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11TH HARDWOOD CONFERENCE PROCEEDINGS

Róbert Németh, Christian Hansmann, Holger Militz, Miklós Bak, Mátyás Báder



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Sopron, Hungary, 30-31 May 2024

**Editors: Róbert Németh, Christian Hansmann, Holger Miltz,
Miklós Bak, Mátyás Báder**



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Polyphenol content of underutilized wood species from Hungary

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ABSTRACT

In the present research the radial variation of the polyphenol content was assessed from various stems of currently underutilized wood species from Hungary. The content and composition of polyphenols are closely related to various wood properties (e.g. color, durability). The sapwood, sapwood/heartwood boundary and various tissues of the heartwood were investigated. The main goal of the research was to find out if there were significant differences in the polyphenol content along the radius which may also affect utilization. Underutilized hardwood species black locust (*Robinia pseudoacacia* L.), Pannonia poplar (*Populus x euramericana* cv. *Pannonia*) and Turkey oak (*Quercus cerris* L.) were investigated which are interesting for future uses and afforestation in Hungary regarding the effects of changing climatic conditions. Quantification of total polyphenol content was accomplished using the Folin-Ciocalteu assay. The identification and radial variation of major polyphenolic compounds was accomplished using high-performance liquid chromatography-photodiode array detection-electrospray ionisation-multistage mass spectrometry detection.

INTRODUCTION

As a consequence of climate change the importance of domestic, drought-tolerant and underused woody species will increase in the future. Wood properties are highly influenced by the content and composition of extractives, out of which polyphenols are especially important as they determine wood color, color stability as well as wood durability. Their concentration can vary between different tissues of the same stem, moreover inside of a given tissue, too. In the present work the radial variation of the polyphenolic composition and content were assessed from various stems of black locust (*Robinia pseudoacacia* L.), Poplar (*Populus x euramericana* cv. *Pannonia*) and Turkey oak (*Quercus cerris* L.) originating from the forests of the Kiskunsági Erdészeti és Faipari Zrt. forestry company (KEFAG). Results contribute to the identification of compounds influencing wood material and technological properties in the future and for the selection of quality wood material.

MATERIALS AND METHODS

Sample collection was carried out in the forests of the Kiskunsági Erdészeti és Faipari Zrt. forestry company in eastern Hungary. From all species 5 representative trees were selected and discs were cut from 1 meter height. Discs were taken to the laboratory and wood chips were cut using a drill from different parts of the discs (sapwood, sapwood/heartwood boundary, middle of heartwood, growth ring 16, growth ring 12 and growth ring 4). Samples were dried at room temperature to even moisture content and extracted (0.2 g) using methanol:water 50:50 (v/v) solution (14 ml) by ultrasonication for 30 minutes. Extracts were centrifuged at 12.000 1/min for 2x10 minutes and taken to analysis.

Polyphenol content and composition was measured with two methods: the Folin-Ciocalteu's total polyphenol content (TPC) and the high-performance liquid chromatography-photodiode array detection-electrospray ionisation-multistage mass spectrometry detection (HPLC-DPA-ESI-MS/MS). The TPC was determined by the Folin-Ciocalteu assay (Singleton and Rossi 1965) and implemented using previously published methodology (Tálos-Nebhaj et al. 2019). Average was determined for each tissue

(n=5) for each species. The HPLC-PDA-ESI-MS/MS separation and identification of polyphenolic compounds was accomplished based on earlier works of the authors (Hofmann et al. 2016; Agarwal et al. 2021; Hofmann et al. 2021a).

RESULTS AND DISCUSSION

Total polyphenol content

According to previous results the significant radial variation was observed within the heartwood of sweet chestnut (*Castanea sativa* L.), where the tissues around the sapwood/heartwood boundary had the highest TPC values and the content of polyphenols decreased while moving towards the innermost tissues of the heartwood (Eichhorn et al. 2017). Comparing to the present results it was found that this tendency must be species-dependent, as different radial variations were observed for all of the three species. Figure 1 depicts the radial variation of the TPC in the three investigates species.

In general a very high standard deviation of the TPC values was reported indicating a very high natural variation of the TPC even within a given species and tissue. In black locust the lowest TPC was found in the sapwood and polyphenol content increased at the sapwood/heartwood boundary and remained very in high in the inner heartwood. Results are in accordance with earlier findings (Magel et al. 1994). Although there is a slight decreasing tendency of the TPC from sapwood/heartwood boundary towards the pith, just like in the case of sweet chestnut (Eichhorn et al. 2017), no significant differences could be evidenced between the individual heartwood tissues primarily as a result of high standard deviation values.

In poplar the highest TPC was determined in the sapwood/heartwood boundary tissue. Both sap and heartwood had significantly lower TPC levels. Interestingly the TPC of heartwood was not significantly higher compared to sapwood as opposed to general findings. The standard deviation of results is even higher compared to black locust samples.

In Tukey oak no significant differences were found between any of the tissues. According to the results there was a uniform distribution along the radius even at the sapwood/heartwood boundary no increase was determined. According to literature data the cold and hot water soluble extractive content of Turkey oak sapwood is higher compared to heartwood (Deaconu et al. 2023). The radial variation of the TPC needs further explanations as it contradicts major findings of the radial variation of polyphenols in tree stems (Magel et al. 1994; Eichhorn et al. 2017; Hofmann et al. 2021b) and can possibly accounted by the presence of other types of extractives.

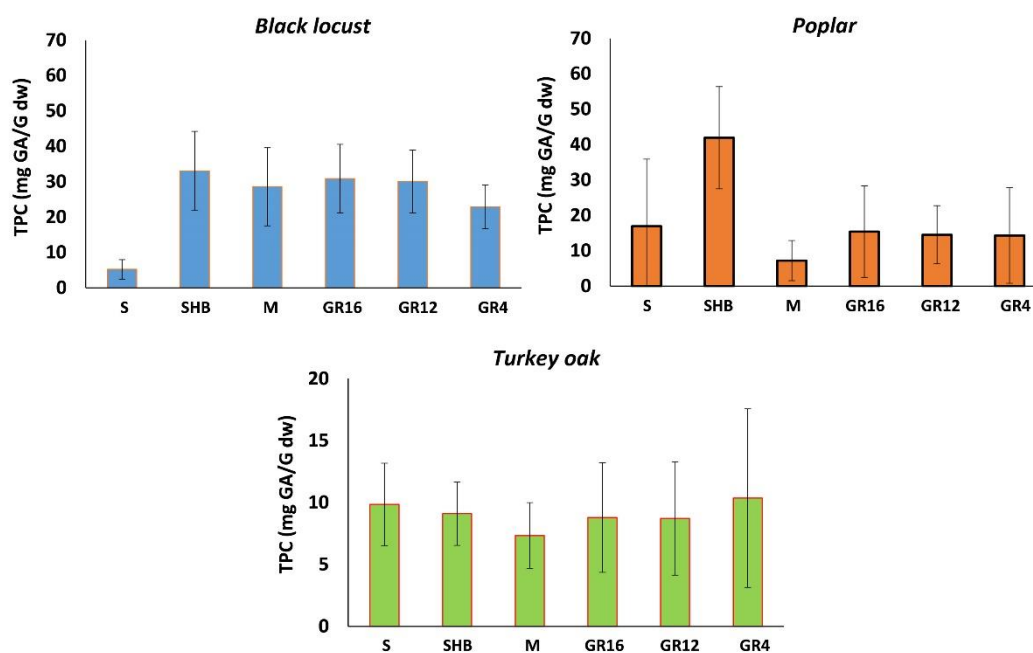


Figure 1: Radial variations of the total polyphenol content (TPC) in different species. S: sapwood, SHB: sapwood/heartwood boundary, M: middle of heartwood, GR16: growth ring 16, GR12: growth ring 12, GR4: growth ring 4

The TPC assay gives a fast quantitative evaluation of the total polyphenol content of samples, however the assay can interfere with some non-polyphenol type compounds, too (e.g. reducing sugars, organic acids, etc.) which influence results (Prior et al. 2005; Everette et al. 2010). Thus, in order to have precise knowledge on polyphenol composition and concentration the separation, identification and quantification of individual compounds has to be assessed additionally.

HPLC-PDA-ESI-MS/MS analysis of polyphenols

In the present work the HPLC-PDA-ESI-MS/MS technique was applied for the separation and identification of the individual polyphenolic compounds from the samples. The PDA (250-380 nm) chromatogram is depicted in Figure 2. Some of the compounds have been tentatively identified, yet the identification of most compounds needs to be carried out in the future.

The most abundant peaks in black locust sample correspond to tetrahydroxy-dihydroflavonol (2), dihydrorobinetin (6), fustin (7), dimeric prorobinetinidin (8) and robinetin (11). According to peak heights the highest concentrations are found in heartwood, especially at the sapwood/heartwood boundary (SHB) and the heartwood tissues next to it (GR16), while inner heartwood (GR4) has decreased concentrations, and lowest values can be measured in the sapwood. These results are in accordance with the TPC measurements.

Contrary to the TPC values, the overall highest peaks were found in the sapwood sample of poplar and not at the sapwood/heartwood boundary. Out of the highest peaks aromadendrin (9) and naringenin (14) have been identified by mass spectra, while the identification of peaks 12 and 15 and 16 require future work. According to the chromatogram concentrations decrease, while moving to inner heartwood tissues, which similarly as with TPC values. It can be assumed that other type of extractives may have been present in poplar sapwood/heartwood boundary which interfered with the TPC measurements giving very high values, which suggests that before evaluation of TPC results the presence of reducing type extractives must be considered.

The overall lowest peaks were determined in Turkey oak samples, which was found interesting also comparing with fairly high TPC values. We assume again that interfering compounds must have influenced the TPC values for Turkey oak samples. Highest peaks were determined at the sapwood/heartwood boundary, while moving toward the inner heartwood tissues peak height decrease indicating lower concentrations. Peak 4 was identified as (+)-catechin, while peak 10 has not yet been identified and requires future work.

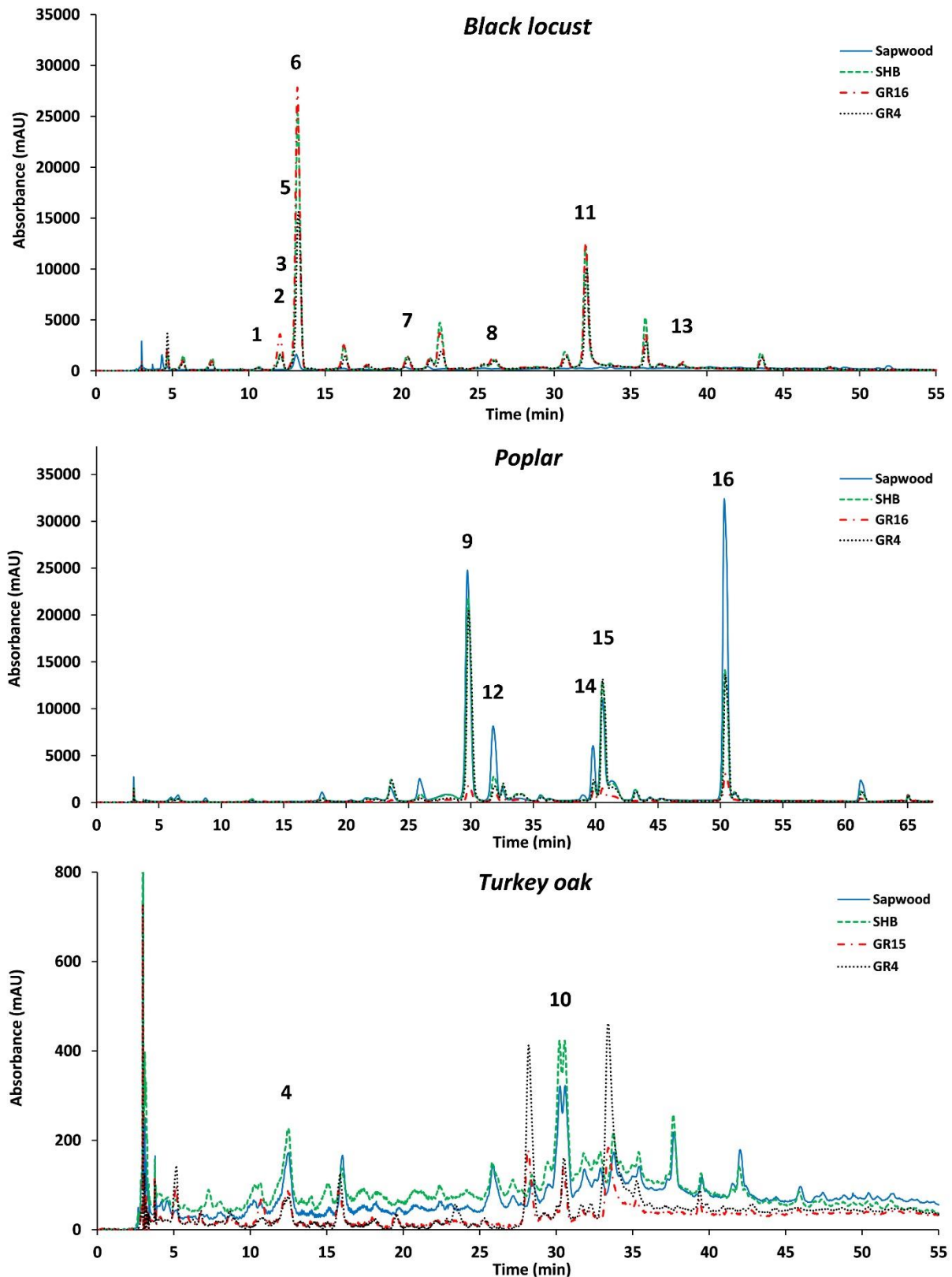


Figure 2: The HPLC-PDA (250-380 nm) chromatogram of selected wood samples (GR4: growth ring 4, RG16: growth ring 16, SHB: sapwood/heartwood boundary). 1: prorobinetinidin, 2: tetrahydrodihydroflavonol, 3: fisetin, 4: (+)-catechin, 5: robtin, 6: dihydrorobinetin, 7: fustin, 8: dimeric prorobinetinidin, 9: aromadendrin, 10: unidentified (m/z 565), 11: robinetin, 12: unidentified (m/z 229), 13: robtein, 14: naringenin, 15: unidentified (m/z 561), 16: unidentified (256)

CONCLUSIONS

Climate change poses a challenge to wood industry and domestic wood resources have to be utilized in a more efficient way, which requires a more extensive use of drought tolerant and underutilized species in the future. Poplar, Tukey oak and black locust provide wood which must be better utilized in the future. Extractive content, especially polyphenols affect various wood properties. Tendencies of the radial variation of polyphenol content was found to be different in the investigated species. Folin-Ciocalteu's total phenol content assay is fast and reliable method and can give a good estimation of the total polyphenol content, yet it can interfere with reducing compounds (sugars, organic acids) thus results must be critically evaluated especially for species and tissues with high reducing sugar and organic acid content. Chromatographic/mass spectrometric determinations are more defined, yet analysis takes more time and implies higher costs.

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