ОРИГИНАЛЬНАЯ СТАТЬЯ

PECULIARITIES OF WILLOW (*SALIX ALBA*) BARK MINERAL COMPOSITION

N. Golubkina[1*], U. Plotnikova[1], Z. Amagova[2], S. Sheshnitsan[3]

[1] Federal Scientific Vegetable Center,
Selectsionnaya 14, pos. VNIISSOK, Odintsovsky district, Moscow region, 143072, Russian Federation
[2] Chechen Agricultural Institute,
Lilovaya 1, Grozny, Chechen republic, 366021, Russian Federation
[3] Voronezh State University of Forestry and Technologies,
Timiryazeva 8, Voronezh, 394036, Russian Federation

ABSTRACT. Willow bark is highly valuable in medicine, different branches of industry and ecology. In this respect antioxidants and minerals distribution between bark tissues seems to be highly valuable. Using ICP-MS mineral composition of willow bark cambium, phloem and periderm was determined for the first time. Predominance of P, K and Ca was recorded for cambium characterized by intensive metabolism. On the contrary, periderm demonstrated the highest concentrations of all heavy metals, except Ni, Al and As, and the highest coefficients of variation compared to phloem and cambium, especially for Al (107.5%), Pb (112.2%), V (133.3%), and Sn (90.9%). Though trace elements also prevail in periderm, low coefficient of variation between tissues indicates the importance of Fe, Mn, B, Zn, Cu and Mo in plants growth. Among tissues studied cambium recorded the highest antioxidant activity with relatively similar values of polyphenol content compared to periderm and phloem data. The results prove high prospects of willow bark cambium utilization as a valuable source of antioxidants and trace elements and of periderm as a natural adsorbent.

KEYWORDS: willow bark, periderm, phloem, cambium, antioxidants, minerals.

INTRODUCTION

Up-to-date about 550 species of willow are identified. White willow (*Salix alba* L.) is one of the most common one, especially in the Northern hemisphere. Powerful root system, quick growth and ornamental value provide its wide utilization in parks, for weaving, production of houseware, and for windbreaks. This plant, and especially bark, has been highly valuable for centuries in traditional medicine as an analgesic, antipyretic and anti-inflammatory drug (Barnes et al., 2007) (Fig. 1). Indeed, willow is known to be a unique natural source of numerous antioxidants, including salicylates and polyphenols, demonstrating a synergism between each other (Shara et al., 2010; Nica et al., 2021) and providing a protection of cell membrane phospholipids against oxidation (Durak, Gawlik-Dziki, 2014). A simple method for separation and purification of catechin, triendrin, picein and salicin from willow bark was developed (Dou et al., 2021). Bark and inner bark are rich in pectin and proteins (Dou et al., 2018).

In modern agriculture willow bark is highly valuable, especially in organic farming as a natural fungicide, suitable for protection of plants against various fungal diseases caused by *Plasmopara viticola*, *Venturia inaequalis*, *Taphrina deformans*, *Erysiphe necator* and *Podosphaera leucotricha* (Deniau et al., 2019). Water extracts of bark record powerful growth stimulation properties due to the presence of indole 3-butyric acid. Application of willow bark extracts provides the significant increase of root growth, protein accumulation and enhancement of antioxidant enzymes activity in stress conditions (Mutlu-Durak et al., 2021).

Special investigations are devoted to willow bark mineral content and adsorption capacity of heavy metals. High adsorption capacity of willow bark was utilized in water purification to remove Ni and Cd (Najama, Andeabib, 2021), Zn and Cu (Rypińska, Biegańska, 2014). Separate data are published on the mineral content of the whole willow bark (Dementieva et al., 2017; Bajraktari et al., 2022).

Complex structure of bark, composed of periderm, phloem and cambium, supposes the significance of mineral distribution between these components in antioxidant and mechanical defense of

* Адрес для переписки:
Голубкина Надежда Александровна
E-mail: segolubkina@rambler.ru

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plants and in practical application for different branches of industry. Taking into account valuable differences between these tissues in the intensity of metabolism, the aim of the present investigation was a comparative evaluation of minerals accumulation and antioxidant status of willow bark periderm, phloem and cambium in the absence of significant oxidant stress.

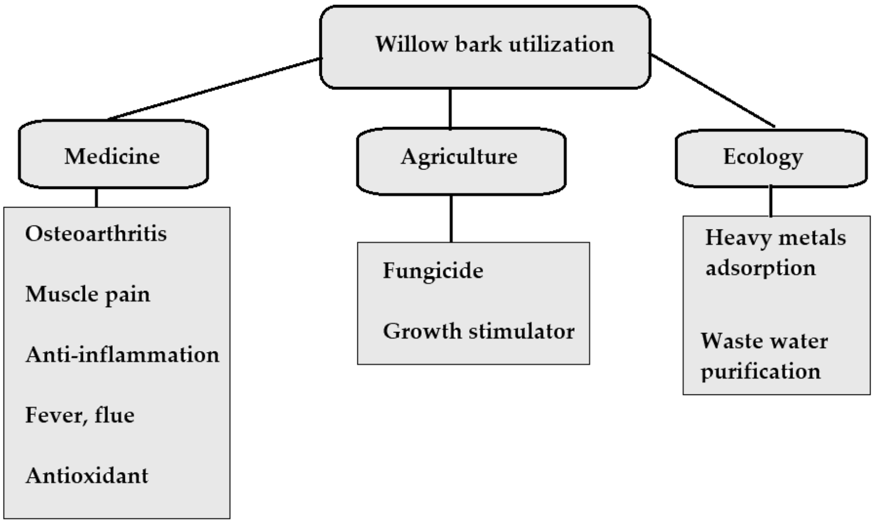

Fig. 1. *Willow bark utilization*

MATERIALS AND METHODS

Sampling and samples preparation

Sampling of willow bark was achieved in September, 2022 at the territory of Balashikha forest park, Moscow region (55°47′23″ N., 37°54′44″ E.), using 10–15 cm diameter tree trunks of five 20–30 old trees felled after a thunderstorm. Cambium, phloem and periderm tissues were separated, dried to constant weight at 80 ^{o}C and homogenized. Mixed samples of tree bark components (300–400 cm^2 bark surface from each tree) were used in the analysis.

Mineral composition

The content of Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Se, Si, Sn, Sr, V, and Zn in dried homogenized samples was assessed using ICP-MS on quadruple mass-spectrometer Nexion 300D (Perkin Elmer Inc., Shelton, CT, USA), equipped with the seven-port FAST valve and ESI SC DX4 autosampler (Elemental Scientific Inc., Omaha, NE, USA) at the 'Micronutirients' Center (Moscow, Russia). Rhodium 103 Rh was used as an internal standard to eliminate instability during measurements. Quantitation was performed using external standard (Merck IV, multi-element standard solution); Perkin-Elmer standard solutions for P, Si, and V, and all the standard curves were obtained at five different concentrations. For quality control purposes, internal controls and reference materials were tested together with the samples daily. Microwave digestion of samples was carried out with sub-boiled HNO_3 diluted 1:150 with distilled deionized water (Fluka No. 02, 650 Sigma-Aldrich, Co., Saint Louis, MO, USA) in the Berghof SW-4 DAP-40 microwave system (Berghof Products + Instruments Gmb H, 72, 800 Eningen, Germany).

Total Polyphenols (TP)

Total polyphenols were determined in 70% ethanol extract using the Folin – Ciocalteu colorimetric method as previously described (Golubkina et al, 2020). Half gram of dry bark homogenates was extracted with 20 mL of 70% ethanol at 80 □ for 1 h. The mixture was cooled down and quantitatively transferred to a volumetric flask, and the volume was adjusted to 25 mL The mixture was filtered through filter paper, and 1 mL of the resulting solution was transferred to a 25 mL volumetric flask, to which 2.5 mL of saturated Na_2CO_3 solution and 0.25 mL of diluted (1 : 1) Folin – Ciocalteu reagent were added. The volume was brought to 25 mL with distilled water. One hour later the solutions were analyzed through a spectrophotometer (Unico 2804 UV, Suite E Dayton, NJ, USA), and the concentration of polyphenols was calculated according to the absorption of the reaction mixture at 730 nm. As an external standard, 0.02% gallic acid was used. The results were expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g d.w).

Antioxidant Activity (AOA)

The antioxidant activity of willow bark periderm, phloem and cambium was assessed using a redox titration method (Golubkina et al, 2020) via titration of 0.01 N $KMnO_4$ solution with ethanolic extracts of dry samples, produced as described in the previous section. The reduction of $KMnO_4$ to colorless Mn^{+2} in this process reflects the quantity of antioxidants dissolvable in 70% ethanol. The values were expressed in mg gallic acid equivalents (mg GAE/g d.w.).

Statistical Analysis

The data were processed by analysis of variance and mean separations were performed through the Duncan's multiple range test, with reference to 0.05 probability level, using SPSS software version 21 (Armonk, 315 NY, USA). The results were expressed as means (three determinations for each sample) and standard deviation (M±SD).

RESULTS AND DISCUSSION

Mineral composition of plants reflects both genetic peculiarities of the species and bioaccessibility of elements in concrete ecological conditions, including soil characteristics, climate and the existence of stress factors. Whole bark of certain willow species gathered at the Northern Caucasus has been extensively characterized by its mineral composition, with special attention to ultra- trace elements (Dementieva et al., 2017). On the other hand, the distribution of elements between bark tissues seems to be highly valuable. Thus, periderm is composed by dead sells with high adsorptive capacity, providing mechanical and antioxidant protection of trees, the intermediate layer phloem (transfers sugars and other carbon-based nutrients throughout the tree) and inner layer with the highest rate of metabolism – cambium.

Data presented in Table 1 indicate the predominance of macroelements accumulation in cambium. It seems significant that the highest coefficients of variation between macro elements concentrations between periderm, phloem and cambium were recorded for P (85.1%) and K (63.3%) – the essential elements for all plants. On the contrary, CV for Ca happened to be less than 2%. Taking into account the significant role of Ca as an important regulator of different processes related to growth (cell division, cell wall synthesis, repair of damage from different biotic and abiotic stresses) and protection against oxidant stress and facts of significant changes of its cambium concentration in spring and due to stress intensity, it seems obvious that Ca distribution between bark tissues will vary greatly in stress conditions (Lautner, Fromm, 2010).

Indeed, the essential nutrients for trees are provided by cambium, which is capable to accumulate the highest levels of P, K and Ca with the predominant differences between tissues. Thus, according to the presented data, cambium concentration of P is more than 5 times higher than in periderm, while K concentration exceeds that of periderm by 3.5 times.

The most significant differences in periderm, phloem and cambium distributions were recorded for Al, As and heavy metals. Indeed, in this group of elements coefficients of variation happened to be the highest reaching up to 133 % for V. Indeed, the differences in V, Pb, Al and Sn concentrations of cambium and periderm were equal to 9.22, 4.57, 5.44 and 5.5 times according. Less intensive differences were indicated for Sr (2.01 times), Cr (2.68 times) and As (1.60 times). The exception was only Ni, whose distribution between bark tissues was uniform. The presented data indicate that heavy metals distribution in willow bark may cause significant problems with the safeness of water extracts used for medicinal purposes in case of whole bark utilization. On the other hand, the above mentioned facts are in good agreement with the well-known high adsorption capacity of bark (Şen et al., 2015). Unfortunately, only scares information is available on the differences in the adsorption capacity of outer and inner layers of bark. Thus, the only work of Aoyama et al. (2004) indicated higher levels of heavy metals adsorption by outer bark layer compared to phloem of Japanese cedar bark. The present results indicate high prospects of willow periderm utilization for waste water purification from various heavy metals such as Pb, V, Cd and Al. Among these elements only Cd was shown to adsorb fluently on willow bark (Najama, Andeabib, 2021).

As far as trace elements are concerned their variations between tissues seem to be less pronounced and more specific compared to heavy metals. Indeed, no trace elements predominate in cambium except Se, Fe, Cu and iodine compared to phloem data. The highest coefficients of variations between periderm, phloem and cambium was shown for Fe and Mo (more than 60%), while the lowest one was found for Se (about 12%). These facts indicate indirectly the significance of trace elements for all bark tissues. One should also pay attention to the fact that environmental pollution with not only Pb, but also with Zn and Cu may be successfully removed via photoremediation with willow bark cuttings (Labrecque et al., 2020). Whether willow phloem may become more desirable for this purpose remains unknown.

Table 1. ***Mineral composition of willow bark tissues (mg/Kg d.w.)***

Element	Periderm	Phloem	Cambium	M±SD	CV, %
Macroelements					
Ca	26597 b	43650 a	36857 a	35701±585	1.6
K	3338 c	5508 b	11689 a	66845±4333	63.3
Na	132 c	248 b	315 a	2231±92	39.8
P	510 c	893 b	2678 a	11360±1157	85.1
Mg	1286 a	744 b	880 b	9970±282	29.1
Trace elements					
B	3.14 a	2.16 b	1.68 c	2.33±0.74	31.8
Co	0.55 a	0.32 b	0.25 c	0.37±0.16	43.2
Cu	8.22 a	2.92 c	4.63 b	5.26±2.71	51.5
Fe	287 a	80.4 c	122 b	163±109	66.9
I	1.40 a	0.77 b	0.84 b	1.00±0.35	35.0
Li	0.16 a	0.09 c	0.13 b	0.13±0.03	23.1
Mn	136 a	91 b	104 b	110±23.2	21.1
Mo	0.14 a	0.05 b	0.05 b	0.08±0.05	62.5
Se	0.09 a	0.07 b	0.08 ab	0.08±0.01	12.5
Si	3.01 a	2.39 b	1.99 b	2.46±0.51	20.7
Zn	177 a	181 a	131 b	163±28	17.2
Al, As and heavy metals					
Al	19.92 a	3.60 b	3.66 b	9.06±9.41	107.5
As	0.08 a	0.05 b	0.05 b	0.06±0.02	33.3
Cd	3.22 a	1.52 b	1.12 c	1.95±1.12	57.4
Cr	0.99 a	0.37 b	0.37 b	0.58±0.36	62.1
Pb	1.69 a	0.17 c	0.37 b	0.74±0.83	112.2
Ni	1.39 a	1.35 a	1.30 a	1.35±0.05	3.7
Sn	0.22 a	0.07 b	0.04 c	0.11±0.10	90.9
Sr	83.81 a	66.42 b	41.68 c	63.97±21.17	33.1
V	0.83 a	0.06 c	0.09 b	0.33±0.44	133.3

N o t e : Values in lines with similar letters do not differ statistically according to Duncan test at $p<0.05$.

Furthermore, evaluation of antioxidant status of willow bark revealed the highest values typical for cambium with the uniform values for polyphenols content between periderm, phloem and cambium. (Fig. 2). The results of willow bark AOA determination, presented on Fig. 2, was in good agreement with previously obtained data for willow bark from the Western part of Moscow region (Golubkina et al., 2022).

In general, according to the literature data both mineral content and antioxidant activity of willow bark may vary considerably demonstrating CV value reaching 33% (Golubkina et al., 2022). And among factors affecting both mineral composition and antioxidant status one may indicate the existence of stress factors, climate, tree age and place of habitat, especially altitude above the sea level reaching 33% (Golubkina et al., 2022). Unfortunately, up-to-date no information exists about variations of minerals and antioxidants accumulation in willow bark tissues of minerals and antioxidants accumulation, which indicates the necessity of further investigations.

Taking into account the antioxidant activity and mineral distribution between willow bark periderm, phloem and cambium it seems highly important to separate cambium, phloem from periderm and use the former for medicinal purposes and the latter for waste water purification.

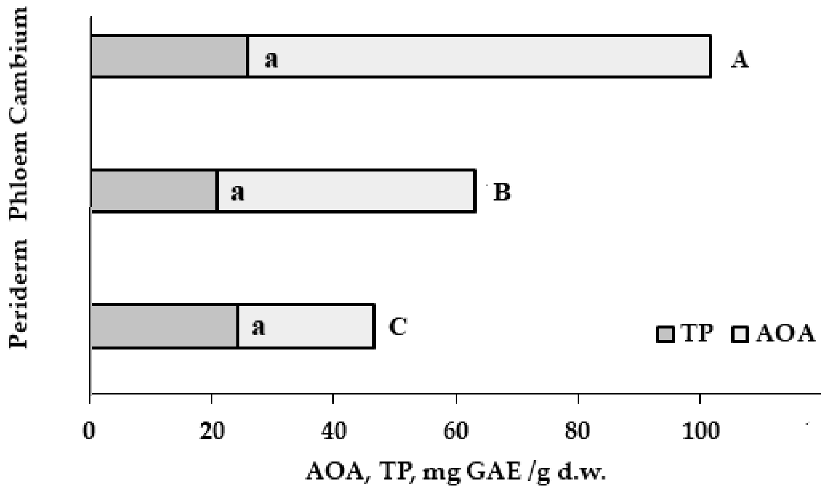

Fig. 2. *Antioxidants distribution between willow bark components. TP- total phenolics; AOA- total antioxidant activity (values with the same letters do not differ statistically according to Duncan test at p<0.050)*

CONCLUSION

The presented results give the first description of macro and trace elements distribution between outer and inner tissues of willow bark indicating the significance of their separation before utilization in medicine and ecology.

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ОСОБЕННОСТИ МИНЕРАЛЬНОГО СОСТАВА КОРЫ ИВЫ (*SALIX ALBA*)

Н. Голубкина[1], У. Плотникова[1], З. Амагова[2], С. Шешницан[3]

[1] Федеральный научный центр овощеводства,
Российская Федерация, 143072, Московская обл., Одинцовский район, пос. ВНИИССОК, Селекционная 14
[2] ФГБНУ Чеченский НИИСХ,
Российская Федерация, 366021, Чеченская республика, г. Грозный, ул. Лиловая 1
[3] Воронежский лесотехнический университет,
Российская Федерация, 394036 Воронеж, Тимирязева 8

РЕЗЮМЕ. Кора ивы высоко ценится в медицине и различных отраслях промышленности и экологии. В связи с этим важным представляется распределение антиоксидантов и минеральных веществ между компонентами коры. Используя ИСП-МС, впервые установлен минеральный состав камбия, флоэмы и перидерма коры ивы. Установлено предпочтительное накопление P, K и Ca в камбии, отличающемся наиболее интенсивным метаболизмом. Напротив, наиболее высокие концентрации тяжелых металлов (за исключением Ni, As и Al) были выявлены для перидерма, причем эта группа элементов отличалась наиболее высоким коэффициентом вариации по сравнению с флоэмой и камбием, составившем 107,5% для Al, 112,2% для Pb, 133,3% для V и 90,9 % для Sn. Хотя микроэлементы также характеризуются предпочтительным накоплением в перидерме, низкие коэффициенты вариации между тканями свидетельствуют о важности Fe, Mn, B, Zn, Cu и Mo для роста ивы. Среди исследованных тканей камбий отличался наиболее высокой антиоксидантной активностью и сравнительно одинаковым содержанием полифенолов по сравнению с соответствующими значениями для перидерма и флоэмы. Полученные результаты свидетельствуют о перспективности использования камбия коры ивы как значимого источника антиоксидантов и микроэлементов, а также перидерма в качестве природного адсорбента.

КЛЮЧЕВЫЕ СЛОВА: кора ивы, перидерм, флоэма, камбий, минеральный состав, антиоксиданты.