

COMPARISON OF PHENOLIC PROFILE AND IN VITRO ANTIOXIDANT CAPACITY OF *SORBUS DOMESTICA* L. LEAF SAMPLES FROM POLAND AND CROATIA

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This work presents a comparative analysis of the phenolic composition (UHPLC-PDA-ESI-MS³, HPLC-PDA-fingerprint, UV-spectrophotometric methods) and antioxidant activity (DPPH, FRAP) of leaf samples from two vegetation seasons of a medicinal and dietary plant *Sorbus domestica* growing in its natural habitat (Croatia, C) and cultivated in Poland (P). The samples from both sources were rich in structurally diverse polyphenols (44 analytes; P: 73.4–76.6 and C: 98.3–106.7 mg GAE/g dry leaves) including the dominating flavan-3-ols and flavonoids. The greatest qualitative and quantitative differences were observed for flavonoids (P: 14.3–20.3%; C: 27.5–34.1% of polyphenols) – in the Polish samples flavonoid diglycosides predominated, in the Croatian samples the contents of both monoglycosides and diglycosides were similar. In the case of dry methanolic extracts, despite the higher extraction efficiency obtained for the Croatian samples (32–36% vs 23–24%), the quality of the extracts was comparable, both in terms of the total phenolic content (P: 269.4–280.0; C: 297.6–304.4; mg GAE/g dry extract) and antioxidant activity parameters (DPPH, EC₅₀, µg/mL. P: 10.5–10.9, C: 10.0–10.3; and FRAP mmol Fe²⁺/g. P: 6.64–7.13, C: 7.06–7.11). As a result, the study confirmed the influence of environmental conditions on the phenolic profile and antioxidant capacity of *S. domestica* leaves, as well as showed that despite some differences, plant materials from both Poland and Croatia might be suitable for production of natural health products.

Keywords: antioxidant activity, extract quality, LC-MS, leaves, polyphenolic composition, *Sorbus domestica* L.

INTRODUCTION

Sorbus domestica L. (service tree) is a deciduous tree from the Rosaceae family, which due to its edible fruits and decorative qualities has been cultivated in the Mediterranean region since Roman times. The main areas of its occurrence are the Balkan Peninsula, the Apennines, southern France and eastern Spain (Rotach, 2003). The species is easily acclimatized to various climate conditions, and its present distribution is to a large extent a result of human influence, as over the centuries it has been locally planted and sub-spontaneously spread to some parts of Central Europe. Although the plant prefers the Mediterranean climate, it can also be cultivated in temperate regions (including Poland) with generally good results, except for diminished flowering and fruit production (Poljak et al., 2015).

Service tree is a source of traditionally used plant materials (mainly fruits and leaves) with anti-inflammatory, anti-atherogenic, anti-diabetic, antidiarrheal and diuretic properties (Kültür, 2007). So far the available literature data concerning biological activity of *S. domestica* have focused mostly on the fruits from southern Europe and indicated promising antioxidant and anti-diabetic potential of the extracts and polyphenolic fractions obtained therefrom (Termentzi et al., 2006, 2008). However, although berries in general are recognized as rich sources of bioactive polyphenols, recent studies underline superiority of leaves obtained from fruit plants, which constitute cost-effective plant materials available throughout the whole growing season (Teleszko and Wojdyło, 2015). Indeed, our previous studies documented that leaves of *S. domestica* cultivated in Poland accumulate an immense diversity of polyphenols,

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and thus constitute a promising herbal raw material that may be used in prevention or adjunctive therapy of oxidative stress/inflammation-related pathologies (Maczak et al., 2018). On the other hand, it is known that therapeutic efficiency of medicinal plants as a function of secondary metabolites production is influenced by numerous factors, including environmental growth conditions (Michel et al., 2017).

Plant secondary metabolites, also known as specialized or specific metabolites, are crucial components of plants adaptive mechanisms and play an important role in defence against various stress factors, such as high or low temperature, solar radiation, drought or humidity, as well as herbivory or pathogenic attack (Ncube et al., 2012). Numerous scientific reports emphasize the influence of climatic factors on the biosynthesis and accumulation of polyphenols (e.g., flavonoids) in plants tissues, depending on the species, organ or stage of plant development (Aninbon et al., 2016; Chen et al., 2018; Zhang, Wu et al., 2017). Apart from their beneficial role for the plants, some of these metabolites are of great pharmaceutical interest because of their health promoting properties (Chen et al., 2018; Ncube et al., 2012). Since the majority of medicinal species grow in wild and their metabolite pools are determined by uncontrolled environmental factors, pharmaceutical raw materials originating from plants from different regions might differ in quality (Ncube et al., 2012). Thus, for each medicinal plant, the studies of its properties in relation to the environmental and/or geographical conditions should be conducted. It is especially important for species such as service tree which are cultivated outside their typical habitat. Unfortunately, there is no previous report on *S. domestica* concerning this issue.

Therefore, the aim of this research was to determine the differences in the phenolic profile, antioxidant capacity and extraction yields of the respective hydroalcoholic extracts between *S. domestica* leaves harvested in Poland (Central Europe) and Croatia (Mediterranean region). The material for the study were dried leaves, as well as dry hydromethanolic extracts prepared thereof as the substances preferred by the pharmaceutical industry due to the improved stability, more favorable physical and biological properties, and simplicity in further processing. The raw materials were collected in two different growing seasons in late summer (August), since the period after flowering and before fruit maturity was reported to be optimal for phenolics accumulation in the leaves of fruit trees, including *Sorbus* species (Cosmulescu and Trandafir, 2011; Olszewska, 2011). The qualitative and quantitative phenolic profiles were monitored by UHPLC-PDA-ESI-MS³, HPLC-PDA-

fingerprint and spectrophotometric methods. The DPPH free radical-scavenging and FRAP (ferric reducing antioxidant power) assays were selected as *in vitro* activity models based on the earlier findings for leaf dry extracts of *S. domestica* (Maczak et al., 2018), which indicated that these two tests are good predictors of antioxidant capacities of the extracts in more complex *in vivo*-relevant models.

MATERIALS AND METHODS

PLANT MATERIAL AND EXTRACTS PREPARATION

Leaves of *S. domestica* L. were collected in August 2014 and 2016 in the Arboretum (51°49'N, 19°53'E), Forestry Experimental Station of Warsaw University of Life Science in Rogow (Poland) from long-term cultivation and authenticated by Piotr Banaszczak (Head of the Arboretum), as well as in Risika on Krk island (Croatia; 45°06'N, 14°38'E) from plants growing in their natural habitat and authenticated by prof. M.A. Olszewska (Department of Pharmacognosy, Medical University of Lodz, Poland). The voucher specimens were deposited in the herbarium of the Department of Pharmacognosy, Medical University of Lodz, Poland (KFG/HB/14001-SDOM:P/C and KFG/HB/16001-SDOM:P/C). The raw materials were dried, powdered with an electric grinder and sieved.

Extracts of raw plant materials were prepared for analysis as follows: accurately weighed samples of the leaf material (100 mg) were refluxed first for 30 min with 30 mL of methanol-water (7:3, v/v), and then twice for 15 min with 20 mL of the same solvent. The obtained extracts were combined, filtered and diluted with the extractant to 100 mL. Each plant material was extracted in triplicate to give the test extracts, which were analyzed for their total phenolic (TPC) and total proanthocyanidin contents (TPA), as well as the activity in DPPH and FRAP tests. For HPLC analysis leaf samples (1.5–2.0 g) were first defatted by pre-extraction with 100 mL chloroform for 45 min (the extracts were discarded), and then extracted with methanol-water (7:3, v/v) as described above. The results were calculated per dry weight (dw) of the leaves.

Dry extracts were prepared as follows: 2.5 g of leaf samples were pre-extracted for 18 h with 1 L chloroform in a Soxhlet apparatus and the pellet was next refluxed three times for 6 h with 100 mL of 70% (v/v) aqueous methanol to give the defatted methanol extracts. The obtained extracts were then evaporated *in vacuo* and lyophilized using an Alpha 1–2/LD Plus freeze dryer (Christ, Osterode am Harz, Germany) before weighing. The results of the TPC test were calculated per dry weight (dw) of the extract.

CHEMICALS AND INSTRUMENTATION

HPLC grade reagents and standards were purchased from Sigma-Aldrich (Seelze, Germany/St. Louis, MO, USA). HPLC grade solvents were obtained from Avantor Performance Materials (Gliwice, Poland). In all analyses, redistilled water was used. The analytical equipment used in the study was the same or of the same origin as employed earlier (Mataczak et al., 2018).

PHYTOCHEMICAL PROFILING
AND ANTIOXIDANT ACTIVITY STUDIES

The UHPLC-PDA-ESI-MS³ was carried out according to Mataczak et al. (2018) with some modifications in the elution system and sample injection. The mobile phase consisted of solvent A (water/acetonitrile/formic acid, 95:5:0.1, v/v/v), and solvent B (acetonitrile/formic acid, 100:0.1, v/v) with the elution profile as follows: 0–40 min, 5–30% B (v/v); 40–55 min, 30–95% B; 55–63 min, 95% B; 63–70 min, 95%–6% B; 70–80 min, 6% B (equilibration). The sample solutions of the extracts were prepared in methanol-water (7:3, v/v) at the concentration 5 mg/mL. The flow rate was 0.3 mL/min. UV-vis spectra were recorded over the range of 200–600 nm. The LC eluate was introduced directly into the ESI interface without splitting and analyzed in a negative ion mode. The analysis was carried out using a scan from m/z 200 to 2200.

The TPC and TPA were quantified by the Folin-Ciocalteu and *n*-butanol-HCl methods, respectively, as described previously (Olszewska et al., 2012). The results were expressed as equivalents of gallic acid (GAE) and cyanidin chloride (CYE), respectively.

The HPLC-PDA-fingerprint assays were performed according to Olszewska et al. (2012) with some modifications in the elution system. The mobile phase consisted of solvent A (0.5% water solution of orthophosphoric acid, w/v) and solvent B (acetonitrile) with the elution profile as follows: 0–1 min, 5% B (v/v); 1–12 min, 5–19% B; 12–18 min, 19% B; 18–19 min, 19–30% B; 19–20 min, 30–50% B; 20–21 min, 50–5% B; 21–25 min, 5% B (equilibration). The flow rate was 1.4 mL/min, and the column was maintained at 30°C. The phenolic analytes were quantified as equivalents of nine external standards: (–)-epicatechin (flavan-3-ols and proanthocyanidins), protocatechuic and *p*-hydroxybenzoic acids (hydroxybenzoic acids), chlorogenic acid (caffeoylquinic acid isomers), caffeic and *p*-coumaric acids (hydroxycinnamic acid derivatives), quercitrin (flavonoid monoglycosides), rutin (flavonoid diglycosides), and quercetin (flavonoid aglycones), depending on the PDA spectra.

The DPPH and FRAP tests were performed according to the method optimized previously (Olszewska et al., 2012) and expressed as normalized EC₅₀ values (calculated from concentration-inhibition curves) and μmol of ferrous ions (Fe²⁺) produced by 1 g of dry leaves or the dry extract (calculated from the calibration curve of ferrous sulphate), respectively.

DATA ANALYSIS

The results are reported as means ± SD (standard deviation) for the indicated number of experiments. The significance of differences between the samples and controls was determined with one-way ANOVA, followed by the post hoc Tukey's test for multiple comparisons. All calculations were performed using Statistica 12Pl software for Windows with *p* values less than 0.05 regarded as significant.

RESULTS

The qualitative UHPLC-PDA-ESI-MS³ analysis of the leaf extracts from *S. domestica* revealed the presence of over forty phenolic constituents (UHPLC peaks 1–44; Fig. 1, Table 1), forty-three of which were fully or tentatively identified by comparing their retention times, UV-Vis spectral data and MS profiles with those of the reference compounds and the literature data (Clifford et al., 2005; Fang et al., 2002; Mataczak et al., 2018; Ncube et al., 2014). According to the identification data, three major groups of compounds could be differentiated, including phenolic acids (peaks 1–5, 7, 8, 10, 13, 14, 17, 30), flavan-3-ols and proanthocyanidins (peaks 6, 9, 11, 12, 15, 16, 18–21, 26, 27), as well as flavonoids (peaks 22–25, 28, 29, 31–43). Direct comparison of the recorded UHPLC profiles revealed only small qualitative differences between the leaf samples from Poland and Croatia, i.e., caffeic acid hexoside (3), one procyanidin dimer B-type (9) and quercetin rhamnoside (35) occurred only in the Polish samples, while two *p*-coumaroylquinic acid isomers (13, 14), 3,4-dicaffeoylquinic acid (17) and kaempferol hexoside (31) were found only in the Croatian samples (Table 1). However, these discrepancies may result from detection limitations, as the mentioned compounds were present in the extracts in small amounts only, and for the majority of the individual analytes and groups of analytes the substantial quantitative variations were indicated.

The levels of total phenolics (TPC), determined by the Folin-Ciocalteu assay and expressed as gallic acid equivalents (GAE), differed significantly (*p* < 0.05) between the leaf materials from Poland (73.4–76.6 mg GAE/g leaf dw) and Croatia

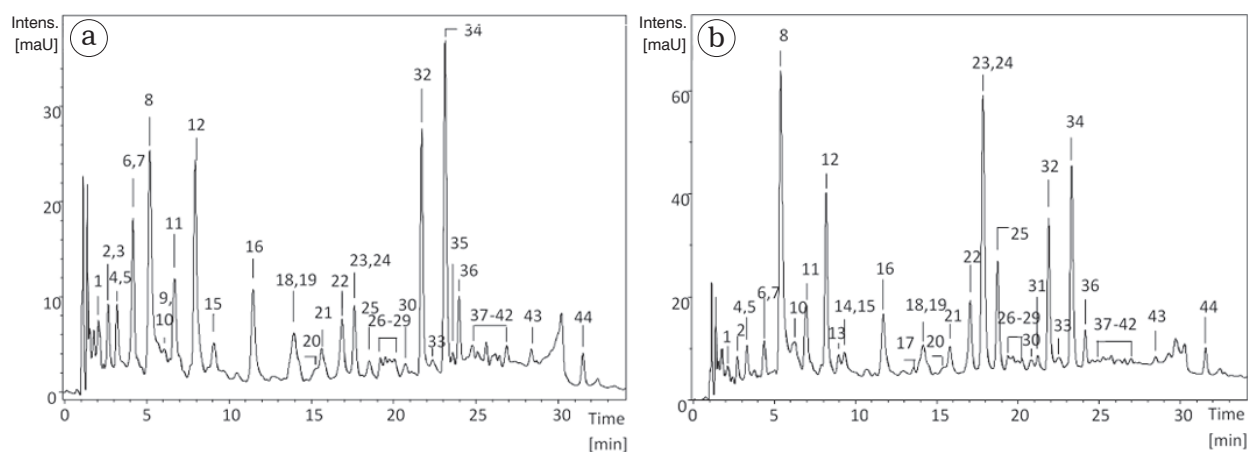


Fig. 1. Representative UHPLC-UV chromatograms of *Sorbus domestica* leaf extracts from Poland (a) and Croatia (b) at 280 nm. Peak numbers refer to those implemented in Table 1.

TABLE 1. UHPLC-PDA-ESI-MS data on polyphenols identified in *Sorbus domestica* leaf extracts.

No	Compound	R _t (min)	UV λ _{max} (nm)	[M-H] ⁻	MS ²	MS ³	Extract
1	chlorogenic acid hexoside	2.2	325	515	353 ,341,323,191	191,179	B
2	neochlorogenic acid hexoside	2.7	325	515	353 ,341,323,191,179	191,179	B
3	caffeic acid hexoside	2.7	325	341	179,161		P
4	3-O-caffeoylquinic acid (NCHA) [*]	3.3	325	353	191,179		B
5	protocatechuic acid hexoside	3.3	270	315	153,219		B
6	procyanidin dimer B-type	4.3	280	577	451, 425 ,407,289	407,273	B
7	<i>p</i> -coumaric acid hexoside	4.3	310	325	163,119		B
8	5-O-caffeoylquinic acid (CHA) [*]	5.4	325	353	191,179		B
9	procyanidin dimer B-type	6.2	280	577	451, 425 ,407,289	407,273	P
10	4-O-caffeoylquinic acid (CCHA) [*]	6.2	325	353	173,191,179		B
11	procyanidin B-2 (PB2) [*]	6.9	280	577	451, 425 ,407,289	407,273	B
12	(-)-epicatechin (ECA) [*]	8.2	280	289	245,205		B
13	<i>p</i> -coumaroylquinic acid isomer	9.0	310	337	191,163		C
14	<i>p</i> -coumaroylquinic acid isomer	9.2	310	337	191,163		C
15	proanthocyanidin dimer B-type	9.2	280	577	425,407,289		B
16	procyanidin trimer B-type (PC1)	10.5	280	865	713 ,695,577	695,425,407,289	B
17	3,4-dicafeoylquinic acid	13.6	280,325	515	353 ,335,299,255,191	173,179,191,135	C
18	procyanidin tetramer B-type	14.1	280	1153	863,739,575,451,425		B
19	procyanidin dimer B-type	14.1	280	577	451,425,407,289		B
20	proanthocyanidin derivative	15.6	280	720	635, 575 ,451,289	289, 279	B
21	procyanidin dimer hexoside	15.8	280	739	587 ,451,339,289	477,417,339,255	B

No	Compound	R _t (min)	UV λ _{max} (nm)	[M-H] ⁻	MS ²	MS ³	Extract
22	quercetin hexoside-rhamnoside	17.1	275,350	609	447,301	301	B
23	rutin (RT)*	17.8	256,355	609	463,301,179		B
24	hyperoside (HY)*	17.8	254,353	463	301	179	B
25	isoquercitrin (IQ)*	18.7	256,353	463	301	179	B
26	procyanidin dimer B-type	19.4	280	577	451, 425 ,407,289	289,341,245	B
27	proanthocyanidin derivative	19.7	280	483	451,341,289		B
28	quercetin hexoside derivative	19.9	275,350	519	357,301	151,135	B
29	reinutrin*	20.2	275,350	433	301		B
30	4,5-dicaffeoylquinic acid	20.8	280,325	515	353,299,255,203,173	173,179,191	B
31	kaempferol hexoside	21.2	275,350	447	285		C
32	quercetin hexoside-rhamnoside (HRQ)	21.8	256,350	609	447,301,179	301	B
33	quercetin hexoside-rhamnoside	22.5	275,350	609	447,301,179	301	B
34	quercitrin (QCT)*	23.3	276,350	447	301,179		B
35	quercetin rhamnoside	23.7	280,350	447	301	179,151	P
36	quercetin pentoside-rhamnoside (PRQ)	41.5	256,353	579	447,301	301	B
37	quercetin hexoside-rhamnoside	25.0	256,350	609	447,301	301	B
38	quercetin hexoside-rhamnoside	25.3	256,350	609	447,301	301	B
39	quercetin hexoside-rhamnoside	25.9	263,350	609	447	301	B
40	quercetin dirhamnoside	26.2	265,347	593	447	301	B
41	quercetin dirhamnoside	26.6	265,335	593	447,301	301	B
42	quercetin derivative	27.0	255,350	591	447,301		B
43	afzelin*	28.4	263,346	431	285,255		B
44	unidentified compound	31.5	280	451	341, 299	189	B

Peak numbers and retention times (R_t) refer to those implemented in Fig. 1. Compounds identified with authentic standards were marked with an asterisk. UV λ_{max}, absorbance maxima in PDA spectra. [M-H]⁻, pseudomolecular ions in MS spectra recorded in a negative mode. MS²/MS³, secondary ions (MS² ions in bold were subjected to MS³ fragmentation). P – Polish sample. C – Croatian sample. B – both extracts.

(98.3–106.7 mg GAE/g leaf dw), which was also reflected in the extraction yields (Figs. 2 and 3). However, it should be noted that the differences between years were for each country significantly smaller than those observed between countries, regardless of the harvest year. In the case of the concentrated phenolic fractions (leaf dry extracts), the differences in the TPC levels were generally less pronounced (P: 269.4–280.0 mg GAE/g extract dw; C: 297.6–304.4 mg GAE/g extract dw), and statistically significant ($p < 0.05$) only between countries (Fig. 3). Flavan-3-ols and proanthocyanidins were the dominant groups of polyphenols in all plant samples with

(–)-epicatechin (12) and procyanidin B2 (11) as prevailing constituents (Figs. 1 and 2). The total proanthocyanidin content (TPA), determined by *n*-butanol/HCl assay and expressed in cyanidin chloride equivalents (CyE), in all plant materials constituted about 30–35% of their TPC levels, while the respective total contents (TLPA) determined by HPLC-PDA represented 59–64% (P) and 45–52% (C) of total phenols calculated as the sum of individual analytes (TPH, Fig. 4), depending on the harvest year. The second relevant group of constituents were flavonoids, which constituted 14–20% (P: 3.61 mg/g dw of the leaf material in 2014 and 4.18 mg/g in 2016) and 28–34% (C: 9.38 mg/g

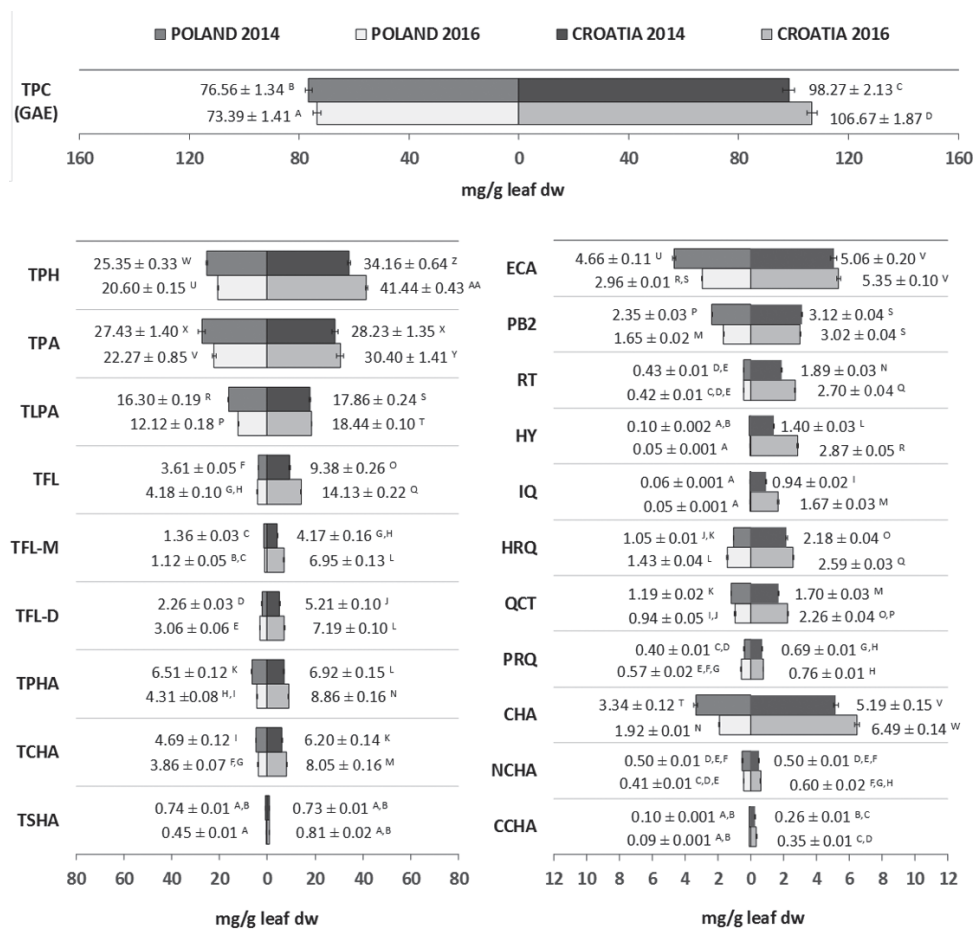


Fig. 2. Quantitative profile of *Sorbus domestica* leaves from Poland and Croatia harvested during two years (2014 and 2016). Data expressed as mean values ± SD (n = 3). For each parameter different capitals indicate significant differences ($p < 0.05$). TPC, total phenolic content in gallic acid equivalent (GAE) determined by the Folin-Ciocalteu assay; TPH, total phenolic content determined by HPLC-PDA; TPA, total proanthocyanidin content determined by the *n*-butanol/HCl assay; TLPA, total content of individual proanthocyanidins assayed by HPLC; TFL, total flavonoid content; TFL-M, total content of flavonoid monoglycosides; TFL-D, total content of flavonoid diglycosides; TPHA, total content of phenolic acids; TCHA, total content of chlorogenic acid isomers and derivatives; SPHA, total content of simple hydroxybenzoic and hydroxycinnamic acids; ECA, (-)-epicatechin; B2, procyanidin B2; RT, rutin; HY, hyperoside; IQ, isoquercitrin; HRQ, quercetin hexoside-rhamnoside; QCT, quercitrin; PRQ, quercetin pentoside-rhamnoside; CHA, chlorogenic acid; NCHA, neochlorogenic acid; CCHA, cryptochlorogenic acid.

in 2014 and 14.13 mg/g in 2016) of TPHs, respectively (Figs. 2 and 4). Interestingly, in the Croatian leaves the total contents of flavonoid monoglycosides (TFL-M) and diglycosides (TFL-D) were similar, while in the plant materials from Poland flavonoid diglycosides predominated and constituted about 62–73% of TFL (Fig. 4). Taking into account the profile of individual compounds, hyperoside (24) and isoquercitrin (25) occurred in the Polish samples in trace amounts only, while in the Croatian samples they were among the main flavonoids (Figs. 1 and 2, Table 1). The total content of phenolic acids (TPHA, Fig. 2) constituted about

20–21% of the TPH levels (Fig. 4). In this group chlorogenic acid isomers and derivatives (TCHA) were predominant and represented 86–91% of TPHA. The major isomer was chlorogenic acid (8) forming about 50–70% of the TCHA content in the Polish samples and 80–84% in the Croatian samples (Figs. 1 and 2). Chlorogenic acid was also the principal polyphenol occurring in the leaves from Croatia (5.19–6.49 mg/g) and the second main phenolic compound present in the Polish samples (1.92–3.34 mg/g) (Fig. 2).

Confirming previous results (Matczak et al., 2018), the analyzed leaf samples exhibited

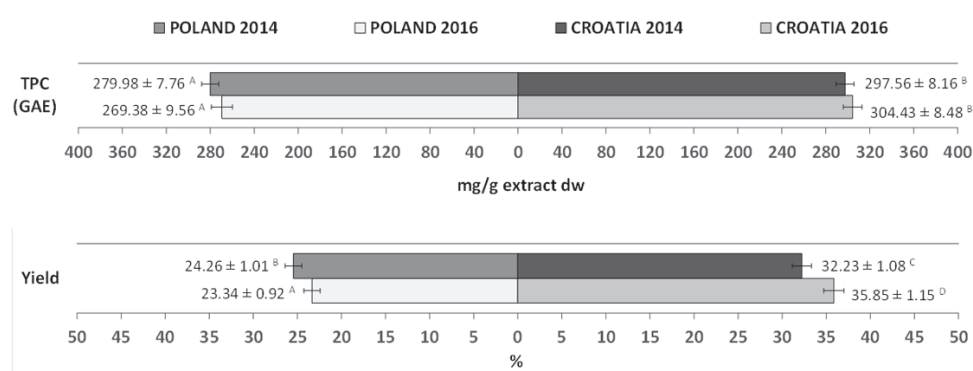


Fig. 3. Extraction yield and total phenolic contents (TPC) of *Sorbus domestica* leaf extracts determined by the Folin-Ciocalteu assay. Data expressed as means ± SD (n = 3). For each parameter different superscripts indicate significant differences ($p < 0.05$) in the Tukey's test.

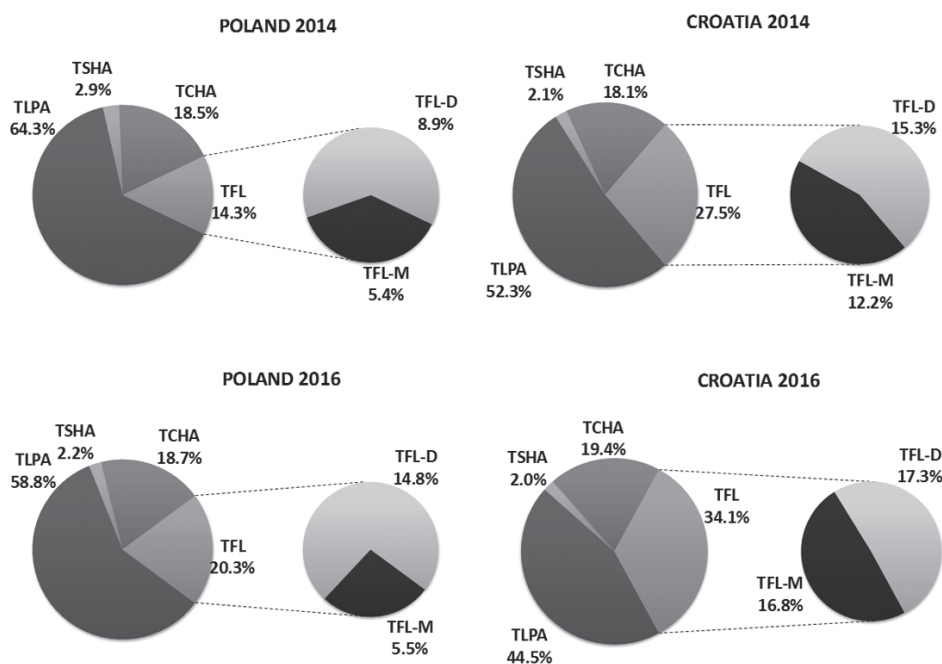


Fig. 4. Contribution of individual groups of compounds to total phenolic contents determined by HPLC-PDA and calculated as the sum of individual analytes (TPH) in *Sorbus domestica* leaves from Poland and Croatia harvested during two years (2014 and 2016). For TLPA, TFL, TFL-M, TFL-D, TCHA, SPHA codes see Fig. 2.

a concentration-dependent activity (Fig. 5), clearly corresponding to the levels of phenolics (Fig. 2). However, the differences in the TPC levels between the dry extracts of different origin (Fig. 3) were too small to be reflected in their DPPH and FRAP parameters, which were thus comparable (DPPH EC_{50} : 10.04–10.89 μg of extract dw/mL; and FRAP: 6.64–7.13 mmol Fe^{2+} /g of extract dw; $p > 0.05$), regardless of the year and country of the harvest (Fig. 5).

DISCUSSION

Variation in the phenolic composition is a common phenomenon in the plant kingdom. The presence of active substances and changes in their content are influenced by various environmental conditions, including climate, soil composition, availability of water and nutrients, as well as the stage of plant development (Ncube et al., 2012). It is known that obtaining a raw material with the desired biological

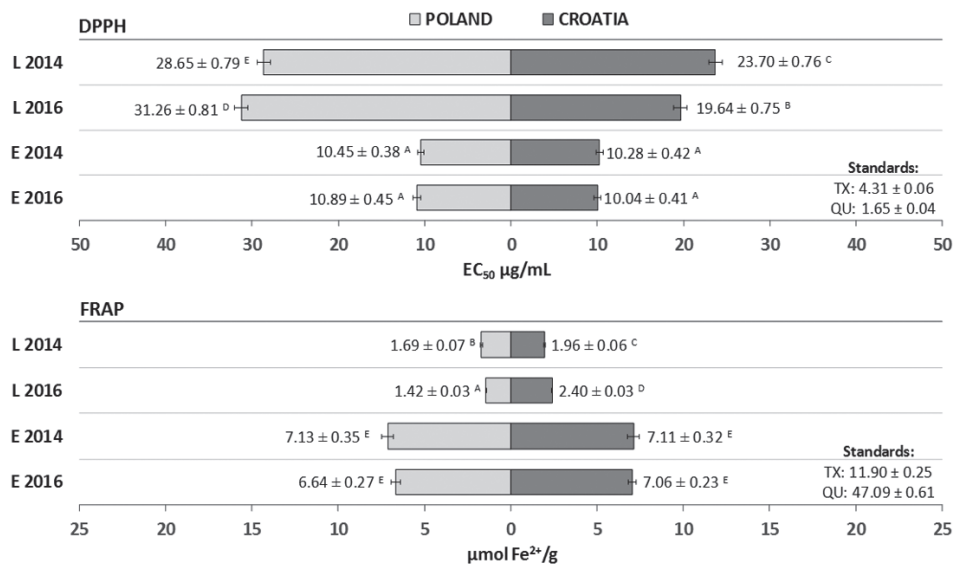


Fig. 5. Antioxidant activity of *Sorbus domestica* leaves and *S. domestica* leaf dry extracts from Poland and Croatia (2014 and 2016 year). Results are presented as mean values ± SD (n = 3) calculated per dry weight of leaves (L) or extracts (E). For each parameter (DPPH and FRAP tests) different letters indicate significant differences ($p < 0.05$) in the Tukey's test. DPPH-scavenging activity: EC₅₀, expressed in μg of dry leaves (L2014/L2016) or dry extract (E2014/E2016) per mL of the DPPH solution. FRAP activity: expressed in μmol of ferrous ions (Fe²⁺) produced by 1 g of dry leaves (L2014/L2016) or dry extract (E2014/E2016).

properties requires its collection at a specific time of the year or, sometimes, even the day. For example, during the development of flowers, the ingredients are often allocated to generative organs, and as a result, their content decreases in other parts of the plant, such as leaves (Duda et al., 2015; Ncube et al., 2012). However, numerous studies showed that changes in the phenolic content over growing season in perennial trees and shrubs are relatively small in comparison to those observed for herbaceous plants (Michel et al., 2017; Olszewska and Kwapisz, 2011). Still, the period after flowering and before fruit maturity is characterized by the strongest accumulation of phenolics in the leaves of fruit trees, including *Sorbus* species (Cosmulescu and Trandafir, 2011; Olszewska, 2011). Indeed, our preliminary investigation on seasonal variation of the phenolic content in *S. domestica* leaves in Poland (data not published) confirmed the above mentioned trends and indicated August as the most advantageous for comparison of the raw material quality from Poland and Croatia.

In general, the total phenolic contents in the tested samples were comparable to those obtained in our previous study for the *S. domestica* leaf dry extracts (Matczak et al., 2018) and confirmed the ability of the plant to accumulate high contents of active compounds in the leaves. They put the investigated extracts in a good position compared to

the leaf and flower dry extracts of other *Sorbus* plants (211.7–306.2 mg GAE/g dw), as well as to the extracts from plants considered important in the prevention of oxidative stress-related ailments, e.g., green tea (450 mg GAE/g dw) (Matczak et al., 2018; Olszewska et al., 2012). Moreover, the remarkable consistency of the polyphenolic composition between the harvest years, i.e., 2015 (Matczak et al., 2018), 2014 and 2016 (present work) was also noticed for the samples of Polish origin.

The comparison of the samples from Poland and Croatia harvested in 2014 and 2016, leads to the conclusion, that the variations in the phenolic profile between the samples from different years were relatively small in comparison to the differences found between the countries. The discrepancies observed in the TPC levels between the leaf samples from Poland and Croatia, and thus in the extraction yields, may result from the well-known tendency of plants to accumulate higher quantities of some secondary metabolites in response to oxidative stress conditions (Ncube et al., 2012; Roux et al., 2017; Wei et al., 2017). It was reported that UV-B radiation and drought stress increase the expression of genes responsible for biosynthesis of polyphenols, mainly flavonoids and hydroxycinnamates (Agati and Tattini, 2010; Ncube et al., 2012; Zhang, Wu et al., 2017). Indeed, higher UV Index and longer sunshine duration (lower cloud percentage and

more sunny days) in the summer months in Croatia (Table 2) resulted in a higher phenolic content in the Croatian samples. However, the differences observed between samples were not as remarkable as one would expect. The explanation might be linked to the influence of water stress on the production of some metabolites, e.g., condensed proanthocyanidins and tannins. According to literature, this correlation can be either positive or negative (Ncube et al., 2012; Zhang, Wu et al., 2017). Considering the fact that *S. domestica* naturally grows in dry to very dry conditions (Poljak et al., 2015), a higher precipitation level in Poland (rainy days: P: ~21–22 vs C: ~9–13) might influence the synthesis of some secondary metabolites.

TABLE 2. Meteorological data for the *Sorbus domestica* harvesting sites in August 2014 and 2016.

Average ^a	Poland ^b		Croatia ^c	
	2014	2016	2014	2016
Temperature (°C)	23	20	23	27
UV Index	5	5	6	7
Cloud (%)	34	39	20	23
Sunny days	7	9	11	22
Sunny hours	331	295	321	338
Humidity (%)	69	69	76	72
Rainy days	22	21	13	9

^a Averages based on World Weather Online forecast analysis. ^b Data for Lodz (about 25 km from Rogow Arboretum). ^c Data for Risika on Krk island.

Further determination of the main phenolic groups and individual compounds, performed using HPLC-PDA and specific UV-spectrophotometric methods, revealed that in fact the greatest qualitative and quantitative differences were observed for flavonoids. According to literature, excessive UV-B radiation increases the biosynthesis of flavonoids, mainly those with catechol group in the B-ring of the flavonoid skeleton (e.g., quercetin derivatives), which reduce photo-oxidative damage and regulate the development of plants via modulation of auxin movement (Agati and Tattini, 2010; Harborne and Williams, 2000; Tattini et al., 2004; Zhang, Chen et al., 2017). One of the molecules, whose content in plant tissues appears to be highly dependent on UV irradiation, is hyperoside (Chen et al., 2018; Zhang, Wu et al., 2017; Zhang, Chen et al., 2017). This compound, being the effective scavenger of reactive oxygen species, is suggested to play a crucial role in plant photoprotection (Agati and Tattini, 2010; Silva et al., 2008; Zhang, Chen et al., 2017). This may be

the reason of the significant increase in the hyperoside content (by about 1.3 mg/g in 2014 and 2.8 mg/g in 2016) in the *S. domestica* samples from Croatia in comparison to the Polish samples (Fig. 2). Another flavonoid, the content of which substantially differed between leaf samples (by about 1.5 mg/g in 2014 and 2.3 mg/g in 2016), was rutin. According to Zhang, Wu et al. (2017), rutin accumulation depends not only on the UV-B irradiation but also on salt and drought stress, which may explain its higher content in the samples from the Mediterranean region (Fig. 2).

The next group of compounds, which seems to be highly dependent on environmental conditions, is formed by chlorogenic acid isomers and derivatives. Numerous studies indicated that the biosynthesis of hydroxycinnamic and hydroxybenzoic acid derivatives manifests an early reaction to UV exposure (Huyskens-Keil et al., 2007; Petrul'ová et al., 2014; Tattini et al. 2004). Considering its molecular structure and properties, chlorogenic acid is considered to be a potent free radical scavenger, as well as strong UV-B attenuator (Agati and Tattini, 2010; Cha et al., 2014; Tattini et al., 2004). On the other hand, it was reported that the hydroxycinnamates to flavonoids ratio decreases upon exposure to strong sunlight, suggesting the increase in the flavonoid biosynthesis at the expense of hydroxycinnamic acid derivatives under UV irradiation (Agati and Tattini, 2010). Indeed, the TCHA/TFL ratio in the Croatian samples (about 0.7 in 2014 and 0.6 in 2016) was lower than in the Polish samples (about 1.3 in 2014 and 0.9 in 2016). However, to better understand the changes in the profiles of the secondary metabolites in the investigated species depending on environmental conditions, further studies are necessary, covering samples from various parts of Europe, including both target countries.

Plant extracts are complex matrices composed of multiple molecules with various mechanisms of activity. Our previous study (Matczak et al., 2018) demonstrated that *S. domestica* leaf extracts are potent antioxidants in both chemical and biological models, and that their concentration-dependent activity is determined by the content of phenolics, primarily flavan-3-ols, proanthocyanidins and flavonoids. The DPPH free radical scavenging activity and ferric reducing ability (FRAP) turned out to be good tools for the preliminary estimation of antioxidant capacity of this plant material and for comparison of the received data with other studies. We showed that the results of DPPH and FRAP tests correlate with the TPC, TPH, TLPA and TFL levels, as well as with antioxidant activity parameters of *S. domestica* leaf extracts in a biological model of human plasma exposed to oxidative stress, and that they can be used as biological activity markers

of *S. domestica* leaf samples (Matczak et al., 2018). Thus, these two chemical tests were selected to compare the antioxidant capacity of the leaves from Poland and Croatia. Interestingly, although the TPC values of dry extracts were slightly higher for Croatian samples, the activity parameters of all defatted leaf extracts did not differ statistically. This may be due to the proportions between the particular groups of compounds, especially flavan-3-ols, proanthocyanidins and flavonoids, and their influence on the plant antioxidant capacity. For instance, in the Polish samples flavan-3-ols and low-molecular-weight proanthocyanidins (TLPA) constituted approximately 59-64% of the TPH level, while in the Croatian samples their percentage contribution (ca. 45-52%) was significantly decreased with the increasing percentage of flavonoids (ca. 28-34%). Indeed, according to our previous research, ECA (the main flavan-3-ol in the *S. domestica* leaves) was stronger antioxidant than flavonoid glycosides in all tests, including FRAP and DPPH (Matczak et al., 2018).

Summarising the results of the present study, it should be expected that extracts obtained from *S. domestica* leaves cultivated in various geographical regions might exhibit comparable therapeutic properties. Despite the differences in the total phenolic contents, as well as individual molecules in the plant raw materials, the defatted dry extracts prepared from the Polish and Croatian leaf samples harvested in different years (and thus exposed to various environmental conditions) were exceptionally rich in highly active polyphenolic compounds, and could be considered as biologically equal. Taking into account the fact that in our previous studies (Matczak et al., 2018) significant differences in the content of polyphenols in various extracts from *S. domestica* resulted in comparable plasma protective activity, both samples from Poland and Croatia could be considered as promising raw materials for future applications. Nevertheless, the economic value of the particular plant material as a source of active extracts may be well defined by the extraction yield.

CONCLUSIONS

Taking into account the observed differences between the samples, as well as climatic changes between years and locations, it can be concluded that the latter have a dominant influence on the variability of the analyzed parameters. However, the present research provides only preliminary information on the variations in the phenolic profile of *S. domestica* leaves. Further research covering samples from different parts of Europe is required to better understand the influence of environmental conditions on the production

of plant secondary metabolites, and thus on the quality of leaves. Nevertheless, the study demonstrated that the leaves of service tree, both from the Polish cultivation and from their natural habitat in Croatia, have a great potential as a source of active polyphenols. Considering both the content of active compounds and the extraction yields, leaves from Croatia seem to be more advantageous for industrial purposes than the Polish samples. However, although environmental conditions depending on the year and/or location had an influence on secondary metabolite production, qualitative and quantitative differences in the phenolic profile of the raw materials did not significantly affect antioxidant parameters of the products derived thereof, i.e., dry extracts. Therefore, the leaves of *S. domestica* from both sources seem appropriate for production of extracts with health-promoting properties.

AUTHOR'S CONTRIBUTIONS

MR: research concept and design, collection and assembly of data, data analysis and interpretation, manuscript preparation, final approval of manuscript; MD: collection and assembly of data, data analysis and interpretation; MAO: research concept and design, data analysis and interpretation, critical revision of the manuscript, final approval of manuscript. The authors declare no conflict of interest.

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