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ORIGINAL PAPER

PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF WILLOW BARK OBTAINED IN AN ANNUAL CUTTING CYCLE*

Kazimierz Warmiński[1,3], Mariusz J. Stolarski[2,3], Łukasz Gil[2,4], Michał Krzyżaniak[2,3]

[1] Department of Chemistry
[2] Department of Genetics, Plant Breeding and Bioresource Engineering
[3] Centre for Bioeconomy and Renewable Energies
University of Warmia and Mazury in Olsztyn, Poland
[4] ChemProf Doradztwo Chemiczne s.c. Michał Łuczyński, Katarzyna Łuczyńska and ChemProf sp. z o.o., Gutkowo, Olsztyn, Poland

Abstract

Willow bark is an interesting source of bioactive substances. However, the extremely wide diversity of willow species and genotypes makes it necessary to search for the most useful ones. Preferably, the bark of the best willow genotypes should come from field plantations, which would guarantee the high quality and stability of this bioresource. Therefore, the aim of this study was to determine the phenolic and flavonoid content, the antioxidant capacity as well as the potential yield of bioactive substances from bark of ten willow genotypes harvested in two annual cutting cycles. The research material consisted of the bark of four genotypes of *Salix purpurea*, two genotypes of *S. americana*, two genotypes of *S. triandra* and two genotypes of interspecies hybrids of *S. purpurea* × *S. daphnoides*. One-year-old willow shoots where harvested in March 2018 and 2019 from a field experiment conducted by the Department of Genetics, Plant Breeding and Bioresource Engineering. The highest total flavonoid content was found in the bark of *S. purpurea* UWM 166, whereas the total phenolic content and total antioxidant capacity were the highest for *S. triandra* UWM 197. The highest potential yield of total phenolic was obtained from *S. americana* UWM 094 and *S. triandra* UWM 197. The highest potential yield of total flavonoids was obtained from *S. purpurea* × *S. daphnoides* UWM 193 genotype.

Kazimierz Warmiński, Ph.D., Department of Chemistry, Faculty of Agriculture and Forestry, University of Warmia and Mazury in Olsztyn, ul. Prawocheńskiego 17, 10-720, Olsztyn, Poland, e-mail: kazimierz.warminski@uwm.edu.pl, ORCID ID: 0000-0003-1674-2319.

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The content of bioactive substances and their potential yields were the highest in the second year of the study.

Keywords: perennial industry crops, *Salix purpurea*, *Salix triandra*, bioactive compounds, polyphenols, flavonoids, free radical scavenging activity.

INTRODUCTION

The lignocellulosic biomass from perennial industry crops could be a source of bioactive substances form multipurpose use (Grzegorczyk et al. 2019, Malm et al. 2021). And willow short rotation coppice is an interesting crop for this reason (Sulima et al. 2006, 2017). The genetic diversity of willow is the underlying cause of the extremely different content of bioactive substances determined among species but also among varieties or genotypes of one species. Willow bark contains multiple secondary metabolites with evidenced bioactivity (Mahdi 2010, Peng et al. 2017). This activity refers to both the plants in which these substances occur and also to other organisms. Bioactive compounds are not indispensable for plant growth. However, some play certain functions in plant response to biotic and abiotic factors. The major bioactive substances found in willow biomass include phenolic compounds, but there are also terpenoids, phytosterols, alkaloids and resins in it. Phenols are plant pigments, which protect plants from pests and pathogens, as well as playing a positive role in oxidative stress (Heiska et al. 2007, Cheynier et al. 2013).

The structure of phenolic compounds includes aromatic rings carrying one or more hydroxyl moieties. They are divided into multiple classes, such as phenolic acids, flavonoids, stilbenes, lignans and tannins (Blomhoff 2010, Falcone Ferreyra et al. 2012). Phenolic acids include hydroxybenzoic acids (salicylic acid, pyrocatechuic acid etc.) and hydroxycinnamic acids (caffeic acid, ferulic acid, and many others). However, it is the flavonoids that are the most diversified group of phenolic compounds, implicating the greatest biological importance. The number of plant flavonoids is estimated at over eight thousands. They are grouped in such sub-classes as flavonols, flavanols, flavones, flavanones, isoflavones and anthocyanins (Blomhoff 2010). Flavonoids occur in greater amounts in flowers and fruit, imparting yellow, orange, red, or purple color to them (Falcone Ferreyra et al. 2012). Besides, they exhibit antioxidative and antimicrobial activities (Joshi et al. 2015). It needs to be emphasized that these properties are exploited in dietetics (human nutrition) and medicine. Flavonoids have also been detected in other morphological parts of plants, e.g. in bark and leaves of willow (Pobłocka-Olech 2006, Paunonen et al. 2009). The aerial biomass of willow contains also other phenols, including lignans that elicit anticarcinogenic and antidiabetic properties (Brereton et al. 2017). Different pharmacological effects are ascribed to phenolic glucosides, also known as salicylic compounds. They

occur mainly in plants from the family *Salicaceae*, and their most known representative is salicin (salicyl alcohol glucoside) that was discovered back in the 19th century. In the human gastrointestinal tract, salicin is hydrolyzed to salicyl alcohol, which is further oxidized to salicylic acid. In humans and animals, salicylic acid exhibits antipyretic, analgesic, anti-inflammatory, antirheumatic and anticoagulant properties (Mahdi 2010, Noleto-Dias et al. 2018).

Noteworthy is that the interest spurred so far by willow bioactive compounds has been associated with phenolic glucosides found in bark, mainly in such species as *Salix purpurea* and *S. alba*. Nevertheless, recent studies have shown that extracts from the whole biomass, i.e. from bark and wood (and not only from bark) of *S. viminalis* willow can demonstrate significant bioactivity (Ostolski et al. 2021).

Using liquid and gas chromatography, it is possible to determine individual bioactive compounds in food, feed and pharmaceutical products as well as raw materials. However, chromatographic methods are expensive and time consuming. Alternative methods used for screening plant materials are spectrophotometric methods. They enable a quick but less accurate assessment of the total content of some groups of bioactive compounds. The best-known parameters are total phenolic (TPC), flavonoid (TFC), tannin (TTC), flavan-3-ol (TF3L), flavanone and dihydroflavonol (FDC), monomeric anthocyanin (TMA) content and total antioxidant capacity (TAC) – Zielińska et al. (2018), Tyśkiewicz et. al. (2019). As mentioned above, willow bark is interesting primarily for its content of salicylic glycosides. However, it can also be a valuable source of bioactive substances with antioxidant activity. Therefore, the novelty of these studies was the phytochemical screening of various willow genotypes. The aim of this study was to determine the phenolic and flavonoid content, the antioxidant capacity as well as the potential yield of bioactive substances from bark of ten willow genotypes harvested in two annual cutting cycles.

MATERIALS AND METHODS

Plant material

The subject of these studies was the bark of ten willow genotypes harvested in two consecutive one-year harvest cycles. There were four genotypes of *S. purpurea* (Bona cultivar, UWM 062, UWM 166, UWM 168), two genotypes of *S. americana* (UWM 031, UWM 094), two genotypes of *S. triandra* (UWM 055, UWM 197) and two genotypes of interspecies hybrids of *S. purpurea* × *S. daphnoides* (UWM 029, UWM 193).

The willow bark was collected from a field experiment conducted by the Department of Genetics, Plant Breeding and Bioresource Engineering,

Faculty of Agriculture and Forestry, University of Warmia and Mazury in Olsztyn, Poland. One-year-old willow shoots where harvested in March 2018 and 2019. Immediately after harvesting, the shoots were debarked and the bark was dried at 40°C. Subsequently, the bark was ground in an mill with a 1 mm mesh sieve.

Total phenolic, flavonoid content and antioxidant capacity determination

The total phenolic content (TPC) was determined by the Folin-Ciocalteu method in methanol-water extract of willow bark (2 g/25 cm^3 MeOH:H_2O 60:40 v/v) – Baba, Malik (2015), Zielinska et al. (2018). In short, extract of the bark (sample) or a blank (0.09 cm^3), the Folin-Ciocalteu's reagent (0.09 cm^3), saturated solution of Na_2CO_3 (0.18 cm^3) and distilled water (1.44 cm^3) were mixed in a test tube. The mixture was incubated for 30 min at room temp. (25°C). Subsequently, absorbance was measured at the wavelength of 725 nm with a Spectrum Two spectrophotometer (Perkin Elmer) against gallic acid as the reference standard. The standard curve was prepared in the concentration range of 8.6-86.0 μg cm^{-3}. The results were expressed as the gallic acid equivalent (GAE) in mg g^{-1} of air-dry bark. The measurements were performed in three analytical replicates.

The total flavonoid content (TFC) in the bark extract was determined by the aluminium chloride colorimetric method (Baba, Malik 2015, Zielinska et al. 2018). In short, bark extract (sample) or a blank (1.23 cm^3) was mixed in a test tube with distilled water (1.25 cm^3) and a 5% (w/v) solution of $NaNO_2$ (0.062 cm^3). Subsequently, 10% solution of $AlCl_3$ (0.123 cm^3) was added. The mixture was incubated for six minutes at room temperature (25°C). Following this, 0.41 cm^3 of NaOH solution (1 mol dm^{-3}) was added. The mixture was centrifuged for 10 min at 2,000 g (Centrifuge MiniSpin® plus, Eppendorf). Subsequently, absorbance was measured at 510 nm with a Spectrum Two spectrophotometer (Perkin Elmer) against (-)-catechin as the reference standard. The standard curve was prepared in the concentration range of 7.6-76.0 μg cm^{-3}. The results were expressed as the catechin equivalent (CE) in mg g^{-1} of air-dry bark. The measurements were performed in three analytical replicates.

The total antioxidant capacity (TAC) was determined as DPPH• scavenging activity (Zielińska et al. 2012, Baba, Malik 2015). The same protocol was used to examine the bark extract and Trolox standard solutions. Trolox was used in the concentration range of 25.2-252.0 μg cm^{-3} in 60% methanol (v/v). In short, bark extracts (0.1 cm^3) were mixed with a methanol solution of DPPH• (0.25 cm^3) and the mixture was incubated for 20 min at room temp. (25°C). The absorbance was measured at 517 nm with a Spectrum Two spectrophotometer (Perkin Elmer). TAC was converted to the Trolox equivalent (TE) in mg g^{-1} of air-dry bark. The measurements were performed in three analytical replicates. Additionally, based on the dry bark yield and

the content of the components, the potential yield of total phenolic and flavonoid content was calculated.

Statistical analysis

The statistical analysis of the results was performed with Statistica PL software. The arithmetic means were calculated for all studied features. Subsequently, homogeneous groups with a significance level of $P<0.05$ were determined using the Tukey's significance (HSD) test. The correlation coefficients between the analyzed features were also calculated.

RESULTS AND DISCUSSION

The flavonoid, phenolic content, antioxidant capacity and potential yield of bioactive substances yield were significantly differentiated by the genotype and the year as well as by their interaction (Table 1). The highest TFC

Table 1

Analysis of variance (*P* values) repeated measure for the analysed features

Source of variation	Total flavonoid content	Total phenolic content	Total antioxidant capacity	Potential yield of flavonoid content	Potential yield of phenolic content
Genotype	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Year	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Year x Genotype	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

* significant values

(7.15 mg g^{-1} CE) was found in the bark of *S. purpurea* UWM 166 (Table 2). The second homogeneous group included *S. purpurea* UWM 168 and the intraspecies hybrid UWM 193. The lowest TFC (1.56 mg g^{-1} CE on average) was found in the bark of *S. triandra* UWM 055. It was also the significantly lowest level in both study years. The average TFC in the second study year (5.65 mg g^{-1} CE) was 25% higher than in the first year. It ranged from 1.10 to 8.10 mg g^{-1} CE throughout the experiment, depending on the genotype and on the study year (Table 2). In studies by other authors, the TFC in the bark of *S. purpurea*, *S. daphnoides* and *S. myrsinifolia* was 3.0-5.3, 8.4-14.4 mg g^{-1} and 7.5-8.5 mg g^{-1}, respectively (Paunonen et al. 2009, Köhler et al. 2020). Brereton et al. (2017) showed that the biomass of two-year-old shoots of *S. viminalis*, *S. dasyclados* and *S. miyabeana* contains 0.01-0.38 mg g^{-1} flavonoids.

Total phenolic content (TPC) in the willow bark from annual shoots was higher than TFC. *S. triandra* UWM 197 contained the highest TPC levels

Table 2

Total flavonoid content (TFC) in the willow bark from annual shoots depending on the genotype and harvest year (mg g^{-1} CE)

Genotype	Harvest year		
	2018	2019	mean
S. purpurea cv. Bona	4.26±0.05 *g*	6.64±0.01 *d*	5.45±0.53 *C*
S. purpurea UWM 062	4.74±0.01 *f*	6.04±0.07 *e*	5.39±0.29 *C*
S. purpurea UWM 166	6.20±0.05 *e*	8.10±0.07 *a*	7.15±0.43 *A*
S. purpurea UWM 168	7.69±0.03 *b*	4.87±0.04 *f*	6.28±0.63 *B*
S. americana UWM 031	1.63±0.03 *i*	4.64±0.07 *f*	3.13±0.68 *E*
S. americana UWM 094	4.60±0.04 *f*	4.33±0.06 *g*	4.46±0.07 *D*
S. triandra UWM 055	1.10±0.02 *j*	2.03±0.02 *h*	1.56±0.21 *F*
S. triandra UWM 197	4.80±0.00 *f*	6.19±0.03 *e*	5.50±0.31 *C*
S. purpurea × *S. daphnoides* UWM 029	4.20±0.03 *g*	7.15±0.02 *c*	5.68±0.66 *C*
S. purpurea × *S. daphnoides* UWM 193	6.01±0.08 *i*	6.50±0.03 *d*	6.25±0.11 *B*
Mean	4.52±0.35 *Y*	5.65±0.3 *X*	5.09±0.24

Capital letters A,B,C… denote homogeneous groups for genotypes; X, Y… denote homogeneous groups for the years; a,b,c….. denote homogeneous groups for genotype x study year interactions; no letters denotes no significant differences; ± standard error of mean.

with an average of 48.16 mg g^{-1} GAE, homogeneous group "A" (Table 3). The second homogeneous group "B" included bark of two genotypes *S. purpurea* × *S. daphnoides* (over 36 mg g^{-1} GAE) and *S. americana* UWM 094

Table 3

Total phenolic content (TPC) in the willow bark from annual shoots depending on the genotype and harvest year (mg g^{-1} GAE)

Genotype	Harvest year		
	2018	2019	mean
S. purpurea cv. Bona	10.38±0.26 *j*	24.78±0.31 *g*	17.58±3.23 *G*
S. purpurea UWM 062	16.13±0.17 *h*	36.00±0.27 *d*	26.07±4.45 *D*
S. purpurea UWM 166	14.62±0.35 *i*	31.79±0.1 *e*	23.2±3.84 *E*
S. purpurea UWM 168	26.43±0.17 *f*	16.74±0.46 *h*	21.58±2.18 *F*
S. americana UWM 031	12.16±0.28 *j*	43.41±0.1 *c*	27.78±6.99 *C*
S. americana UWM 094	37.34±0.45 *d*	32.71±0.27 *e*	35.02±1.06 *B*
S. triandra UWM 055	18.92±0.47 *h*	23.45±0.28 *g*	21.18±1.04 *F*
S. triandra UWM 197	41.97±0.55 *c*	54.36±0.58 *b*	48.16±2.79 *A*
S. purpurea × *S. daphnoides* UWM 029	13.73±0.28 *i*	59.57±1.34 *a*	36.65±10.27 *B*
S. purpurea × *S. daphnoides* UWM 193	28.24±0.35 *f*	44.21±0.83 *c*	36.23±3.59 *B*
Mean	21.99±1.94 *Y*	36.70±2.42 *X*	29.35±1.81

Explanation see Table 2

Table 4

Total antioxidant capacity (TAC) of willow bark from annual shoots depending on the genotype and harvest year (mg g^{-1} TE)

Genotype	Harvest year		
	2018	2019	mean
S. purpurea cv. Bona	7.38±0.13 *j*	14.47±0.29 *i*	10.92±1.59 *I*
S. purpurea UWM 062	14.02±0.31 *i*	30.55±0.33 *g*	22.29±3.7 *G*
S. purpurea UWM 166	15.36±0.11 *i*	20.88±0.51 *h*	18.12±1.26 *H*
S. purpurea UWM 168	17.92±0.19 *h*	7.72±0.14 *j*	12.82±2.28 *I*
S. americana UWM 031	14.04±0.36 *i*	57.53±0.26 *d*	35.78±9.73 *D*
S. americana UWM 094	64.38±0.52 *c*	54.81±1.49 *d*	59.60±2.25 *B*
S. triandra UWM 055	46.64±0.8 *e*	35.85±0.47 *f*	41.24±2.45 *B*
S. triandra UWM 197	78.95±0.43 *b*	99.83±1.35 *a*	89.39±4.71 *A*
S. purpurea × *S. daphnoides* UWM 029	11.95±0.15 *i*	54.81±0.52 *d*	33.38±9.59 *E*
S. purpurea × *S. daphnoides* UWM 193	19.55±0.33 *h*	38.08±0.64 *f*	28.82±4.15 *F*
Mean	29.02±4.42 *Y*	41.45±4.73 *X*	35.24±3.31

Explanation see Table 2

Table 5

Simple correlation coefficient between the bioactive substances

Item	Total antioxidant capacity	Total flavonoid content	Total phenolic content
Total antioxidant capacity	1.00		
Total flavonoid content	-0.02	1.00	
Total phenolic content	0.79*	0.45*	1.00

* significant values (P<0.05)

(over 35 mg g^{-1} GAE). The lowest TPC was found in *S. purpurea* cv. Bona bark, 17.58 mg g^{-1} GAE. The mean TPC in the second year (36.70 mg g^{-1} GAE) was almost 67% higher than in the first year (21.99 mg g^{-1} GAE). A particularly high TPC (59.57 mg g^{-1} GAE) was found in the second study year in the bark of *S. purpurea* × *S. daphnoides* UWM 029 and *S. triandra* UWM 197 (54.36 mg g^{-1} GAE). In German studies it was shown that the bark of *S. purpurea* and *S. daphnoides* contains 47.5-81.3 and 67.8-84.8 mg g^{-1} total phenols, respectively (Köhler et al. 2020). TPC in the bark of annual *S. pyrolifolia* shoots was 289 mg g^{-1} (Lavola et al. 2018).

The TAC (DPPH assay) of the willow bark was the highest for *S. triandra* UWM 197 with an average of 89.39 mg g^{-1} TE (Table 4). The second homogeneous group "B" included bark of two genotypes *S. americana* UWM 094 (59.60 mg g^{-1} TE) and *S. triandra* UWM 055 (41.24 mg g^{-1} TE). The lowest TAC was found in *S. purpurea* cv. Bona bark, 10.92 mg g^{-1} TE. The mean TAC in the second year (41.45 mg g^{-1} TE) was significantly higher, by almost

43%, than in the first year. A particularly high TAC was found in the second study year in the bark of *S. triandra* UWM 197 (99.83 mg g^{-1} TE). Whereas the lowest value was found in *S. purpurea* cv. Bona bark, in the first study year, only 7.38 mg g^{-1} TE (Table 4). The TAC was significantly positively correlated with the TPC (0.79) and TPC was significantly positively correlated with the TFC (Table 5). A positive and significant correlation between the TAC and TPC of plant raw materials and extracts has been found in many studies (Kiselova et al. 2006, Jeż et al. 2018, Zielińska et al. 2018, Ostolski et al. 2020). This is because phenolic compounds are the main plant antioxidants, in addition to compounds such as ascorbic acid, tocopherols and carotenoids (Blomhoff 2010, Shahidi, Zhong 2015, Orsavová et al. 2019).

Based on the dry bark yield and the content of the components, the potential total phenolic yield (TPY) and total flavonoid yield (TFY) was calculated. In general, the potential TPY was several times higher than that of TFY (Tables 6, 7). The highest potential TPY was obtained from *S. americana* UWM 094 and *S. triandra* UWM 197, nearly 137 kg ha^{-1} (Table 6).

Table 6

Potential yield of total phenolic (TPY) depending on the genotype and harvest year (kg ha^{-1})

Genotype	Harvest year		
	2018	2019	mean
S. purpurea cv. Bona	21.72±0.94 *e*	47.32±1.69 *d*	34.52±5.79 *C*
S. purpurea UWM 062	33.43±1.34 *d*	62.73±0.97 *c*	48.08±6.59 *B*
S. purpurea UWM 166	37.35±4.18 *i*	78.3±7.73 *c*	57.83±9.96 *B*
S. purpurea UWM 168	72.35±3.58 *c*	42.82±2.42 *d*	57.59±6.88 *B*
S. americana UWM 031	29.5±4.07 *d*	94.15±9.04 *c*	61.82±15.12 *B*
S. americana UWM 094	153.81±4.88 *b*	119.8±18.34 *b*	136.81±11.39 *A*
S. triandra UWM 055	50.71±1.4 *d*	57.82±4.29 *d*	54.26±2.57 *B*
S. triandra UWM 197	128.22±2.53 *b*	145.53±2.23 *b*	136.87±4.16 *A*
S. purpurea × *S. daphnoides* UWM 029	50.34±3.94 *d*	180.21±4.56 *a*	115.27±29.16 *A*
S. purpurea × *S. daphnoides* UWM 193	99±5.05 *c*	146.07±5.56 *b*	122.53±11.05 *A*
Mean	67.64±8.01 *Y*	97.47±8.67 *X*	82.56±6.16

Explanation see Table 2

The two hybrids of *S. purpurea* × *S. daphnoides* were also included in the first homogeneous group "A". The next five genotypes were included in the second homogeneous group. The lowest TPY (34.52 kg ha^{-1}) was produced by *S. purpurea* cv. Bona. The TPY was higher in the second year (97.47 kg ha^{-1}) than in the first experimental year by 44%. Also, the total phenolic content was higher in the second year and, in consequence, the potential TPY was affected by the substance content. In the whole experiment, TPY ranged from 21.72 to 180.21 kg ha^{-1} for *S. purpurea* cv. Bona in the first year and *S. purpurea* × *S. daphnoides* UWM 029 in the second year, respectively.

Table 7

Potential yield of total flavonoid (TFY) depending on the genotype and harvest year (kg ha^{-1})

Genotype	Harvest year		
	2018	2019	mean
S. purpurea cv. Bona	8.91±0.39 *c*	12.69±0.45 *c*	10.80±0.89 *C*
S. purpurea UWM 062	9.82±0.39 *c*	10.52±0.16 *c*	10.17±0.25 *C*
S. purpurea UWM 166	15.85±1.78 *b*	19.96±1.97 *b*	17.91±1.5 *B*
S. purpurea UWM 168	21.06±1.04 *b*	12.46±0.7 *c*	16.76±2 *B*
S. americana UWM 031	3.94±0.54 *e*	10.07±0.97 *c*	7.01±1.46 *C*
S. americana UWM 094	18.95±0.6 *b*	15.85±2.43 *b*	17.40±1.31 *B*
S. triandra UWM 055	2.94±0.08 *e*	5.00±0.37 *d*	3.97±0.49 *D*
S. triandra UWM 197	14.66±0.29 *b*	16.58±0.25 *b*	15.62±0.46 *B*
S. purpurea × *S. daphnoides* UWM 029	15.40±1.2 *b*	21.64±0.55 *a*	18.52±1.52 *B*
S. purpurea × *S. daphnoides* UWM 193	21.08±1.08 *a*	21.46±0.82 *a*	21.27±0.61 *A*
Mean	13.26±1.19 *Y*	14.62±1.01 *X*	13.94±0.78

Explanation see Table 2

The highest potential TFY was obtained from *S. purpurea* × *S. daphnoides* UWM 193 genotype, 21.27 kg ha^{-1} (Table 7). The next five genotypes were included in the second homogeneous group "B" (range from 15.62 to 18.52 kg ha^{-1}). The next three genotypes were included in the third homogeneous group "C". The lowest TFY (3.97 kg ha^{-1}) was produced by *S. triandra* UWM 055. TFY was also higher in the second year (14.62 kg ha^{-1}) than in the first experimental year by 10%. In the whole experiment, TFY ranged from 2.94 to 21.64 kg ha^{-1} for *S. triandra* UWM 055 in the first year and *S. purpurea* × *S. daphnoides* UWM 029 in the second year, respectively. Whereas the TFY was more stable in both years for *S. purpurea* × *S. daphnoides* UWM 193, over 21 kg ha^{-1}. The yield of bioactive compounds is a very important parameter that determines the use of genotypes in cultivation for pharmaceutical and/or dietary purposes. The yield of the main sub-classes of bioactive compounds in willow biomass was investigated. Paunonen et al. (2009) assessed the yield of phenolic glycosides in *S. myrsinifolia* at the level of 122-825 kg ha^{-1}. The yield of total flavonol of *S. dasyclados* and *S. miyabeana* was 0.1 and 2.25 kg ha^{-1}, respectively (Brereton et al. 2017). It should be emphasized that flavonols are a sub-class of flavonoids, therefore these values are much lower than the TFY obtained in our research.

CONCLUSIONS

This study confirmed that the diversity of willow species and genotypes and year of the plants harvest are very important factors in terms of using

bark as a source of bioactive substances. The highest TFC was found in the bark of *S. purpurea* UWM 166, whereas TPC and TAC was the highest in bark of *S. triandra* UWM 197. The potential TPY was several times higher than TFY. The highest potential TPY was obtained from *S. americana* UWM 094 and *S. triandra* UWM 197, nearly 137 kg ha^{-1}. The highest potential TFY was obtained from *S. purpurea* × *S. daphnoides* UWM 193 genotype, 21.3 kg ha^{-1}. The mean TFC, TPC, their potential yields and TAC in the second year were higher than in the first year.

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