TPC levels, the resulting antioxidant capacities of the phenolic fractions in the *S. torminalis* extracts were lower than those from *S. aucuparia* by a factor of 1.1-1.6, suggesting that the distinctive chemistry of the phenolic constituents also influences the antioxidant power of the two species.

Keywords: *Sorbus torminalis* (L.) Crantz, antioxidant activity, DPPH, ABTS, lipid peroxidation, phenolic content

Sorbus torminalis (L.) Crantz (syn. *Torminalis clusii* (Roem.) Robertson & Phipps) represents one of the most important genera in the Rosaceae family (1). The genus *Sorbus* L. in the broad sense (s.l.) includes up to 250 species growing in mainly temperate areas in the Northern Hemisphere, and is commonly divided into five subgenera (1, 2), grouped around five model species. One of these is the diploidal *S. torminalis*, which is either classified in the monotypic subgenus *Torminaria* Roem. (1) or together with the two hybrid species *S. latifolia* (Lam.) Pers. and *S. semiincisa* Borbás in the subgenus *Torminaria* (DC.) Rchb. (2).

S. torminalis (otherwise known as the wild service tree or the chequer tree) is a medium-sized deciduous tree native to central and southern Europe, north-western Africa, western Asia, and the Caucasus (2). It can be distinguished from all other *Sorbus* species by its simple, deeply lobed leaves and brown fruits with many lenticels and a multilayered epidermis (2).

Different tissues (fruits, inflorescences, leaves and bark) of various *Sorbus* taxa have a wide variety of ethnomedical properties, such as anti-diarrhoeal, diuretic, anti-inflammatory, anti-atherogenic, anti-diabetic, vasoprotective and vasorelaxant activities, and are also potent antioxidant agents (3–8). The fruits of *Sorbus* species, including the *S. torminalis* fruits, are an important source of vitamin C and can be consumed fresh after exposure to the first autumn frost, when overripe, or processed as jams, syrups and wines (9). Some of the biological activities, especially the antioxidant capacity of *Sorbus* species have been attributed to the high levels of phenolic

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compounds present in these plants (3, 4, 10–12). High antioxidant activity has previously been reported for numerous *Sorbus* taxa, including *S. aria* (L.) Crantz (10, 12), *S. domestica* L. (4) and *S. aucuparia* L. (10, 11, 13), the model species for the subgenera *Aria*, *Cormus* and *Sorbus* s.s., respectively. However, the activity of *S. torminalis* has not yet been investigated.

The known phytochemical constituents of *S. torminalis* include the same groups of phenolic compounds as found in other members of the genus, such as flavonoids, caffeoylquinic acids and condensed tannins (6, 14). Given that phenolics from these groups are known to be potent and safe antioxidants (15–17), the potential activity of plant tissues derived from *S. torminalis* could show similar promise to the proven activity of other *Sorbus* species. On the other hand, the flavonoid chemistry of *S. torminalis* is distinctive within the genus due to the presence of a series of methoxylated flavones and flavonols, glucosides of chrysoeriol, limocitrin, isorhamnetin and sexangularetin (14), which could imply differences in the antioxidant activity between *S. torminalis* and other *Sorbus* species, which contain mainly free hydroxy flavonols, derivatives of quercetin and kaempferol (11). Thus, a detailed study on the activity of *S. torminalis* is required to verify its potential value as a source of natural antioxidants.

The search for antioxidants from natural sources has developed over the past two decades into an important field of research (18). Many natural compounds, especially plant phenolics, have been identified as potent antioxidants (19), functioning as free-radical scavengers and reducing agents (quenchers of reactive oxygen species, ROS) and protecting living cells against lipid peroxidation *in vitro* and *in vivo* (18, 20). It is believed that oxidative stress induced by ROS is a primary factor in various degenerative diseases, such as cancer, atherosclerosis, coronary diseases and age-related degenerative brain disorders (21). Epidemiological studies have revealed that a diet rich in fruits, vegetables and other plant products containing phenolics is beneficial to human health and disease prevention (22–24), mostly owing to the ability of plant constituents to neutralize ROS. Interest in the antioxidant properties of plant phenolics also derives from their strong activity and low toxicity compared with those of synthetic phenolic antioxidants, such as BHA (butylated hydroxyanisole, 2-*tert*-butyl-4-hydroxyanisole and 3-*tert*-butyl-4-hydroxyanisole), BHT [butylated hydroxytoluene, 2,6-bis(1,1-dimethylethyl)-4-methylphenol], or TBHQ [*tert*-butylhydroquinone, 2-(1,1-dimethylethyl)-1,4-benzenediol], which are commonly used as antioxidants in processed foods (18).

Therefore, the aim of this project was to study the antioxidant activity and total phenolic content of the inflorescences, leaves and fruits of *S. torminalis*. The activity of the plant extracts was assayed using three *in vitro* complementary tests (the DPPH and ABTS free radical-scavenging methods, and the AAPH-induced linoleic acid peroxidation test). The results were compared with the activity of the extracts obtained from inflorescences, leaves and fruits of the most common rowan *S. aucuparia*, and also with the activity of simultaneously analyzed standard antioxidants, such as quercetin, Trolox, BHA, BHT and TBHQ. The total phenolic content in the extracts was determined using the Folin-Ciocalteu method, and the relationship between the antioxidant capacity and the phenolic content was investigated.

MATERIAL AND METHODS

Plant material

Samples of *S. torminalis* and *S. aucuparia* were collected in 2009 in the Arboretum (51°49'N, 19°53'E), Forestry Experimental Station of Warsaw University of Life Sciences (SGGW) in Rogów (Poland). The inflorescences and leaves were collected in June, and the fruits in October, after the first slight frost, as recommended by traditional medicine (9, 25). Voucher specimens (KFG/HB/07010-STOR and KFG/HB/07001-SAUC) were authenticated by the author, and were deposited at the Department of Pharmacognosy, Medical University of Łódź, Poland.

Chemicals and instrumentation

HPLC or GC grade purity reagents and standards, such as 2,2-diphenyl-1-picryl hydrazyl (DPPH); 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS); 2,2'-azo-bis-(2-amidinopropane) dihydrochloride (AAPH); (±)-6-hydroxy-2,2,7,8-tetramethylchroman-2-carboxylic acid (Trolox); linoleic acid; chlorogenic acid hemihydrate; gallic acid monohydrate; and quercetin trihydrate were purchased from Sigma-Aldrich Inc. (Germany/USA). Analytical grade antioxidant standards, such as BHA, BHT and TBHQ were obtained from the same supplier. Redistilled water and analytical grade chemicals and solvents (POCh S.A., Poland) were used for other analyses.

Absorbance was measured in 10 mm quartz cuvettes using a Lambda 25 spectrophotometer (Perkin-Elmer, USA) (DPPH and LA-peroxidation

tests) or using a Specol spectrophotometer (Carl Zeiss, Germany) (all other assays). Samples for the tests were incubated in a constant temperature using a BD 23 incubator (Binder, Germany).

Preparation of plant extracts for testing antioxidant activity and phenolic profile

The samples of plant material were air-dried under normal conditions, powdered with an electric grinder, and sieved through a 0.315 mm sieve. An accurately weighed mass (3.0 g for the LA-peroxidation test, and 100–300 mg for the other assays) was refluxed first for 30 min with 70% (v/v) aqueous methanol (30 mL), and then twice for 15 min with the same solvent (20 mL). The obtained extracts were combined, filtered and diluted with methanol to 100 mL to give the test extracts (TE).

DPPH free radical-scavenging test

The scavenging activity was determined as described before (see preceding paper).

ABTS free radical-scavenging test

The scavenging activity was also determined using the TEAC method, according to Re et al. (27), with some variations. The $ABTS^{+}$ working solution was prepared through the reaction between potassium persulfate and ABTS as described earlier (27), and then equilibrated to the absorbance of the negative control of 0.700 ± 0.030 at 734 nm. The negative control prepared by mixing the equilibrated ABTS solution (2 mL) with methanol (1 mL). The assays were made for the same concentrations of TE and the standard compounds as prepared for the DPPH tests. An aliquot of the diluted sample (1 mL) was added to the equilibrated $ABTS^{+}$ solution (2 mL) and vigorously shaken. After 15 min of incubation in screwcap vials at room temperature and in the dark, the decrease in the absorbance was measured at 734 nm. The mixtures of the diluted samples (1 mL) and methanol (2 mL) were used as the blanks. The scavenging percentage of $ABTS^{+}$ by the samples was estimated as the percentage decrease of absorbance, as calculated using the formula: % decrease = $100 \times (1 - A_{sample}/A_{control})$. The concentration of the analytes (standards or plant materials used for extract preparation) in the reaction medium (in µg/mL) was plotted against the scavenging percentage of $ABTS^{+}$, and the EC_{50} values were calculated from the obtained scavenging curves. The activity of the plant materials was then expressed in terms of Trolox® (TEAC) equivalent antioxidant capacity in µmol/g d.w.

Linoleic acid (LA) peroxidation test

The ability of TE to inhibit AAPH-induced LA-peroxidation was assayed according to the method of Azuma et al. (28) with some modifications. Six different concentrations of all TE and standards were prepared in 70% (v/v) aqueous methanol. An aliquot of the diluted sample (0.30 mL) was placed in a screw-cap vial and mixed with 1.40 mL of 1.3% (w/v) LA in methanol, 1.40 mL of 0.2 M phosphate buffer (pH 7.0), and water (0.70 mL). The negative control was prepared using methanol (0.30 mL) instead of the sample. Peroxidation was initiated by the addition of 0.20 mL of 46.35 mM AAPH solution in phosphate buffer. The vial was incubated at 50.0 ± 0.1°C in the dark, sampling being carried out every hour until the absorbance of the control reach the value of 0.550 ± 0.020. The degree of oxidation was measured by the ferric thiocyanate method (29). The reaction mixture (0.10 mL) was diluted with 9.70 mL of 75% (v/v) methanol and mixed with 0.10 mL of 20 mM $FeCl_2$ solution in 3.5% (w/w) HCl and 0.10 mL of 10% (w/w) aqueous NH_4SCN solution. After precisely 3 min the absorbance was measured at 500 nm versus 75% methanol. The inhibition ratio (I%) of the peroxidation process was calculated as follows: $I\% = 100 \times (1 - DA_{sample}/DA_{control})$, where *DA* is the difference between the absorbances measured at the end and the start of the test. The concentration of the analytes in the reaction medium (expressed in µg/mL for the standards, and in mg/mL for the plant materials used for extract preparation) was plotted against the inhibition ratio (I%), and the IC_{50} value was read from the obtained regression curve.

Determination of total phenolic content

The total phenolic content in TE was determined according to Folin-Ciocalteu (FC) method (10) as decribed previously (see preceding paper).

Statistical analysis

The statistics (calculation of standard deviation, analysis of variance) were performed using the software StatisticaPl for Windows (StatSoft Inc., Poland).

According to our previous study (Olszewska & Michel, 2009), methanol:water (70:30, v/v) was found to be the best initial solvent for extracting quantitatively multiple antioxidants from *S. aria* leaves.

RESULTS AND DISCUSSION

The antioxidant effects of 70% (v/v) methanolic extracts from various organs (inflorescences,

leaves and fruits) of *S. torminalis* were studied in comparison with those of *S. aucuparia*, the model species being one of the most commonly occurring (1) and one of the most active antioxidant species within the genus *Sorbus* (11). The initial solvent was chosen according to the results of previous optimization studies (10). The activity of the extracts obtained from the two investigated species (Tab. 1) was compared with that of several potent standard antioxidants, both natural (quercetin) and synthetic (Trolox, BHA, BHT and TBHQ). For chemical characterization, the level of total phenolic compounds was determined using the standard Folin-Ciocalteu method (FC), and was expressed in gallic acid equivalents (GAE, Tab. 1), a unit that has previously been proven to be an accurate estimate of the total level of real phenolic metabolites in *Sorbus* extracts (10–13). Before the analysis, the structure and chemical character of the main phenolic metabolites of *S. torminalis* were identified by isolation and chromatographic studies as proanthocyanidins, caffeoylquinic acids and flavonoids (14). Since the reactivity of these bioactive constituents in radical reactions differs significantly depending on the structure of the radical, the basic reaction mechanism, and a wide range of reaction conditions (30), the antioxidant capacities of the tested extracts could not be evaluated using only one method (31). Therefore, three complementary *in vitro* models utilising different reaction mechanisms were employed.

The free radical scavenging activity of the analytes was tested by two UV-photometric decolorization methods, the DPPH (26) and ABTS (TEAC III) (27) assays. Both tests predominantly utilize a single electron transfer (SET) redox reaction mechanism (18, 31). The results obtained from the DPPH (Fig. 1) and ABTS tests were strongly dependent on incubation time and concentration of the antioxidant analyte in the reaction medium. The antiradical capacities of the analytes were expressed as the percentage decrease in the initial concentration of the DPPH radical or the initial absorbance of the solution of the $ABTS^{+}$ radical cation, and were further characterized by the EC_{50} values (Tab. 1). The consistency of the antiradical effects determined by the DPPH and ABTS assays was confirmed by statistically significant ($p < 0.05$) linear correlation between the EC_{50} values (µg/mL), and was characterized by high correlation ($r = 0.9993$) and determination coefficients ($R^2 = 0.9986$). A slightly lower correlation ($r = 0.8535$ and $R^2 = 0.7285$, $p < 0.05$) was found between Trolox equivalent parameters for the DPPH (TEAA) and ABTS tests (TEAC).

A wide range of antiradical capacity was observed among the different extracts. The values of TEAA ranged from 62.0 to 782.8 µmol Trolox/g d.w. of plant material, whereas the TEAC values ranged from 56.3 to 536.4 µmol Trolox/g d.w.. The plant tissues derived from *S. torminalis* exhibited lower antiradical properties than those obtained from *S. aucuparia*. Among the extracts obtained from the same species, the highest antiradical activity was observed in those from the inflorescences, followed by those from the leaves, while the fruits exhibited the lowest antioxidant potential. This observation is consistent with previous studies of the free radical scavenging activity of *Sorbus* plants (10, 11).

The TEAA and TEAC values of the extracts were significantly correlated ($p < 0.05$) with the total contents of phenolic compounds as measured using the FC method, confirming that the phenolics are responsible for the activity tested. The observed correlation was strong for both the DPPH ($r = 0.9888$ and $R^2 = 0.9778$) and ABTS tests ($r = 0.9974$ and $R^2 = 0.9949$).

The antiradical capacities of the extracts presented in Table 1 were calculated according to the dry weight of plant material used for extract preparation. The determined activity is thus a function of two main factors, the antiradical effect of the active biomolecules in the tested extracts and the extraction yield or concentration of these compounds in the plant tissues. As shown in Table 1, the total phenolic levels measured in the organs derived from *S. torminalis* (2.14–5.75% GAE) were lower than those of *S. aucuparia* (2.58–11.02% GAE) by a factor of 1.2-1.9. In order to minimize the influence of different extraction yields on the antioxidant results, and to compare thereby the antiradical properties of phenolic fractions occurring in the tested plant materials, the original values of EC_{50} (µg plant material/mL) were converted into the effective concentration of phenolics (µg phenolics/mL) with the total phenolic contents expressed in GAE (%). The recalculated values of EC_{50} are listed in Table 2. The subsequent differences found between the activities of the phenolic fractions derived from both species were remarkably lower than those found between the respective plant extracts. All phenolic fractions (PFs) exhibited significant antiradical activity, which was comparable or even higher than the activity of the simultaneously assayed standards (Tab. 1). The ranking of the DPPH free radical scavenging activity ($1/EC_{50}$, µg/mL, $p < 0.05$) of PFs and standard antioxidants was as follows: quercetin > PF of *S. aucuparia* inflorescences > PF of *S. aucuparia* leaves > BHA > TBHQ > PF of *S. torminalis* leaves > PF of *S. torminalis* inflorescences > Trolox > PF of *S. aucuparia* fruits > PF of *S. torminalis* fruits >

Table 1. Antioxidant capacities of standard phenolic compounds and 70% methanolic extracts from *S. torminalis* and *S. aucuparia*.

Sample	Total phenolics	DPPH radical scavenging test			ABTS radical scavenging test		Linoleic acid (LA) peroxidation test	
	(GAE) [a]	EC_{50} [b]	EC_{50} [c]	TEAA [d]	EC_{50} [e]	TEAC [f]	% Inhib. [g]	IC_{50} [h]
S. torminalis								
Inflorescences	5.75 ± 0.09	53.49 ± 0.65	2.26 ± 0.03	244.1	213.50 ± 5.93	224.5	68.34 ± 0.48	0.986 ± 0.031
Leaves	4.28 ± 0.09	66.08 ± 0.91	2.79 ± 0.04	197.6	263.60 ± 5.82	181.9	58.69 ± 0.49	1.354 ± 0.016
Fruits	2.14 ± 0.10	210.60 ± 1.61	8.90 ± 0.07	62.0	851.20 ± 22.24	56.3	41.65 ± 0.32	2.778 ± 0.066
S. aucuparia								
Inflorescences	11.02 ± 0.25	16.69 ± 0.24	0.71 ± 0.01	782.8	89.38 ± 1.92	536.4	89.52 ± 0.66	0.376 ± 0.012
Leaves	8.23 ± 0.09	24.10 ± 0.28	1.03 ± 0.01	541.2	121.93 ± 1.40	393.2	78.54 ± 0.72	0.566 ± 0.018
Fruits	2.58 ± 0.08	146.51 ± 0.80	6.25 ± 0.03	89.1	582. 47 ± 17.76	82.3	53.39 ± 0.72	1.821 ± 0.038
Standards								
BHA	-	2.35 ± 0.09	0.099 ± 0.004	1.00	6.97 ± 0.12	1.24	81.25 ± 2.30	14.31± 0.57
BHT	-	6.54 ± 0.12	0.283 ± 0.005	0.44	19.26 ± 0.23	0.55	80.50 ± 2.54	21.48 ± 0.66
TBHQ	-	2.73 ± 0.10	0.115 ± 0.004	0.79	8.23 ± 0.15	0.97	100.00 ± 2.70	38.53 ± 0.85
Quercetin	-	1.63 ± 0.08	0.071 ± 0.003	2.41	3.98 ± 0.08	3.64	88.38 ± 1.15	49.41± 1.29
Trolox	-	3.27 ± 0.10	0.136 ± 0.004	1.00	12.00 ± 0.09	1.00	92.74 ± 1.10	23.43 ± 1.76

Results are the means from triplicate experiments ± SD. Values in each column are significantly different at $p < 0.05$. [a] Total phenolic content expressed in gallic acid equivalents (GAE. %),. [b,c] Scavenging strength; amount of antioxidant needed to reduce the initial concentration of $DPPH^{\cdot}$ by 50%, expressed as follows: [b] in µg/mL of $DPPH^{\cdot}$ solution; [c] in g/g $DPPH^{\cdot}$. [d] Micromolar Trolox® (TEAA) equivalent antioxidant capacity expressed in µmol/g for the plant material or in µmol/µmol for the standard, resp. [e] Scavenging strength, µg/mL; amount of antioxidant needed to decrease the initial absorbance of $ABTS^{\cdot+}$ solution by 50%. [f] Micromolar Trolox (TEAC) equivalent antioxidant capacity expressed in µmol/g for the plant material or in µmol/µmol for the standard, resp. [g] Inhibition ratio of LA oxidation after 5 h incubation with the final antioxidant concentration 2.25 mg/mL for the plant materials or 0.20 mg/mL for the standards. [h] Inhibition concentration; amount of antioxidant needed to decrease the LA oxidation by 50%, expressed in mg/ml of LA solution for the plant materials or in µg/ml of LA solution for the standards. [b-h] Amount of antioxidant was calculated for the weight of the dry plant material used for extract preparation or for the weight of the standard, respectively.

Table 2. Antioxidant efficiencies of phenolic fractions of the extracts from *S. torminalis* and *S. aucuparia*.

Sample	EC_{50} (μg/mL) [a]		IC_{50} (μg/mL) [b]
	DPPH radical scavenging test	ABTS radical scavenging test	Linoleic acid (LA) peroxidation test
S. torminalis			
Inflorescences	3.07	12.28	56.69
Leaves	2.83	11.28	57.95
Fruits	4.51	18.21	59.45
S. aucuparia			
Inflorescences	1.84	9.85	41.43
Leaves	1.98	10.03	46.58
Fruits	3.78	15.03	46.98

[a, b] Values recalculated from the original mean antioxidant capacities of the extracts (EC_{50} or IC_{50}, expressed in μg/mL) and the total phenolic contents expressed in GAE (%, gallic acid equivalents). For the original data and definition of the parameters of EC_{50} and IC_{50} see Table 1.

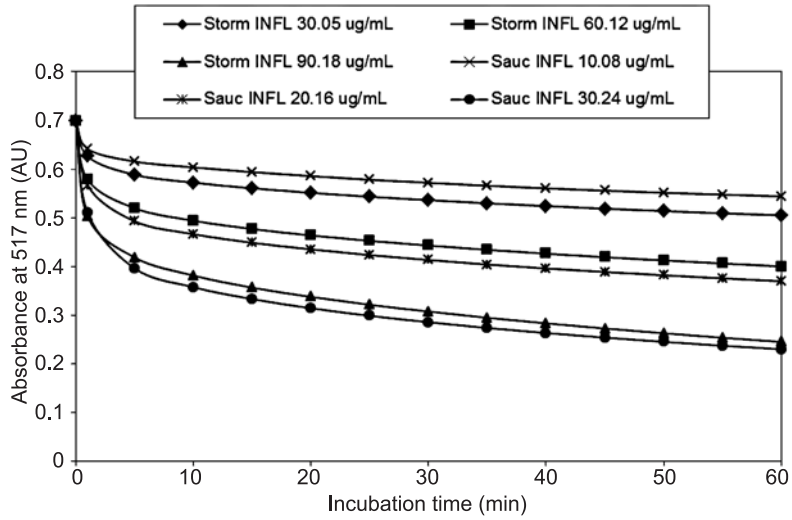

Figure 1. Decay of the absorbance of a DPPH˙ solution (60 μM) in methanol (25°C) in the presence of different concentrations of the extracts from the inflorescences (INF) of *S. torminalis* (Storm) and *S. aucuparia* (Sauc). The numbers in the legend indicate the concentration of plant material used for extract preparation in the reaction medium (μg/mL). Each point on the graph represents the mean value of triplicate experiments (RSD < 5%)

BHT. In the ABTS test, the magnitudes of the observed activities were similar with the exception of BHA and TBHQ, which both were more active in this test than in the DPPH assay.

The ability of *Sorbus* extracts to react *via* the hydrogen atom transfer (HAT) mechanism was studied by testing the inhibition of linoleic acid peroxidation (31). In this test, linoleic acid (LA) was oxidized in a chain reaction initiated by peroxy radicals generated through thermal decomposition of 2,2'-azobis-(2-amidinopropane) dihydrochloride (AAPH). This chain reaction can be retarded by an antioxidant donor of hydrogen atom, which scavenges the chain-carrying peroxy radical (32). The degree of oxidation (level of developed lipid peroxides) was measured using the ferric thiocyanate (FTC) method (29). The chain-breaking antioxidant activity of the analytes was expressed as the percentage inhibition of LA-oxidation and was characterized by the IC_{50} value. As shown in Figures 2a

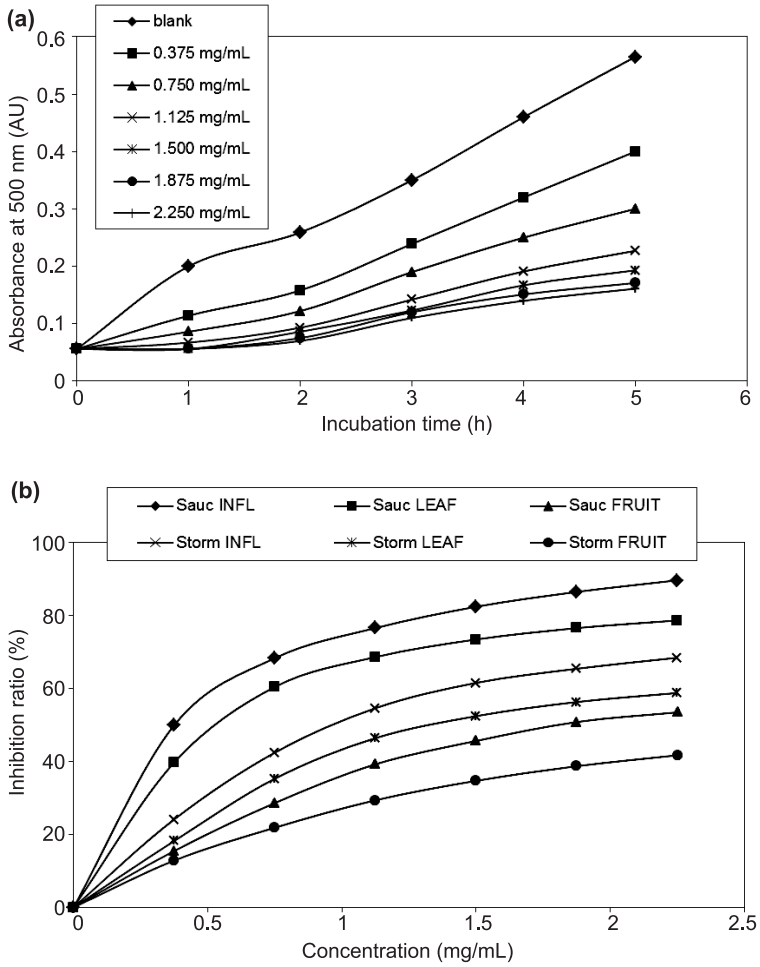

Figure 2. Inhibition of linoleic acid peroxidation by the *Sorbus* extracts: (a) effect of the extract from the *S. torminalis* inflorescences at different concentrations (mg/mL, calculated for the plant material used for extract preparation) on the formation of lipid peroxides (FTC method); (b) dependence of the inhibition ratio of the peroxidation process after 5 h of incubation at 50°C on the concentration (mg/mL) of the extracts from the inflorescences (INF), leaves (LEAF) and fruits (FRUIT) of *S. torminalis* (Storm) and *S. aucuparia* (Sauc). Each point on the graph represents the mean value of triplicate experiments (RSD < 5%)

and 2b, the chain-breaking antioxidant activity of the analyzed *Sorbus* extracts was strongly dependent on incubation time and dose. The results obtained after 5 h of incubation are listed in Table 1. The IC_{50} values ranged from 0.38 mg/mL for the inflorescences of *S. aucuparia* to 2.78 mg/mL for the fruits of *S. torminalis*. Statistically significant ($p < 0.05$) linear correlations were observed between the IC_{50} values for the LA-peroxidation test and the total phenolic levels expressed in GAE ($r = –0.8998$ and $R^2 = 0.8097$), as well as between the IC_{50} values and the antiradical efficiencies EC_{50} (μg/mL) found in the DPPH ($r = 0.9831$ and $R^2 = 0.9666$) and ABTS tests ($r = 0.9799$ and $R^2 = 0.9602$), respectively. These correlations confirmed that the assayed extracts utilize both HAT and SET reaction mechanisms, and that they could act simultaneously as direct free-radical scavengers and chain-breaking antioxidants. The phenolic constituents of the extracts could also be considered to be responsible for both kinds of antioxidant activity. However, the lower correlation between the GAE and the IC_{50} values for the LA-peroxidation test than that observed between the GAE and EC_{50} values for both radical scavenging tests suggests that some non-phenolic compounds could be partially responsible for the chain-breaking antioxidant effect of the *Sorbus* extracts. A plot of the capacity to inhibit LA-peroxidation (percentage) *vs.* the GAE of the extracts (Fig. 3) gave an ordinate value (y-intercept) of 29.7%, which indicates that, theoretically, 29.7% of the prevention of peroxidation is due to non-pheno-

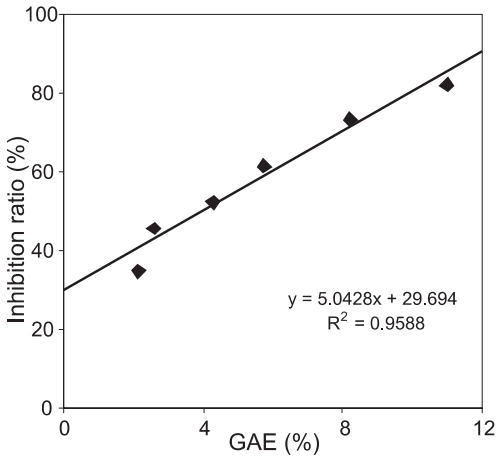

Figure 3. Inhibition of linoleic acid peroxidation by the *Sorbus* extracts: dependence of the inhibition ratio on the total phenolic content (GAE, gallic acid equivalents) of the plant material used for extract preparation. Each point on the graph represents the mean value of triplicate experiments. For SD values see Table 1

lic constituents. Some of the natural non-phenolic compounds known for their antioxidant activity, such as ascorbic acid and carotenoids (33), have indeed been reported previously in *Sorbus* plants, especially in the fruits (34).

In order to evaluate the chain-breaking antioxidant effect of the phenolic fractions in the tested extracts, the original values of IC_{50} (Tab. 1) were converted into the effective concentration of phenolics (µg phenolics/mL), with the total phenolic content expressed in GAE (%). The recalculated values of IC_{50} (Tab. 2) were compared with those obtained for the simultaneously assayed standards (Tab. 1). The resulting antioxidant activity of the phenolic fractions (PFs) derived from the tested plant tissues was comparable (*S. torminalis* fractions) or higher (*S. aucuparia* phenolics) than the activity of quercetin, a natural phenolic antioxidant, but it was lower than that of synthetic antioxidants (BHA, BHT, Trolox, TBHQ). The chain-breaking antioxidant activity ($1/IC_{50}$, µg/mL, $p < 0.05$) of PFs and standard antioxidants was ranked as follows: BHA > BHT = Trolox® > TBHQ > PF of *S. aucuparia* inflorescences > PF of *S. aucuparia* leaves = PF of *S. aucuparia* fruits > quercetin > PF of *S. torminalis* inflorescences > PF of *S. torminalis* leaves > PF of *S. torminalis* fruits. It should be pointed out that all synthetic standards showed remarkably higher activities in the LA-peroxidation tests than in both free-radical scavenging tests, and no significant correlation was found between their IC_{50} and EC_{50} values ($R^2 < 0.28$). On the other hand, although the studied *Sorbus* extracts are better free-radical scavengers than chain-breaking antioxidants, the high and significant correlations found between their IC_{50} and EC_{50} values (described above) indicate that these extracts are universal antioxidants.

As shown in Table 2, the phenolic fractions derived from *S. torminalis* exhibited slightly lower antioxidant capacities (by a factor of 1.1–1.6, depending on the test) than those from *S. aucuparia*. The observed differences are probably due to the distinctive chemistry of the phenolic compounds, especially flavonoids, occurring in these species. As reported previously (6, 14), *S. torminalis* contains a series of glycosides of flavones and methoxylated flavonols, such as apigenin, luteolin, chrysoeriol, isorhamnetin, limocitrin and sexangularetin, which have been proven to be less active in several *in vitro* antioxidant tests (35–37) than derivatives of quercetin, the main flavonoid aglycone found in *S. aucuparia* (10, 11). On the other hand, methoxylated flavones have been reported to exhibit outstanding *in vivo* pharmacological activities compared with their hydroxylated counterparts. The most important differentiating features are as follows: a) improved resistance to intestinal and hepatic metabolism: the process of phase II conjugation *via* the glucuronic acid and/or sulfation pathway is slowed down for methoxylated compounds, which can thus be accumulated in mammalian tissues at levels exceeding those of free hydroxy flavonoids by as much as 350% (38); b) improved intestinal absorption: methoxylated flavones show 5- to 8-fold higher apparent permeability across the Caco-2 cell monolayers than the corresponding hydroxylated ones (39); c) improved safety: 3'- or 4'-*O*-methylation of catechol-type flavonoids prevents their mutagenic and prooxidant activity *in vivo* (40). Moreover, methoxylated compounds, especially 3'-*O*-methylated conjugates such as isorhamnetin, the main flavonoid aglycone of the *S. torminalis* inflorescences (14), have been identified among the main metabolites of free hydroxyflavones, such as quercetin (41), thus being in part responsible for their antioxidant effects *in vivo*. Therefore, it might be concluded that *in vitro* tests could underestimate the *in vivo* antioxidant capacity of methoxylated flavones, and overestimate that of free hydroxy ones. Given the relatively low differences in antioxidant activities observed in the present study between phenolic fractions derived from *S. torminalis* (isorhamnetin abundant) and those from *S. aucuparia* (quercetin abundant), further research on the *in vivo* antioxidant effects of *S. torminalis* extracts would be useful.

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REFERENCE

1. Robertson K.R., Phipps J.B., Rohrer J.R., Smith P.G.: Syst. Bot. 16, 376 (1991).
2. Aldasoro J.J., Aedo C., Garmendia F.M., de la Hoz F.P., Navarro C.: Syst. Bot. Monogr. 69, 189 (2004).
3. Hukkanen A.T., Pölönen S.S., Kärenlampi S.O., Kokko H.I.: J. Agric. Food Chem. 54, 112 (2006).
4. Termentzi A., Kefalas P., Kokkalou E.: Food Chem. 98, 599 (2006).
5. Sohn E.J., Kang D.G., Mun Y.J., Woo W.H., Lee H.S.: Biol. Pharm. Bull. 28, 1444 (2005).
6. Tsitsa-Tzardi E., Loukis A., Philianos S.: Fitoterapia 63, 189 (1992).
7. Yin M.H., Kanga D.G., Choi D.H., Kwon T.O., Lee H.S.: J. Ethnopharmacol. 99, 113 (2005).
8. Kang D.G.; Sohn E.J.; Lee A.S.; Kim J.S.; Lee D.H.; Lee H.S.: Am. J. Chin. Med. 35, 265 (2007).
9. Facciola S.: Cornucopia II: A source book of edible plants. p. 211. Kampong Publications,Vista 1998.
10. Olszewska M.A., Michel P.: Nat. Prod. Res. 23, 1507 (2009).
11. Olszewska M.A., Nowak S., Michel P., Banaszczak P., Kicel A.: Molecules 15, 8769 (2010).
12. Olszewska M.A., Michel P.: Nat. Prod. Res. (2011, in press, doi: 10.1080/14786419.2010.537271).
13. Olszewska M.A.: Acta Pol. Pharm. Drug Res. 68, 937 (2011), (preceeding paper).
14. Olszewska M.A., Roj J.M.: Phytochem. Lett. (2011, in press, doi: 10.1016/j.phytol.2011.02.005).
15. Aron P.M., Kennedy J.A.: Mol. Nutr. Food Res. 52, 79 (2008).
16. Galati G., O'Brien P.J.: Free Radic. Biol. Med. 37, 287 (2004).
17. Watanabe T., Arai Y., Mitsui Y., Kusaura T., Okawa W., Kajihara Y., Saito I.: Clin. Exp. Hypertens. 28, 439 (2006).
18. Ndhlala A.R., Moyo M., Van Staden J.: Molecules *15*, 6905 (2010).
19. Sroka Z.: Z. Naturforsch. 60c, 833 (2005).
20. Rice-Evans C.A., Miller N.J., Paganga G.: Free Rad. Biol. Med. 20, 933 (1996).
21. Aruoma O.I.: Food Chem. Toxicol. 32, 671 (1994).
22. Chong M.F., Macdonald R., Lovegrove J.A.: Br. J. Nutr. 104, S28 (2010).
23. Pandey K.B., Rizvi S.I.: Oxid. Med. Cell Longev. 2, 270 (2009).
24. Willcox B.J., Curb J.D., Rodriguez B.L.: Am. J. Cardiol. 101, S75 (2008).
25. Strzelecka H., Kowalski J.: Encyclopedia of hebral medicine and herbalism (in Polish). p. 202. PWN, Warszawa 2000.
26. Brand-Williams W., Cuvelier M.E., Berset C.: Lebensm. Wiss.Technol. 28, 25 (1995).
27. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C.A.: Free Radic. Biol. Med. 26, 1231 (1999).
28. Azuma K., Nakayama M., Koshioka M., Ippoushi K., Yamaguchi Y., Kohata K., Yamauchi Y., Ito H., Higashio H.: J. Agric. Food Chem. 47, 3963 (1999).
29. Chen H.-M., Muramoto K., Yamauchi F., Nokihara K.: J. Agric. Food Chem. 44, 2619 (1996).
30. Denisov E.T., Denisova T.G.: Russ. Chem. Rev. 78, 1047 (2009).
31. Prior R.L., Wu X., Schaich K.: J. Agric. Food Chem. 53, 4290 (2005).
32. Roginski V., Lissi E.A.: Food Chem. 92, 235 (2005).
33. Foti M.C., Amorati R.: J. Pharm. Pharmacol. 61, 1435 (2009).
34. Kampus K., Kampuse S., Berna E., Kruma Z., Krasnova I, Drudze I.: Latv. J. Agron. 12, 59 (2009).
35. Yokozawa T., Chen C.P., Dong E., Tanaka T., Nonaka G.-I., Nishioka I.: Biochem. Pharmacol. 56, 213 (1998).
36. Seyoum A., Asres K., El-Fiki F.K.: Phytochemistry 67, 2058 (2006).
37. Kim J.-S., Kwon Y.-S., Sa Y.-J., Kim M.-J.: J. Agric. Food Chem. 59, 138 (2011).
38. Cai H., Boocock D.J., Steward W.P., Geschler A.J.: Cancer Chemother. Pharmacol. 60, 257 (2007).
39. Wen X., Walle T.: Drug Metab. Dispos. 34, 1786 (2006).
40. Zhu B.T., Ezell E.L., Liehr J.G.: J. Biol. Chem. 269, 292 (1994).
41. Van der Woude H., Boersma M.G., Vervoort J., Rietjens I.M.C.M.: Chem. Res. Toxicol. 17, 1520 (2004).

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