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ORIGINAL PAPER

Pro-health potential of *Prunus avium* L. and *Prunus domestica* L. leaves cultivated in different water conditions

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Abstract

Leaves can be a valuable source of biologically active compounds that effectively protect the body against oxidative stress and resulting disease. The aim of the experiment was to determine the effect of a genotype and water conditions on the phytochemical substances and antioxidant activity of leaves of sweet cherry cv. Vanda and plum cv. Amers. The study was conducted at the Experimental Station in Lipnik, Poland, in 2017 and 2018. The soil on which the experiment was conducted belongs to the typical rusty soil group and is classified as a Haplic Cambisol. In the Ap level (arable-humus horizon), it has the granulometric composition of clay with a slightly acidic pH. The first experimental factor was a genotype (sweet cherry and plum). The second factor was under-crown watering; half of the trees from each variety were subjected to irrigation (W), and half were used as the control group (O), without irrigation, and with the soil water potential below – 0.01 MPa. Hadar micro-sprinklers were used for watering, sprinkler range r=1 m and efficiency of 2.5l h^{-1}; one sprinkler per tree. It was found that the leaves of both species are abundant in compounds (macro- and microelements, crude protein, antioxidants, vitamins) with pro-health activities, including antioxidant, antibacterial, and anti-inflammatory effects. They are especially characterized by high antioxidant activity. By-products of plant production, such as cherry and plum leaves, can be a valuable and relatively cheap raw material for the production of food, pharmaceuticals or nutraceuticals, and, in times of insufficient feed supply, a valuable supplement to the feed of grazing animals.

Keywords: antioxidants, chemical composition, leaves, *plum*, sweet cherry, vitamins

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INTRODUCTION

In many countries around the world, the processing of food raw materials has contributed to lower consumption of certain nutrients. At the same time, among a significant number of consumers, there is a growing interest in maintaining good health and slowing the aging process. Therefore, based on the idea that food can be a medicine, functional foods (bioactive, nutraceutical, probiotic), enriched with healthful components that are not naturally present (vitamins, fiber, phenolic compounds, phytosterols or minerals), were created (Błaszczak, Grześkiewicz 2014). The increase in consumer awareness in the field of health has also translated into an interest in herbal medicines, those that are less invasive and cause only mild adverse side effects. These bioactive foods and medicinal components originate from different parts of plants.

Leaves are a valuable source of biologically active compounds that effectively protect the body against oxidative stress and resulting disease. Leaves of the black currant plant are abundant in antioxidants (Cyboran et al. 2014), as are leaves of the grapevine (Bodó et al. 2017) and white mulberry (Jeszka et al. 2009). High concentrations of polyphenolic compounds bestowing antioxidant, anti-inflammatory, hypoglycemic, and antimicrobial effects are also present in apple, quince, and cranberry leaves (Teleszko, Wojdyło 2015).

Unused plum leaves left after pruning and harvesting can also be a valuable source of phytochemicals (Stierlin et al. 2018) owing to their content of quercetin, isoquercetin, kaempferol, chlorogenic and quinic acid (Lenchyk 2015) and minerals (Miloševic et al. 2013). Plum leaves as a potential source of bioactive substances have not yet been thoroughly examined (Stierlin et al. 2018). Additionally, in the literature, there is little information available about the chemical composition and antioxidant properties of cherry leaves. A few up-to-date publications confirm that cherry leaves, owing to their high level of antioxidants, can also be a potential source of biologically active substances for the production of new functional foods, as well as for the treatment of cardiovascular disease, diabetes, obesity, and certain types of cancer (Dziadek et al. 2018).

In addition to the pro-health benefits of plant by-products, their use also provides an economic and ecological advantage. The use of agricultural and industrial waste as raw materials can help reduce production costs as well as the burden of environmental pollution. Annually, the agri-food industry generates around 800,000 tonnes of waste (Barbulova et al. 2015). Agricultural waste mainly takes the form of post-harvest residues (stems, straw, leaves, pits, roots, shells, etc.) and animal waste (fertilizers). Such waste products are widely available, renewable and virtually free and thus, they serve as a viable alternative source for the production of, among others, biofuels, biofertilizers, and biogas (Deng et al. 2012). Agricultural residues are abundant in bioactive compounds, hence some remains of the agricultural industry can also be used as animal feed (Temel, Pehluvan 2015). The majority of waste is unused and left to rot or burned, especially in developing countries (Sabiiti 2011).

Due to the influence of genetic and environmental factors, as well as applied agrotechnology, the antioxidant and nutritional properties of plants may undergo modifications (Martinez-Ballesta et al. 2010). Water scarcity is one of the most common causes of reduced plant production (Estrada-Campuzano 2012). The negative impact of water stress on plant production is well documented, but its impact on crop quality is less thoroughly explored. Lack of water leads to the activation of plant defense systems with physiological stress reactions that cause significant changes in the production of metabolites, changing the nutritional and health value of the harvested products (dos Santos et al. 2022).

The main objective of the study was to determine the content of minerals, proteins, vitamins, antioxidants, and the antioxidant activity in the leaves of *Prunus avium* L. and *Prunus domestica* L. cultivated under various water conditions.

MATERIALS AND METHODS

The experiment was conducted at the Agricultural Experimental Station in Lipnik (53°20′35″N 14°58′10″E, altitude 7 m.a.s.l.), Poland, in 2017 and 2018. The study material included sweet cherry cv. Vanda and plum cv. Amers trees. The aim of the experiment was to determine the effect of irrigation on the mineral composition, crude protein, vitamins and antioxidants of cherry and plum leaves. The soil on which the experiment was conducted belongs to the typical rusty soil group and is classified as a Haplic Cambisol. In the Ap level (arable-humus horizon), it has the granulometric composition of clay with a slightly acidic pH. Content analysis of soil minerals showed moderate levels of magnesium and potassium and high levels of phosphorus. The first experimental factor was a genotype (sweet cherry and plum). The second factor was under-crown watering; half of the trees from each variety were subjected to irrigation (W), and half were used as a control group (O), without irrigation, with soil water potential below – 0.01 MPa. Hadar micro-sprinklers were used for watering, sprinkler range r = 1 m and efficiency of 2.5l h^{-1}; one sprinkler per tree. Determination of the doses and times of irrigation was performed using tension meters buried 20 cm below the soil surface. The amount of water applied to the trees ranged between 20-80 mm ha^{-1} for sweet cherry and 30-100 mm ha^{-1} for plum (Table 1), depending on the amount of precipitation in the particular study period (Table 2). The experiment was designed using the randomized block method in 5 replications for sweet cherry and in 4 replications for plum (one

Table 1

Water dose applied in years of experiment

Years		Sweet cherry	Plum
2017	m^3/tree	0.36	0.48
	mm ha^{-1}	20.0	30.0
2018	m^3/tree	1.44	1.60
	mm ha^{-1}	80.0	100.0

Table 2

Sum of rainfall (mm) and mean air temperature (°C) in 2017-2018 years compared to the long-term averages from 1961-2004

Month	Long-term averages 1961-2004		Precipitation		Air temperature	
	precipitation	temperature	2017	2018	2017	2018
April	34.9	8.9	43.6	13.6	7.2	13.3
May	48.6	13.2	85.0	40.6	13.8	16.7
June	61.7	16.2	109.0	36.8	17.0	18.6
July	70.9	18.1	183.6	137.0	17.6	20.1
August	54.1	18.1	74.0	6.5	18.6	20.5
September	51.6	13.6	30.2	16.5	13.6	15.6
Apr - Sept	321.8	14.7	525.4	251.0	14.6	17.5

tree- one repeat). The trees grew on rows of lawn separated by herbicide fallow. The distance between cherry trees in a row was 3 m and 3.5 m between the rows; the corresponding distances for plums were 4 m and 4.5 m.

Leaves for analysis were collected after harvest. Leaves were collected from the outside of the tree crown, at half height. The leaves were taken from 1-year-old shoots without any signs of aging or mechanical damage. After cleaning and cutting into 1 cm pieces, the collected material was placed in an oven at 30-35°C, with a constant air flow rate of 0.5 m s^{-1} for 48 hours. The dried plant material was packed into paper bags (thickness of the dried material layer was 1 cm) and stored in the dark at 15°C and 65% humidity until chemical analyses were performed. Before the analysis, the material was ground in a Knifetec 1095 Sample Mill.

The concentration of total nitrogen (N) was determined in samples mineralized in sulfuric(VI) acid with H_2O_2 – the Kjeldahl method. The concentration of phosphorus (P) was determined by the colorimetric method using a Specol 221 apparatus. An Atomic Absorption Spectrometer (ASA) was used to determine potassium (K), sodium (Na), and calcium (Ca) by means of emulsion flame spectroscopy, and magnesium (Mg), iron (Fe), zinc (Zn), and manganese (Mn) by means of absorption flame spectroscopy. Total protein was determined by the Kjeldahl method (N×6.25).

For total polyphenols, total flavonoids, and antioxidant activity determination, methanol extracts were prepared according to the method presented by Kumaran and Karunakaran (2007). The total phenolic content of plant extracts was determined using Folin-Ciocalteu reagent (Yu et al. 2002). Carotenoids were extracted with an 80% solution of acetone and determined according to Lichtenthaler, Wellburn (1983). The antioxidant capacity was assayed by the Trolox Equivalent Antioxidant Capacity (TEAC) method (Re at al. 1999). Tocopherols were extracted from plant samples with hexane and were determined according to the method described by Prieto et al. (1999). The content of vitamins thiamine (B1), riboflavin (B2), and ascorbic acid (C) in extracts were measured according to the method described by Kłódka et al. (2008).

Experimental results were analyzed using Statistica version 13.3 (StatSoft, Poland). Two-factor analysis of variance (ANOVA) was conducted. The significance of differences between means was compared by the Tukey's multiple range test. Statistical significance was considered at $P \leq 0.05$. Results were presented as the mean ± SD (standard deviation) from three independent determinations (n=3).

RESULTS AND DISCUSSION

The sweet cherry leaves contained 11.2 g kg^{-1} (9%) more protein than the plum leaves. On average, 142.5 and 131.3 g kg^{-1} of protein were recorded in the leaves of sweet cherries and plums, respectively (Table 3). Protein content in sweet cherry fruit is on average 10 g kg^{-1} (USDA 2006), i.e. several times less than in the analyzed leaves. In plum pomace, Milala et al. (2013) reported 39 to 82 g kg^{-1} protein, which is from 48 to 70% less than in the analyzed plum tree leaves. More protein was found in leaves of non-irrigated trees (Table 3). According to other researchers (Tian et al. 2017), controlled irrigation (irrigation deficit), or even its complete lack, can improve the nutritional value, including the concentration of protein, in plants.

The analyzed leaves contained between 4.71 and 7.14 g kg^{-1} phosphorus (Table 3). In plum leaves, it was on average 52% (4.43 g kg^{-1}) higher than in cherry leaves. The applied supplemental irrigation increased the concentration of phosphorus by 0.55 g kg^{-1} (10%), which agrees with the results of studies by Bekmirzaev et al. (2019) and Zuazo et al. (2011).

The average potassium concentration in mango leaves is 24.0 g kg^{-1} (Zuazo et al. 2011) while the leaves of cherries and plums were found to contain 38.2 and 44.4 g kg^{-1}, respectively (Table 3). Significantly more potassium was found in plum leaves (by 6.2 g kg^{-1}, i.e. 16%). Leaves of irrigated trees were characterized by lower concentrations of potassium (by 2%) compared to the control, which is in contrast to the study of Zuazo et al. (2011).

Table 3

Crude protein and macroelement content in sweet cherry and plum leaves (g kg^{-1} DW)

Item	Crude protein	N	P	K	Ca	Mg	Na
Genotype							
Sweet cherry	142.5±1.9^{a}	22.8±0.4^{a}	4.71±0.7^{b}	38.2±0.8^{b}	5.94±0.2^{b}	4.38±0.3^{a}	0.12±0.0^{b}
Plum	131.3±5.9^{b}	21.0±0.9^{b}	7.14±0.1^{a}	44.4±1.9^{a}	6.13±0.2^{a}	4.09±0.2^{b}	0.14±0.0^{a}
Irrigation							
O	140.6±4.6^{a}	22.5±0.7^{a}	5.65±1.8^{b}	41.8±4.9^{a}	5.91±0.2^{b}	4.21±0.3^{b}	0.12±0.0^{b}
W	133.8±8.7^{b}	21.4±1.4^{b}	6.20±1.0^{a}	40.8±2.2^{b}	6.15±0.1^{a}	4.27±0.1^{a}	0.13±0.0^{a}

DW – dry weight, O – without irrigation, W – with irrigation;
Mean values followed by the same letter in each column are not significantly different at $P \leq 0.05$.

In leaves of *Spinacia oleracea* L., which are known to be abundant in minerals and vitamins, the calcium content is 6 g kg^{-1} (Martinez-Ballesta et al. 2010). In this study, leaves of the tested species contained on average 5.94 (sweet cherry) and 6.13 (plum) g kg^{-1} (Table 3) of calcium. The concentration was significantly higher in plum leaves (by 3%, i.e. 0.19 g kg^{-1}) and in irrigated trees (by 4%, i.e. 0.24 g kg^{-1}). Zuazo et al. (2011) did not detect any higher amounts of calcium in irrigated mango leaves.

The analyzed sweet cherry leaves contained 4.38 g kg^{-1} of magnesium (Table 3). The concentration of this element in the leaves of sweet cherry according to Miloševic et al. (2015) ranges from 2.8 to 4.1 g kg^{-1}, depending on the cultivar. In the plum tree leaves, 4.09 g kg^{-1} magnesium was found (Table 3), which agrees with the study by Mayer et al. (2018) reporting a magnesium content between 2.7 and 4.3 g kg^{-1}. The concentration of magnesium under the influence of irrigation increased slightly yet significantly (by 0.06 g kg^{-1}, i.e. 1%). Similarly, Hepaksoy et al. (2016) reported an increase in the magnesium concentration due to irrigation in *Punica granatum* L. leaves.

The leaves of *Moringa oleifera*, a plant valued in the pharmaceutical industry, contain on average 2.55 g kg^{-1} of sodium (Biel et al. 2017). Leaves of sweet cherry and plum contained several times less sodium; on average between 0.12 and 0.14 g kg^{-1} (Table 3). Irrigation increased the sodium concentration by 0.01 g kg^{-1} (8%). Likewise, a decrease in the concentration of sodium in the leaves of *Tetragonia tetragonioides*, along with an increase in water deficit in the soil, was confirmed by Bekmirzaev et al. (2019).

The proportion of individual mineral components in the products consumed is crucial for the absorption by the human body; when proportions are not adequate, consuming even the most highly nutritious products will be insufficient.

Previous studies have shown that a calcium-magnesium ratio (Ca:Mg) < 2.8 is critical for optimal health and its ideal level is close to 2 (Rosanoff et al. 2016). In sweet cherry leaves, the ratio was 1.4, and in plum tree leaves, it was 1.5. Irrigation did not affect the Ca:Mg ratio (Table 4).

According to the RDA, the correct ratio of Ca:P in a human diet is 1.5. Leaves of sweet cherry were characterized by the most advantageous ratio of Ca:P owing to relatively high calcium and low phosphorus content (Grzegorczyk et al. 2017). Moisture conditions did not significantly affect this feature (Table 4).

According to WHO recommendations, the Na:K ratio should be < 0.49 in healthy people (Aburto et al. 2013). The data from Table 4 shows that both sweet cherry and plum leaves satisfy the above condition. Values of the Na:K ratio for all tested samples were 0.003, owing to high potassium and low sodium concentrations.

The concentration of zinc in plant-based foods generally varies from 0.05 to 11.8 mg 100 g^{-1}. The sweet cherry leaves were characterized by a significantly higher concentration of zinc and manganese than plum leaves, by 3.2 (17%) and 20.9 (29%) mg kg^{-1}, respectively (Table 5). There was no significant difference in the iron content in both species tested. Leaves of irrigated trees contained less zinc, iron, and manganese than leaves from control trees, by 3.1 mg kg^{-1} (4%), 4.4 mg kg^{-1} (6%), and 1.5 mg kg^{-1} (2%), respectively.

Table 4

Selected nutrient ratios in leaves of sweet cherry and plum (DW)

Item	Ca:Mg	Ca:P	Na:K
Genotype			
Sweet cherry	1.4	1.26	0.003
Plum	1.5	0.9	0.003
Irrigation			
O	1.4	1.0	0.003
W	1.4	1.0	0.003

DW – dry weight, O – without irrigation, W – with irrigation

Table 5

Microelements content in sweet cherry and plum leaves (mg kg^{-1} DW)

Item	Zn	Fe	Mn
Genotype			
Sweet cherry	21.8±3.6^{a}	76.3±4.6^{a}	93.4±0.6^{a}
Plum	18.6±0.1^{b}	76.5±0.6^{a}	72.5±1.6^{b}
Irrigation			
O	21.7±3.7^{a}	78.6±2.1^{a}	83.7±11.4^{a}
W	18.6±0.1^{b}	74.2±2.1^{b}	82.2±12.8^{b}

DW – dry weight, O – without irrigation, W – with irrigation;
Mean values with the same letter in each column are not significantly different at $P \leq 0.05$.

Complementary irrigation can affect the soil pH (Li et al. 2018). Macroelements are best absorbed at a near-neutral pH. An acidic environment, on the other hand, favors the availability of micronutrients. The uptake of zinc, iron, and manganese is less intensive at pH above 6.5, which explains why under experimental conditions (slightly acidic soil reaction), a higher concentration of these elements was found in the leaves of non-irrigated trees. In turn, significantly more macronutrients were recorded (phosphorus, calcium, magnesium, sodium) in the leaves of irrigated trees.

Sweet cherry leaves contained significantly more polyphenols (by 4%), whereas plum leaves were characterized by a higher concentration of flavonoids and carotenoids, by 48 and 12%, respectively (Table 6). In comparison

Table 6

Content of antioxidants and antioxidant activity in sweet cherry and plum leaves (DW)

Item	Polyphenols (mg GAE kg^{-1})	Total flavonoids (mg QE kg^{-1})	Carotenoids (mg kg^{-1})	ABTS (umol Trolox kg^{-1})
Genotype				
Sweet cherry	8915.9±475.9^{a}	2777.8±104.5^{b}	408.1±39.4^{b}	958.2±32.3^{a}
Plum	8588.1±508.3^{b}	4111.6±291.1^{a}	458.4±31.3^{a}	930.7±26.6^{b}
Irrigation				
O	8319.5±260.3^{b}	3312.8±608.6^{b}	402.2±33.1^{b}	920.0±17.5^{b}
W	9184.5±180.5^{a}	3576.6±866.6^{a}	464.3±25.2^{a}	968.9±21.3^{a}

DW – dry weight, GAE – gallic acid equivalent, QE – quercetin equivalent;
Mean values with the same letter in each column are not significantly different at $P \leq 0.05$.

to sea buckthorn, valued as a good source of leaf antioxidants (Khan et al. 2012), the concentration of polyphenols in the leaves of the studied trees was at a similar level, and flavonoids (in the plum tree leaves) were almost twice as abundant. Irrigation increased the content of all analyzed antioxidants, by 10% (polyphenols), 8% (flavonoids), and 15% (carotenoids), which is in agreement with the results of studies by Khan et al. (2012). There exists a certain water level, according to researchers, at which there is an increase in the content of antioxidants. If a plant faces too much water stress, it can cause a reduction in antioxidants (Bekmirzaev et al. 2019).

Significantly higher antioxidant activity was found in the leaves of sweet cherry, on average by 3%, compared to plum leaves (Table 6). Increasing the water deficit reduced the antioxidant activity (Khan et al. 2012). Leaves from irrigated trees showed higher antioxidant levels, on average by 5%.

Vitamin deficiency, referred to as avitaminosis, can lead to many serious diseases and disorders in the functioning of the body. A greater amount of tocopherols was recorded in plum tree leaves (by 57%), while the sweet cherry leaves contained more L-ascorbic acid (by 27%) – Table 7. Compared to sea buckthorn leaves, the examined leaves contained similar amounts of L-ascorbic acid, almost twice as much thiamine, and several times more

Table 7

Content of vitamins in sweet cherry and plum leaves (mg kg^{-1} DW)

Item	Tocopherols	L-ascorbic acid	Thiamine	Riboflavin
Genotype				
Sweet cherry	12.5±1.5[b]	2556.2±481.8[a]	12.6±0.4[a]	26.9±1.3[a]
Plum	19.6±2.3[a]	2008.9±136.1[b]	12.6±0.8[a]	27.5±2.0[a]
Irrigation				
O	14.7±3.7[b]	2014.5±143.0[b]	12.4±0.6[a]	28.0±1.6[a]
W	17.4±4.4[a]	2550.0±487.2[a]	12.8±0.5[a]	26.5±1.5[a]

DW – dry weight
Mean values with the same letter in each column are not significantly different at $P \leq 0.05$.

riboflavin (Jaroszewska, Biel 2017). More tocopherols and L-ascorbic acid were found in the leaves of irrigated trees, as confirmed in studies by Osuagwu, Edeoga (2012). The genotype and humidity conditions did not affect the thiamine and riboflavin levels in leaves.

CONCLUSIONS

Leaves of sweet cherry and plum trees are a good source of bioactive compounds that, owing to their easy accessibility, high renewal, and relatively low cost of harvesting, can serve as a source of desirable components for the production of food, pharmaceuticals, and nutraceuticals. In periods of insufficient production of fodder, they can also serve as a valuable feed supplement for grazing animals.

The chemical composition of plant-derived components depends on many factors, including the genotype, applied agrotechnical measures, and climatic and soil conditions, as confirmed by the above studies. Plum leaves were characterized by a higher content of macroelements (phosphorus, potassium, calcium, sodium), total flavonoids, carotenoids, and tocopherols. In turn, the sweet cherry leaves contained more zinc and manganese, proteins, polyphenols, and L-ascorbic acid, and showed greater antioxidant activity. The proportions of individual mineral components proved to be beneficial in the leaves of both species. Leaves collected from irrigated trees contained more macronutrients, antioxidants, and vitamins (tocopherols, L-ascorbic acid). They were also characterized by greater antioxidant activity.

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