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NATURAL BORON-CONTAINING COMPOUNDS FROM HERBAL MEDICINAL RESOURCES

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ABSTRACT

Boron (B) has an important role in the proper growth and development of plants. In nature, B is found mostly in the form of boric acid (BA) or borate and can interact with organic molecules from the plants' composition (carbohydrates, polyols, polyphenols, glycoproteins). Depending on their concentration in plant tissue, many organic compounds can form complexes with BA. Natural B-containing compounds have been identified by high-performance thin-layer chromatography (HPTLC), after derivatization with azomethine H, in the following medicinal plant species: Artemisia annua, Coffea arabica, Equisetum arvense, Linum usitatissimum, Persea americana, Stevia rebaudiana, Taraxacum officinale, and Urtica dioica.

INTRODUCTION

Boron (B) is an essential element for plant growth. It normally exists in the soil as boric acid (BA) or borate, but both forms react readily with a variety of molecules to form esters and complexes with numerous mono-, di-, and polyhydroxy compounds. Organic molecules in plants that could bind with B include ribose, apiose, polyols (sorbitol, mannitol), simple sugars (glucose, fructose), phenols, amino acids, glycoproteins, glycolipids, organic acids, phenolic acids. Given the diversity of functional groups with which B can bind, a wide range of B containing organic molecules are likely to be present in all biological systems. The concentration of sugars, sugar alcohols, and other molecules that may form *bis*-hydroxy acid–borate complexes has been determined in many plants. Amounts of B complexing sugar molecules vary depending upon species, plant organ and age. In apple fruit juice, the B-complexing sugars fructose and sorbitol are abundant (4.12–6.76 g/100 mL and 0.11–0.51 g/100 mL, respectively) (Matsunaga & Nagata 1995). B–saccharide complexes may involve sugar alcohols (mannitol, galactitol, sorbitol) and mono-saccharides (fructose, glucose, galactose), depending on their relative abundance in the plant. In peach nectar, fructose–B–fructose is dominant, followed by fructose–

B–sorbitol, and sorbitol–B–sorbitol (Hu et al. 1997). B-containing complexes are found in numerous plant sources, including pome fruits, with high concentrations of sorbitol or fructose, which are a normal part of human diet, consumed both frequently and in relatively large quantities.

In this respect, the identification of new B-containing natural compounds, their synthesis and physico-chemical analysis is a highly innovative research area. After the extraction phase, we will focus mostly on the B compounds with sugars and phenolic acids (Bita et al. 2020).

MATERIAL AND METHODS

The initial plant list contained a larger variety of plants: *Artemisia annua*, *Coffea arabica*, *Echium vulgare*, *Epilobium hirsutum*, *Equisetum arvense*, *Galeopsis speciosa*, *Hypericum perforatum*, *Linum usitatissimum*, *Marrubium peregrinum*, *Melissa officinalis*, *Mentha longifolia*, *Persea americana*, *Prunella vulgaris*, *Salvia verticillata*, *Scutellaria hastifolia*, *Sideritis montana*, *Stachys sylvatica*, *Stevia rebaudiana*, *Taraxacum officinalis*, *Thymus pannonicus*, *Urtica dioica*, *Ziziphora capitata*. The native plants were harvested between June 2016 and July 2020 from the South-West Region of Romania: Bucovăţ, Radovan, Scăeşti, Vârtop, Vulpeşti (Dolj County), Reşca Forest (Olt County), Stroeşti (Vâlcea County), Băile Herculane (Caraş Severin County). The confirmation of the plant species was offered by the Department of Pharmacognosy & Phytotherapy, University of Medicine and Pharmacy of Craiova, Romania. The plant material that was not native was purchased from local and foreign sources.

To obtain the extracts, we used the ultrasound (US)-assisted extraction. US-assisted solid–liquid extraction has been found to be a powerful tool in the analysis of bioactive compounds in herbs and phyto foods (Teixeira et al. 2006, Wongkittipong et al. 2004). By inducing cavitation, the method results in collapse of the material matrix and enhanced recovery of analytes from matrix to the extraction solvent. Approximately 3–4 g of each selected plant material was weighed and added to 30 mL of solvent. We used two different solvents, water and acetonitrile (LiChrosolv®), both purchased from Merck (Darmstadt, Germany). Ultrasonication was performed using a Bandelin Sonoplus Ultrasonic Homogenizer HD3200 (Bandelin Electronic GmbH & Co. KG, Berlin, Germany) for 20 minutes. After ultrasonication of the plant material, the whole sample was centrifuged at 10 000 rpm for 20 minutes with an Eppendorf 5804 centrifuge (Eppendorf, Hamburg, Germany) to properly separate the solid phase. The B-containing fraction was determined as BA by high-performance thin-layer chromatography (HPTLC) with azomethine H derivatization (Bita et al. 2017).

The HPTLC analysis was performed using a CAMAG system (Muttenz, Switzerland). The BA reference sample was prepared in deionized water; 0.5716 g BA was dissolved in 1 L water to obtain a 100 ppm B standard. This stock solution was diluted accordingly to obtain a concentration range of 10–50 ppm. The HPTLC Si 60 F_{254} 20×10 cm glass plates were obtained from Merck. The plates were developed in twin-trough glass chambers (20×10 cm). The derivatization reagent was azomethine H and was prepared by dissolving 1 g of azomethine H and 1 g of ascorbic acid in 200 mL deionized water. As derivatization method, we chose the immersion by the means of the CAMAG Chromatogram Immersion Device 3 for automated dipping of the plate (the setting for speed was 1 and five seconds for immersion time).

Two microliters of reference solutions and 4 µL of sample solutions were

each applied with the CAMAG Linomat 5 as 8 mm bands, at 11.4 mm apart and 8 mm from the lower edge. The developing solvent that was employed was a mixture of 2-propanol–water (8:2, *v/v*), 10 mL developing solvent in front trough, and 20 mL in the rear trough. The developing distance was 50 mm from the lower edge of the plate (42 mm from the application position). Prior to elution, the chamber was saturated with the mobile phase for 20 minutes. After the elution, the plate was dried for 10 minutes using an air dryer at room temperature. The densitometric investigation was performed using a CAMAG TLC Scanner 3, in the absorbance mode. The wavelength used for the scan was 420 nm, which is the absorbance maximum for azomethine H, with a deuterium and tungsten lamp as radiation source (Figure 1). The settings for the TLC Scanner were as follows: absorption measurement mode, 20 mm/s scanning speed, data resolution of 100 µm per step, and a slit dimension of 5×0.2 mm. All the data acquired by the scanner were processed *via* the visionCATS v2.5 software package (Figure 2).

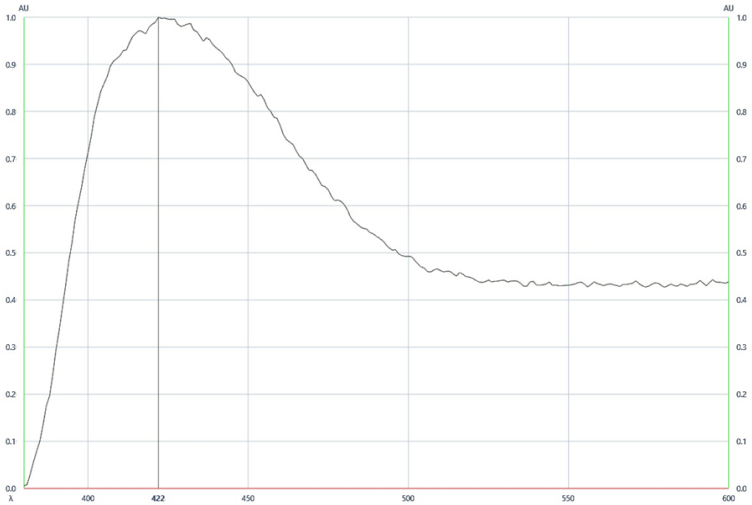

Figure 1. Azomethine H spectrum (420 nm chosen wavelength for analysis).

Figure 2. Boric acid densitogram.

RESULTS AND DISCUSSIONS

To properly select only the plants that contained a higher amount of B-containing compounds, the scientific literature (Naghii et al. 1996) was consulted and using our own B determination method (Bita et al. 2017), we selected only the plants that contained a B concentration greater than 1 ppm/g in the wet phase (green plant). The final herbal products that remained for extraction were: *Artemisiae herba*, *Coffeae semen*, *Equiseti herba*, *Lini semen*, *Perseae fructus*, *Steviae folium*, *Taraxaci radix*, and *Urticae folium*.

Both calibration curves (the one on the water extracts and the other on the acetonitrile extracts) obtained were excellent. The regression mode was linear ($y=1.078\times10^{-4}x - 4.373\times10^{-4}$ for water extracts and $y=9.625\times10^{-5}x - 7.491\times10^{-4}$ for acetonitrile extracts, respectively), with a correlation coefficient (R^2) of 0.9919 for water extracts and 0.9939 for acetonitrile extracts, respectively. The coefficient of variation (CV) of the calibration function was 5.03% and 5.11%, respectively. BA R_f was 0.61±0.03 (Figures 3 and 4).

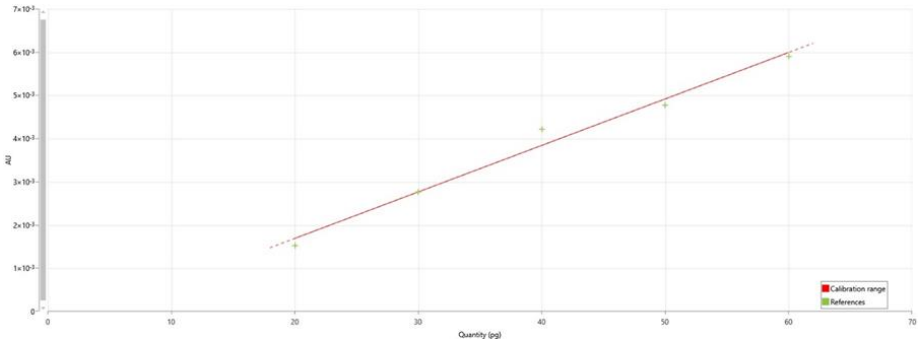

Figure 3. Boric acid calibration curve for water extracts.

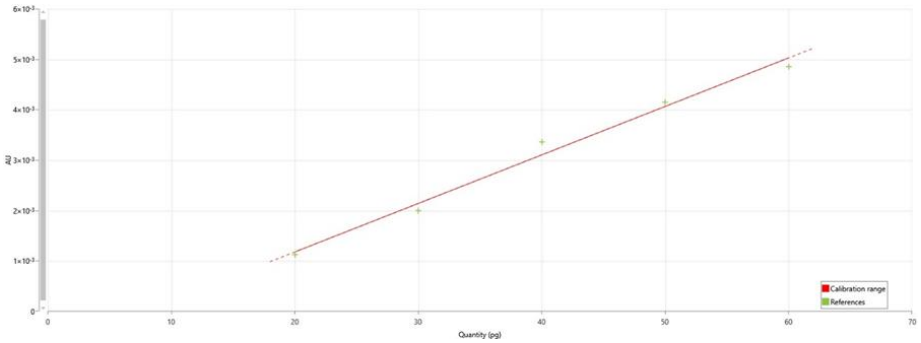

Figure 4. Boric acid calibration curve for acetonitrile extracts.

Not all chosen samples exhibited readable B concentrations mainly because in the plant we can distinguish two forms of B, a water-soluble B in the aqueous sap and structural B fixed in the cells (Sun et al. 2017). Through our method we are focusing only on the water-soluble B and organic-soluble B (acetonitrile) fractions. Preliminary tests showed that BA and borates are soluble both in water and acetonitrile, and practically water-soluble compounds can hydrolyze to BA under the hydro-extraction conditions, while all B compounds of sugars and polyphenols are

also soluble in organic medium without the hydrolysis. The plant material that contained the most B were *Steviae folium* (346.8 ng B in 1 g of raw material), *Coffeae semen* (95.58 ng B in 1 g of raw material) and *Perseae fructus* (16.11 ng B in 1 g of raw material). On the HPTLC plate with acetonitrile samples, the same more pronounced or diminished bands appeared compared to the aqueous extracts. In total, we identified the presence of five bands ranging from R_f 0.2 to 0.86. Bands that are specific to B-containing compounds (azomethine H derivatization) were also visualized in ultraviolet (UV) light, at 254 nm and 365 nm, to identify and appreciate the class of compounds. Specific carbohydrates and phenolic acids identification tests were also used. The result was that out of the five bands of B organic compounds we identified that they are part of the phenolic acids and sugar class (Figures 5 and 6).

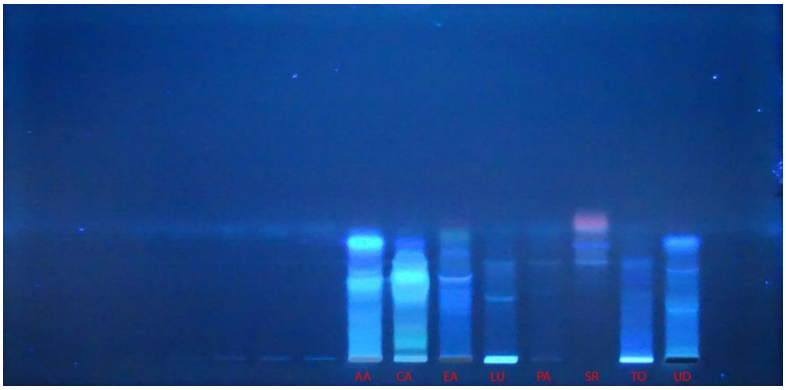

Figure 5. Water extracts HPTLC chromatogram for *Artemisia annua* (AA – *herba*), *Coffea arabica* (CA – *semen*), *Equisetum arvense* (EA – *herba*), *Linum usitatissimum* (LU – *semen*), *Persea americana* (PA – *fructus*), *Stevia rebaudiana* (SR – *folium*), *Taraxacum officinale* (TO – *radix*), *Urtica dioica* (UD – *folium*). Visualization under UV light (365 nm).

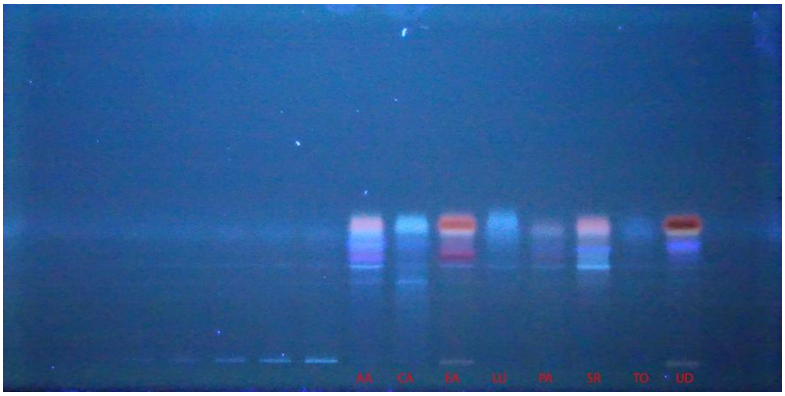

Figure 6. Acetonitrile extracts HPTLC chromatogram for *Artemisia annua* (AA – *herba*), *Coffea arabica* (CA – *semen*), *Equisetum arvense* (EA – *herba*), *Linum usitatissimum* (LU – *semen*), *Persea americana* (PA – *fructus*), *Stevia rebaudiana* (SR – *folium*), *Taraxacum officinale* (TO – *radix*), *Urtica dioica* (UD – *folium*). Visualization under UV light (365 nm).

We were interested in B-containing molecular forms both in water and in acetonitrile because aqueous extracts can degrade B compounds, especially those compounds that are stable in the organic environment. We obtained yellow bands at the same R_f as BA only in the water extracts and in acetonitrile. An explanation for this phenomenon could be that the B compounds could have hydrolyzed during the extraction phase and in the presence of water. On the other hand, the presence of several distinct bands in the acetonitrile extracts might suggest that we can isolate these B-containing compounds relatively easy, and we can further test and identify their structure in the upcoming research stages.

Artemisiae herba, *Coffeae semen*, *Equiseti herba*, *Lini semen*, *Perseae fructus*, *Steviae folium*, *Taraxaci radix* and *Urticae folium* herbal medicinal products and two classes of B-containing compounds (B-containing sugars and B-containing phenolic acids) have been identified and are to be further isolated and physico-chemically characterized.

Proof exists related to the favorable effects of B in humans regarding the calcium metabolism and bone health, most likely since B increases the effectiveness of vitamin D utilization (Hunt 2012, Nielsen 2008). Furthermore, B can counteract the deficiency of vitamin D (Hunt 2012), and it can also increase the serum concentrations of steroid hormones by modulating their metabolism (Devirian & Volpe 2003). Even if a recommended B intake for humans is not officially specified, reports that an administration of 1.0 mg to 3.0 mg of B per day could be deemed satisfactory to achieve positive effects on aging prevention and longevity (Nielsen 2018).

B is vital for vegetable growth even though a specific biochemical function of has not been identified in plants (Landi et al. 2019, Marschner 1995). B is regularly delivered as a foliar fertilizer to increase the nutritional quality of rice (Jin et al. 2008), and as an element of nutrient solution for growing soilless vegetables (Johnson et al. 1957). Concurrently, it should be noted that a high B intake in plants, in relation to high amounts in soil, growing substrate, and/or fertilization practice, could cause toxicity symptoms, such as chlorosis and necrosis of the leaves, as well as the inhibition of plant growth (Shah et al. 2017). It is remarkable to underline that the use of soilless systems permits to precisely measure the concentration of each element in the nutrient solution, potentially preventing any excess or deficiency of nutrients in plants. Additionally, by modulating the mineral composition of the nutrient solution, it is possible to increase or reduce the concentration of target ions in plant tissue to obtain tailored vegetables for specific nutritional requirements (D'Imperio et al. 2016, Di Gioia et al. 2019, Gonnella et al. 2019, Montesano et al. 2016, Smoleń et al. 2019).

This method could also be used to enhance the biosynthesis of specific natural B-containing compounds. Species like *Portulaca oleracea* and *Lemna minor* (D'Imperio et al. 2020, Tatar & Öbek 2014), with already relatively high concentrations of B, could be studied from this point of view.

CONCLUSIONS

In our study, we demonstrated the presence of bioactive B-containing compounds in several medicinal plant products from local or foreign species. Future studies to increase the concentration of these bioactive compounds, *e.g.*, by growing the plants on soilless nutrient solution or by foliar application with B might help to identify their exact structure.

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