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Research article

Antioxidative capacity of birch saps

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Abstract: *In our subsequent studies of birch tree saps we focused on assessing their antioxidant capacity. For research we chose four groups of silver birch trees (Betula pendula Roth.), consisting of five individuals. Antioxidant capacity was examined using spectrophotometric technique. The highest antioxidant capacity, tested by the Folin-Ciocalteau method and expressed as a total phenolics content was 6.59 mg GAE/100 ml of tree sap, and in turn the lowest one 0.88 mg GAE/100 ml. The highest antioxidant capacity determined ABTS method and expressed as radical scavenging activity (RSA) was 30.9% and lowest 5.38% The average values of antioxidant capacity both expressed as a phenolic compounds content and as radical scavenging activity for tree saps collected from four particular locations did not differ significantly. Based on the obtained results of tree sap analyses, it can be claimed that compared to other food products, e.g. fruit and vegetable juices, birch saps are not a rich source of phenolic compounds and when compared e.g. to tee infusions, they have low antioxidant capacity. Although it can be increased by the introduction of functional additives, such as herbal extracts or concentrated fruit juices.*

Keywords: *silver birch, birch tree saps, antioxidant capacity.*

Introduction

Tree saps of the genus *Betula* are obtained at many places throughout the northern hemisphere. In Central and Northern Europe birch tree saps are collected mainly from the trees of two species, ie. *B. pendula* Roth. [1, 2] and

B. pubescens Ehrh [3]. In eastern Asia the source of saps are species *B. platyphylla* var. *japonica* Sukaczev, *B. costata* Trautv., *B. schmidtii* Regel, *B. davurica* Pallas and *B. ermanii* Cham. [4], and in turn, in North America, *B. populifolia* Marsh., *B. alleghaniensis* Britton and *B. papyrifera* Marsh. [5].

Birch tree saps are collected generally for two purposes. Firstly, in order to produce birch syrup [3,5] and, secondly, to consume them directly, as a refreshing drink, to whom folk medicine for a centuries assigns different therapeutic effects. According to folk medicine birch tree saps helped, among others, for external and internal use to strengthen hair and accelerate their growth, for the control of renal diseases including kidney stones, against gastrointestinal disorders, anemia, infectious and parasitic diseases, as well as in order to enhance immune system and even as an antitumor agent [6].

Contemporary science, equipped with the modern analytical apparatus, tries to explain such wide and varied application of birch tree sap, and the tree saps at all. One of the most frequently investigated group of chemical elements are phenolic compounds. Their content was tested both in the birch tree sap [1, 2, 7], as well as maple tree sap [8,9]. Antioxidant capacity of plant product is one of the most frequently examined parameters, proving the health-promoting properties of plant raw materials, plant-derived foodstuffs and plant-based dietary supplements [10]. Consumption of food products rich in phenolic compounds, which are responsible for high antioxidant capacity, counteracts cardiovascular diseases, cancer and ulcers of the stomach and duodenum [11].

In our previous studies, we have compared tree saps of different species of trees and we have demonstrated favorable biological properties, manifested by a high mineral content, with the absence or with the low content of toxic components, such as inorganic anions [12, 13]. We have also examined the antioxidant capacity of tree saps from eight species of trees, and it was very low, regardless of the species. It should be pointed that this researches has been conducted for the few trees and from the one location [7]. Meanwhile, our previous investigation of the composition of tree sap of many individuals of trees clearly indicate a large intraspecies variability. Therefore, in order to thoroughly examine the antioxidant capacity, we have decided to investigate tree saps of one species, but from different localizations. Into the study we have chosen birch species, due to its large acreage in Poland, which poses the greatest prospects for mass use, as well as due to the fact that birch sap is collected and consumed most often.

Experimental

Materials

Tree saps were collected in winter 2014, in South-East Poland, Subcarpathian Voivodeship, in agricultural Niwiska municipality, without any industrial plants or busy roads, with close to one half covered with woods. The collection took place in high ambient pressure, without any precipitation, with the consent of the owners of areas where the studied trees grew. During the collection, the mean

day-time temperatures were 10ºC and at night did not fall below -1ºC. The saps were collected from 10.00 a.m. to 2.00 p.m. Four groups of trees were selected: unproductive land about 50 m from farmland and 20 m from non-busy municipal road, temporarily used for cattle grazing (A, five trees); land about 10-20 meters from a busy farm with a small water course collecting wastewater from the farm (B, five trees); woods - several hundred meters from roads and farm buildings (C, five trees) and land in immediate vicinity of farmland, about 30 m from a non-busy municipal road (D, five trees). These sites were not from each other further as about one kilometer.

Each tree selected for sap tapping had diameter exceeding 25 cm. Sample collection protocol is generally based on the local tradition. Trees were cut with a small axe at the height of about 50 cm, and then a steel tube was firmly put in the base of the cut. In this study sterile centrifuge tube (50 ml volume) was container where tree sap was collected. After collecting the birch sap, which usually took a few minutes, the tubes were closed instantly.

Methods

Tree sap samples, right after collection, were frozen in -20ºC and then, once the whole batch was collected, transported to the Malopolska Centre of Food Monitoring of the University of Agriculture in Kraków where the samples were thawed and analyzed. Total antioxidant capacity of birch saps were measured using Folin-Ciocalteu colorimetric method described previously by Swain and Hillis [14], by UV-VIS Cary 50 spectrophotometer (Varian, USA). The results were expressed as a total phenolics content (TPC) and indicated as mg/L of gallic acid equivalents (GAE). Each sample was analyzed twice.

The antioxidant capacity was also determined by ABTS radical cation decolorization method [15]. ABTS(2,2`azinobis-3-ethylbenzthiazoline-6-sulfonic acid) purchased from Sigma–Aldrich (Steinheim, Germany) was dissolved in water to a 7 μM concentration. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting $ABTS^{\bullet+}$ stock solution with 2.45 μM potassium persulfate (final concentration) purchased from Sigma–Aldrich (Steinheim, Germany) and kept in the dark at room temperature for 12–16 h before use. The radical was stable in this form for more than two days when stored in the dark at room temperature. For the study of birch tree saps, the samples containing the $ABTS^{\bullet+}$ solution were diluted with redistilled water (Milli-Q Millipore 18.2 MΩ/cm resistivity) to an absorbance of 0.74-0.75 (±0.02) at 734 nm and equilibrated at 30°C. A reagent blank reading was taken (A_0). After addition of 3.0 ml of diluted $ABTS^{\bullet+}$ solution (A_{734nm}= 0.74-0.75 (±0.02)) to 30 μl of phenolic extracts, the absorbance reading was exactly 6 min after initial mixing (A_6). The absorbance of the resulting colour was measured with a using a UV-VIS Cary 50 spectrophotometer (Varian, USA). Each sample was analyzed twice and the results were presented as:

%RSA (Radical Scavenging Activity) = $[(A_0 – A_6)/ A_0]*100$ [%], wherein:

A_0 – blank reading;

A_6 – sample absorbance 6 min. after initial mixing.

The Radical Scavenging Activityresults were corrected for the dilution and expressed as TEAC (Trolox Equivalent Antioxidant Capacity) inμmol Trolox/ 100 mL based on a calibration curve for dependence the absorbance of Trolox standard solution with $ABTS^{\bullet+}$reagent mixture on the concentration of Trolox standard solution.

The statistical analysis was carried out using one-way analysis of variance (one-way ANOVA). The differentiator adopted was the tree location. The post-hoc Tukey's Honestly Significant Difference test for equinumerous assays was used for determining the statistically significant differences. All calculations and diagrams were made using Statistica ver. 12.0 software. The differences with the significance of $\alpha < 0.05$ were considered statistically significant.

Results and Discussion

The values obtained for the parameters examined, that is the antioxidant capacity expressed as total phenolics content (TPC) and antioxidant capacity expressed as a percent of radical scavenging activity (% RSA) are presented in figure 1 as the average for the results from five trees.

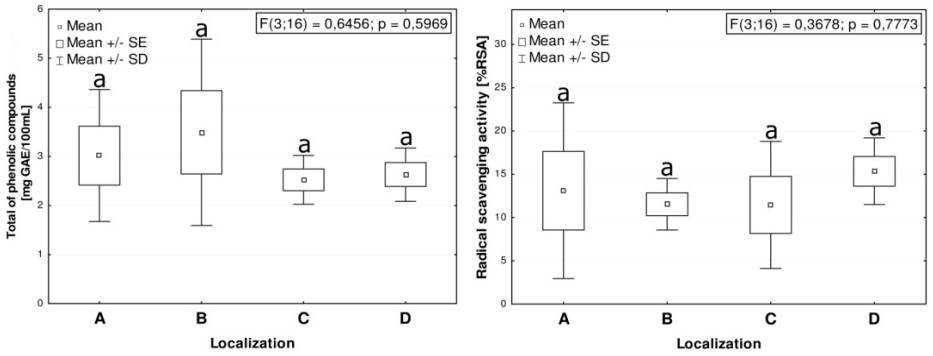

Figure 1. The average antioxidant capacity expressed as a TPC and % RSA. A – unproductive land, temporarily used for cattle grazing; B – land close to a busy farm with a small watercourse collecting wastewater from the farm; C – woods several hundred meters from roads and farm buildings; D – land in immediate vicinity of farmland.
a – the same letters denote the absence of any statistically significant differences between experimental groups ($\alpha < 0.05$)

For the localization A, ie. unproductive land, antioxidant capacity expressed as TPC ranged from 0.88 to 4.51 mg GAE/100 ml with an average 3.02 mg GAE/100 ml, and the antioxidant capacity expressed as radical scavenging activity ranged from 5.38 to 30.9% RSA with an 13.1% RSA as an average. On the other hand, for the B sites, it means land close to a busy farm, TPC is from 1.69 to 6.59 mg GAE/100 ml with an average of 3.49 mg GAE/100 ml, while

radical scavenging activity from 7.29 to 14.4% RSA with an average 11.5% RSA. In the case of position C range of TPC varied from 1.74 to 3.09 mg GAE/100 ml with an average 2.52 mg GAE/100 ml, while the radical scavenging activity from 5.69 to 24.3% RSA and with an average 11.5% RSA. For the localization D TPC was between 2.04 and 3.47 mg GAE/100 ml with an average of 2.63 mg GAE/100 ml, while the radical scavenging activity between 11.4 to 19.9% RSA and with 15.3% RSA as an average.

Birch saps are not outstanding in terms of antioxidant capacity when compared to other tree species saps. In this study the average antioxidant capacity expressed as TPC measured for the 20 individuals of birch trees was 2.91 mg GAE/100 ml, while for groups of five individuals ranged between 1.56 mg GAE/100 ml for European hornbeam and 3.70 mg GAE/100 ml for black walnut. Interestingly, the average TPC of five individuals of silver birch, which are located a few kilometres from the described place of research, was 2.39 mg GAE/100 ml and it was similar to the averages obtained in the present study that for four localizations ranged from 2.52 to the 3.49 mg GAE/100 ml [7].

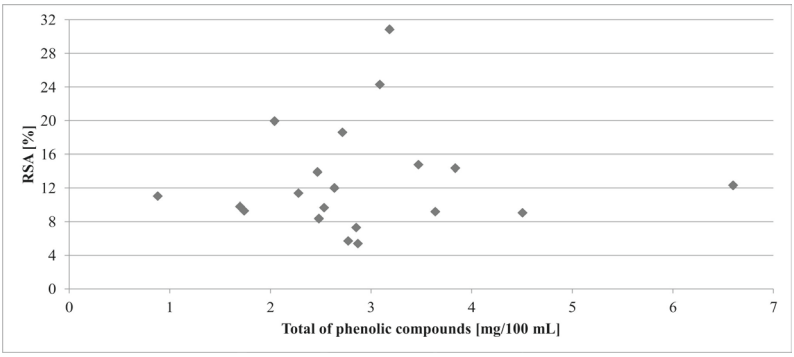

Figure 2. A scatterplot for the total phenolics content and the antioxidant capacity for the examined birch saps

However, the values of the antioxidant capacity expressed as radical scavenging activity of the examined birch saps are higher than those determined in our previous work. For five trees belonging to different species, the antioxidant capacity determined the same ABTS method ranged between 1.37% RSA for common hornbeam (*Carpinus betulus* L.) and 8.82% RSA for black walnut (*Juglans nigra* L.). In the present study, in turn, the average for twenty tree species birch was 12.9% RSA. On the other hand, comparing the average antioxidant capacity of the saps of five distant birch trees RSA was 3.02%, whereas in these studies the averages for the four equally numerous groups of trees ranged between 11.5% and 15.3% RSA [7]. Conclusion can be drawn, therefore, that differences in antioxidant capacity can be traced between the

spaced positions, while they are much smaller when comparing highly diverse habitats, but close to each other.

It must also be emphasized that for the examined birch saps, no correlation was found between the antioxidant activity expressed as total phenolics content and the antioxidant capacity expressed as radical scavenging activity (Figure 2), which was determined in our previous studies for the maple ash saps [7].

Based on the obtained results of tree sap analyses, it can be claimed that compared to other food products tested by the Folin-Ciocalteau method and expressed as a total phenolics content, e.g. fruit and vegetable juices, birch saps are not a rich source of phenolic compounds, and also when compared antioxidant capacity determined ABTS method and expressed as radical scavenging activity e.g. to tee infusions, they have low antioxidant capacity (Figure 3) [16, 17, 18, 19, 20].

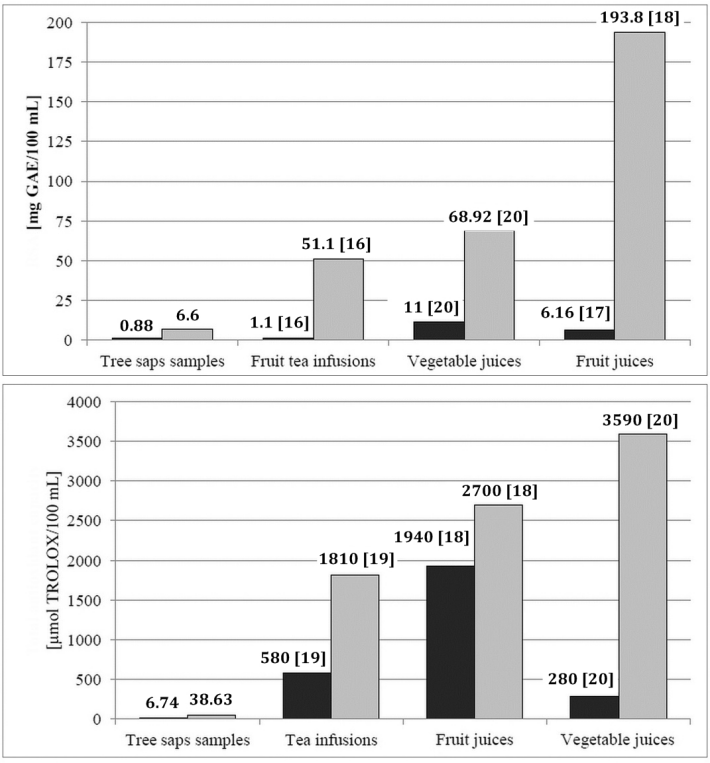

Figure 3. Comparing the antioxidant capacity expressed as total phenolics content and trolox equivalent antioxidant capacity of birch saps with other foodstuffs. Black bars represent minimum values for the tested birch saps and obtained in the cited studies, gray bars represent the maximum values for the tested birch saps and obtained in the cited studies

Conclusions

Our results are especially relevant in relation to the Central Europe fast growing "organic products" market [21, 22]. Increasingly important part of its assortment in recent years become bottled birch tree saps. These drinks are considered by consumers as the source of many health-promoting compounds [23]. Our results indicate that the most favorable to the health of consumers are not birch tree saps marked as a "clean" and with "no additives", but those with the addition of e.g. herbal extracts or fruit juices. These additives may play the role of functional additives, which significantly increase the antioxidant capacity of birch sap and, at the same time, improve sensory properties [10, 24]. However, appropriate information about the health benefits of the functional additives should be placed on the labels of bottled tree saps [25]. The addition of herbal extracts or concentrated fruit juices may also be used by a consumers collecting and consuming birch tree saps on their own. Thanks to this, pro-health value of birch tree saps will increases, as well as the additional effect of correcting the taste, that is not accepted by some consumers, is achieved [23].

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