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ORIGINAL

Efficiency of three conventional methods for extraction of dihydrorobinetin and robinetin from wood of black locust

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Abstract
The aim of the study was to examine the amounts of extractives in sapwood and heartwood of black locust recovered using three different conventional extraction techniques and three different solvent compositions. Heartwood contained larger amounts of total phenols, dihydrorobinetin and robinetin than sapwood, irrespective of the extraction technique and solvent used. Dihydrorobinetin and robinetin were the characteristic phenolic compounds of black locust wood, whereas the concentration of dihydrorobinetin was significantly higher. The highest concentrations of examined extractives were obtained by Soxhlet extraction. More than ninety percent of extractives were leached from wood in a Soxhlet apparatus in less than 2 hours. Maceration with stirring and ultrasonic extraction gave smaller yields of extractives. The amounts of total extractives, total phenols and robinetin leached with the three solvents were comparable. Extraction of heartwood with acetone yielded significantly larger amounts of dihydrorobinetin than extraction with methanol or ethanol. Four hours extraction of wood meal with aqueous acetone in a Soxhlet apparatus was found to be the optimal extraction procedure for the recovery of dihydrorobinetin.

1 Introduction

Extractives are a large and heterogeneous group of lower molecular weight compounds. They are reported to be non-structural components of wood that are located in the lumina of cells and extraneous to the lignocellulosic cell wall (Fengel and Wegener 1989; Rowe 1989). These compounds have a significant impact on wood color, odor, density, equilibrium moisture content, dimensional stability and the natural resistance of wood (Fengel and Wegener 1989; Harju et al. 2003; Holmbom 2011; Kai 1991; Pearce 1996; Rowe 1989).

Extractives can be classified according to their chemical similarities, with respect to the biochemical paths of their synthesis or in relation to the solvent in which they are soluble (Fengel and Wegener 1989; Kai 1991; Rowe and Conner 1979; Umezawa 2000). Based on their solubility, extractives can be divided into classes of lipophilic and hydrophilic extractives (Jansson and Nilvebrant 2009; Willför et al. 2006). Lipophilic extractives are soluble in less polar solvents, such as toluene, hexane or diethyl ether, while hydrophilic extractives are a group of compounds soluble in more polar solvents, for example, acetone, methanol or ethanol.

Analysis of wood extractives starts with correct sample preparation, followed by mass transfer of the targeted compounds to a liquid phase by solid–liquid extraction (Luque de Castro and Priego-Capote 2010). In terms of the conditions of the extraction process, wood can be extracted under less (e.g., room temperature) or more severe (e.g., temperature of solvent above its boiling point) conditions. However, maceration and sonication are usually performed in ambient conditions, while in modern extraction systems, or even in a Soxhlet apparatus, extraction is carried out under more severe conditions, viz. increased temperature and/or pressure.

According to the literature, wood of black locust has often been extracted by maceration, sonication or Soxhlet extraction (Fan et al. 2010; Magel et al. 1994; Meszaros et al. 2007; Sanz et al. 2011, 2012; Sergent et al. 2014; Smith et al. 1989). Examples of wood extraction with advanced and automatized extraction techniques have also recently been reported (Bostyn et al. 2018; Dunisch et al. 2010; Sablik et al. 2016). A shorter extraction time and lower solvent consumption are reported to be the main advantages

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of automatized extraction techniques (Sablik et al. 2016; Schwanninger and Hinterstoisser 2002; Thurbide and Hughes 2000). Bostyn et al. (2018) extracted dihydrorobinetin and robinetin from black locust heartwood with a mixture of ethanol and water (80:20, w/w), thereafter, the extraction was optimised by varying just two extraction parameters, i.e. temperature and a wood/solvent ratio (w/v). The authors concluded that the effect of temperature can be neglected when extracting the robinetins from black locust wood (Bostyn et al. 2018). Nonetheless, it is still unclear which conventional extraction technique and solvent provides the most suitable approach for the extraction of black locust wood on a laboratory scale. Optimal conditions of the extraction procedure are very important for investigating the applicative potential of wood extractives. An effective methodology for extracting value-added compounds from wood must therefore be established.

The aim of the present work was to compare the amounts of extractives present in wood extracts of black locust (hydrophilic extractives, total phenols and two targeted compounds: dihydrorobinetin and robinetin), recovered using three different extraction techniques (Soxhlet apparatus, maceration and sonication) and three different solvent compositions (acetone, ethanol and methanol).

Wood of black locust (*Robinia pseudoacacia* L.) was used in the present investigation. This tree species, which is also called false acacia, locust or simply *robinia*, is known to be a very invasive and widely planted tree species (Rademacher et al. 2016; Vítková et al. 2017). Moreover, wood of black locust is characterized by high natural durability, which is explained by the presence of extractives (Destandau et al. 2016; Sergent et al. 2014). Flavanonol dihydrorobinetin, flavonol robinetin and hydroxycinnamic acid are the three main wood extractives of black locust, as reported by Magel et al. (1994) (Fig. 1). Compounds that occur in black locust have been described as bioactive compounds with antimicrobial, antifungal and antioxidant properties (Katiki et al. 2013; Marinas et al. 2014; Sablik et al. 2016). Wood extractives of black locust thus clearly show great potential in the field of wood preservatives as natural fungicides. As reported by Singh and Singh (2012), the main benefits of using natural compounds for wood protection is their minimum environmental impact at the end of their service life in comparison to synthetic substances.

2 Materials and methods

2.1 Chemicals

Methanol and ethanol, both of analytical grade, formic acid (puriss. p.a., 98%), Folin-Ciocalteu phenol reagent (2 N), sodium carbonate (anhydrous) and gallic acid monohydrate (HPLC assay, ≥ 99%) were purchased from Merck (Sigma–Aldrich Chemie). HPLC grade water and acetone were provided by J.T. Baker. Analytical standards used for chromatographic analysis, i.e., robinetin (HPLC assay, ≥ 95%) and dihydrorobinetin (HPLC assay, ≥ 99%) (Fig. 1), were supplied by Extrasynthese.

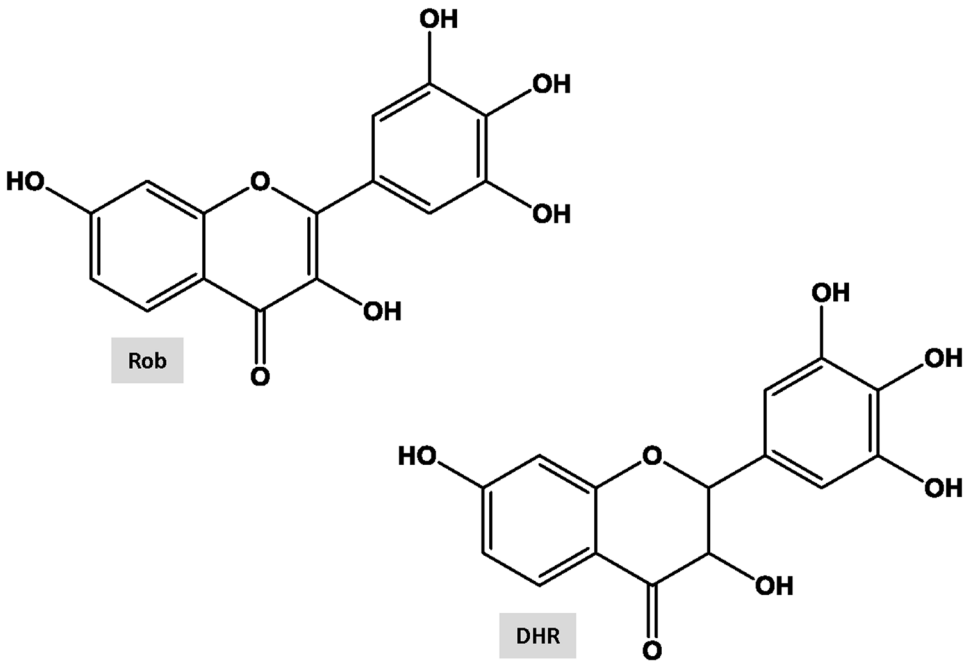

Fig. 1 Structure formulas of robinetin (Rob) and dihydrorobinetin (DHR)

2.2 Material

The investigation was performed on wood of black locust (*Robinia pseudoacacia* L.). Six mature trees were felled in the urban forest of Panovec in Nova Gorica. The sample trees measured on average 21.83 (SD; 1.125) m in height, with a diameter of 26.08 (SD; 2.764) cm at breast height (Table 1). After the trees were felled, sample discs were sawn from each harvested stem at four different heights: 0.20, 3.30, 6.40, and 9.50 m (Fig. 2). Cross sections of sample discs were carefully reviewed. The diameters measured and the age of each disc were determined on the basis of the number of annual rings (Table 1).

Radial profiles were then sawn from each of the sample discs. One sample of sapwood (sw) and several samples of heartwood (hw), were sawn from each radial profile. However, only two samples per stem disk were used for the present investigation, i.e., sw sample a few rings wide and hw sample aged 24–33 years (Fig. 2). Samples were ground using a Retsch cutting mill SM 2000, producing wood meal that passed through a 1.0 mm sieve. By taking volume equivalents of each sapwood and heartwood meal, respectively, one sample containing a mixture of all sapwoods (sw) and one sample containing a mixture of all heartwoods (hw) were obtained. These two samples were subjected to further chemical analysis. Samples were properly stored at − 24 °C until further processing.

2.3 Extraction protocols

All samples were freeze-dried in a Telstar LyoQuest lyophylizator at 0.040 mbar and − 82 °C for 24 h before extraction. Three extraction techniques were used for the extraction: Soxhlet apparatus, sonication and maceration. The applied techniques are reported to be the conventional methods of extraction of wood and bark on a lab scale (Hofmann et al. 2015; Schwanninger and Hinterstoisser 2002; Sergent et al. 2014).

The solvents used for the extraction were acetone, methanol and ethanol. 10% volume of water (v/v) was added to each solvent in order to increase the penetration of the solvent into the wood matrix (Willför et al. 2006). Suitable concentrations of wood extracts were defined by a sample-to-solvent ratio (w/v) of 1:100 (Naczk and Shahidi 2004).

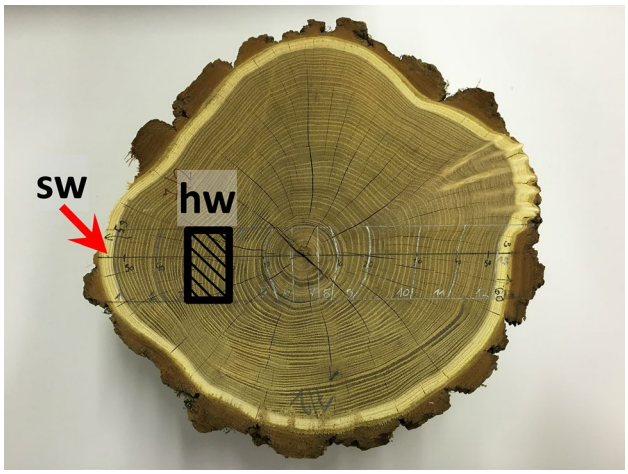

Fig. 2 Cross section of a stem disc with the marked locations of sampling. The black box on the sample disc indicates a position of heartwood samples (hw) that were included in the extraction process. A position of sapwood samples (sw) is indicated with an arrow

2.3.1 Extraction of wood in a Soxhlet apparatus

Two and a half grams of a freeze-dried wood sample were extracted with 250 ml of solvent in a Soxhlet apparatus at 110 °C for 6 h. According to the TAPPI testing method T 264 cm-97 (Preparation of wood for chemical analysis), a standard Soxhlet extraction was carried out with 200 mL of solvent for 6–8 h, keeping the liquid boiling briskly so that the siphoning from the extractor is no less than 4 times per hour. However, in order to determine the optimal duration of Soxhlet extraction, a freeze-dried sample of heartwood was also extracted in 12 successively placed Soxhlet apparatuses with acetone/water (9:1, v/v) at 110 °C. Extracts were collected after the 1st, 2nd, 3rd, 4th, 5th, 6th, 8th, 10th, 12th, 14th, 16th and 18th cycle of extraction. One extraction cycle was defined as the time in which condensed fresh solvent filled a thimble holder and a siphon aspirated the solvent

Table 1 Average dimensions and age of trunk discs taken from six sampled black locust trees (*Robinia pseudoacacia* L.)

Stem disc (no.)	Sampling height (m)	Age of tree* (no. of annual rings)		Heartwood* (no. of annual rings)		Sapwood* (no. of annual rings)		Diameter (cm)	
		avg.	SD	avg.	SD	avg.	SD	avg.	SD
1	0.20	54	10.5	50	10.7	4	0.9	27.0	1.86
2	3.30	50	5.0	46	5.2	4	0.8	22.5	2.91
3	6.40	45	1.3	41	1.5	4	1.0	20.1	3.65
4	9.50	40	2.3	36	2.9	3	1.6	16.0	1.59

*The age of the tree at four different sampling heights was determined according to the number of annual rings counted on a cross section of the stem disc

from the thimble-holder back into the distillation flask (Luque de Castro and Priego-Capote 2010).

2.3.2 Ultrasonic extraction of wood

Sonication extraction was carried out in a Bandelin electronic RK 512 H ultrasonic bath at room temperature for 1 h. Half a gram each of dried sw and hw sample were extracted with 50 ml of solvent.

2.3.3 Maceration with stirring the wood

Maceration with stirring the wood was performed using an IKA RO 15 power magnetic stirrer at room temperature for 24 h. One gram each of sw and hw meal were macerated by stirring in 100 ml of solvent.

2.4 Preparation of wood extracts for spectrophotometric and chromatographic analysis

Prior to the spectrophotometric and chromatographic investigation, the wood extracts were dried in a vacuum chamber at 100 mbar. The dry matter was then dissolved in methanol. Methanol solutions were filtered through a polyamide syringe filter. Prepared samples were stored in darkness at − 24 °C until chemical analysis.

2.5 Chemical analysis

2.5.1 Gravimetric analysis

After extraction, the content of total, hydrophilic, extractives was measured gravimetrically by drying 10 ml of wood extract to a constant mass (mg/g dw).

2.5.2 Spectrophotometric analysis (UV–Vis)

Total phenols were measured according to the protocol already described in Vek et al. (2013, 2014). Scalbert et al. (1989) and Singleton and Rossi (1965). Diluted 2 N Folin-Ciocalteu phenol reagent (*aq*) and an aqueous solution of sodium carbonate (75 g/l) were added to each wood extract. The reaction was performed in a 4.5 ml disposable macro cell closed with a 10 × 10 mm polyethylene lid. After incubation of the reaction mixtures, the absorbance was measured at 765 nm by Perkin-Elmer Lambda UV–Vis. Gallic acid was used as a reference for semi-quantitative evaluation of total phenols. The results were determined by standard curve of gallic acid (concentration range between 0 and 500 mg/l) and expressed in milligrams of gallic acid equivalents per gram of dried wood sample (mg GAE/g).

2.5.3 Chromatographic analysis (HPLC)

Chromatographic analysis was performed on a Thermo Scientific system for high performance liquid chromatography (Accela HPLC). The HPLC was equipped with a quarter 600 pump and a photodiode array detector (PDA). Separation of samples was done on a Thermo Accucore ODS column (4.6 id × 150 mm, 2.6 µm). Water (A) and methanol (B), both containing 0.1% of formic acid, served as a mobile phase. The flow rate of the mobile phase was set at 1000 µl/min. The gradient used was 5–95% of solvent (B). Both the autosampler containing sample trays and the column oven were thermostated at 5 °C and 30 °C, respectively. Three microliters of wood extract was injected onto the column for each HPLC run. Absorbance was measured at 275 nm and UV spectra were recorded from 200 nm to 400 nm. Peak identities were investigated by comparison of retention times and UV spectra of separated compounds with those of analytical standards (Figs. 1 and 3). The chromatographic method was linear in the selected concentration range ($R^2 \geq 0.99$). The samples were measured in triplicate. The contents were expressed in milligrams of dihydrorobinetin (DHR) and robinetin (Rob), respectively, per gram of dry wood (mg/g dw).

2.6 Estimation of extraction efficiency

The efficiency of the above described extraction protocols was compared after gravimetrical, UV–Vis and HPLC analysis by measuring the amounts of total extractives, total phenols and the concentrations of dihydrorobinetin (DHR) and robinetin (Rob) in the extracts.

2.7 Statistics

Basic statistical analysis was performed with Statgraphics software. The data were first checked for normal distribution, and analysis of variance (ANOVA) and Fisher's least significant difference (LSD) procedure at a 95.0% confidence level were performed. Structural formulas of compounds were prepared with PerkinElmer's ChemDraw software.

3 Results and discussion

The amounts of total extractives obtained with the three different extraction techniques significantly differed (ANOVA; $p < 0.05$). The highest amounts of total extractives were gained with Soxhlet extraction (avg. 93.01 mg/g), irrespective of the solvents used. Significantly lower concentrations of wood extractives were obtained by maceration with stirring (avg. 68.41 mg/g) and sonication extraction (avg.

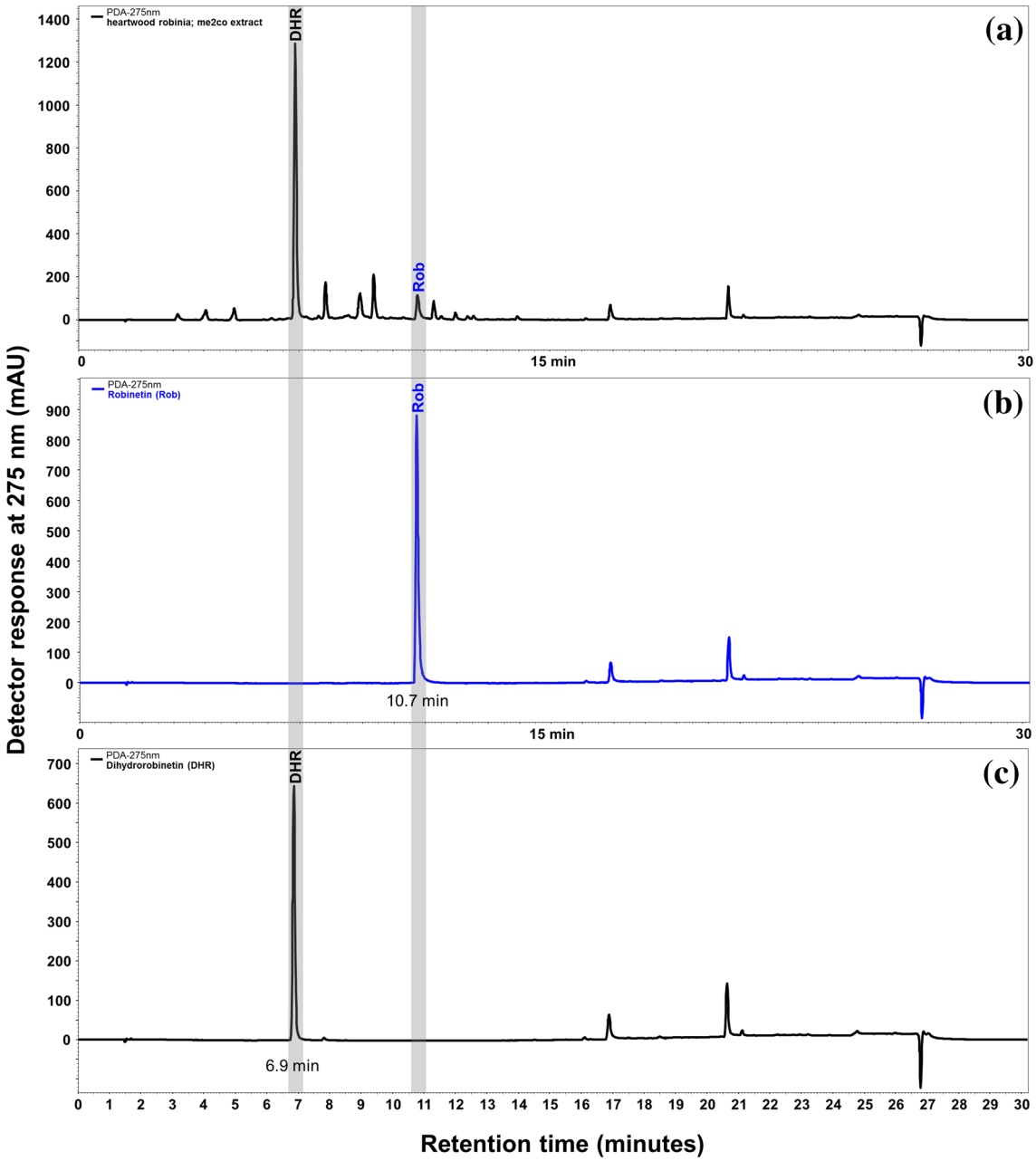

Fig. 3 HPLC-PDA chromatograms of *Robinia pseudoacacia* wood extract and reference compounds, monitored at 275 nm. **a** Acetone extract of black locust heartwood (hw). Solutions of reference compounds (standards) used for identification and chromatographic analysis, **b** solution of robinetin (Rob) and **c** solution of dihydrorobinetin (DHR)

51.92 mg/g) (Fig. 4a). The amounts of extractives in acetone (me2co), ethanol (etoh) and methanol (meoh) were approximately the same (ANOVA $_{\text{Ext, TP, DHR, Rob}}$; $p > 0.05$).

It was confirmed that heartwood of black locust contains higher amounts of phenolic extractives than sapwood (Table 2, Fig. 4b–d). The applied extraction methods gave an average of 73.1 mg/g (sw samples) and 69.1 mg/g (hw samples) of total extractives (Table 2). Similar amounts of extractives after methanol/water (1:1, v/v) extraction of black locust heartwood were reported by Sablik et al. (2016). Reinprecht et al. (2010) reported that wood of black locust includes a higher proportion of tannin and other polyphenolic substances, ranging up to 4%, or even up to 8.3%.

The presence of gallic acid, ellagic acid, tetrahydroxy and trihydroxymethoxy dihydroflavonol, dihydrorobinetin, robinetin, liquiritigenin, isoliquiritigenin, leucorobinetinidin, fustin, fisetin, dihydrofisetin, robtein, butein, robtin, butin, robinin, dihydromyricetin, myricetin, piceatannol,

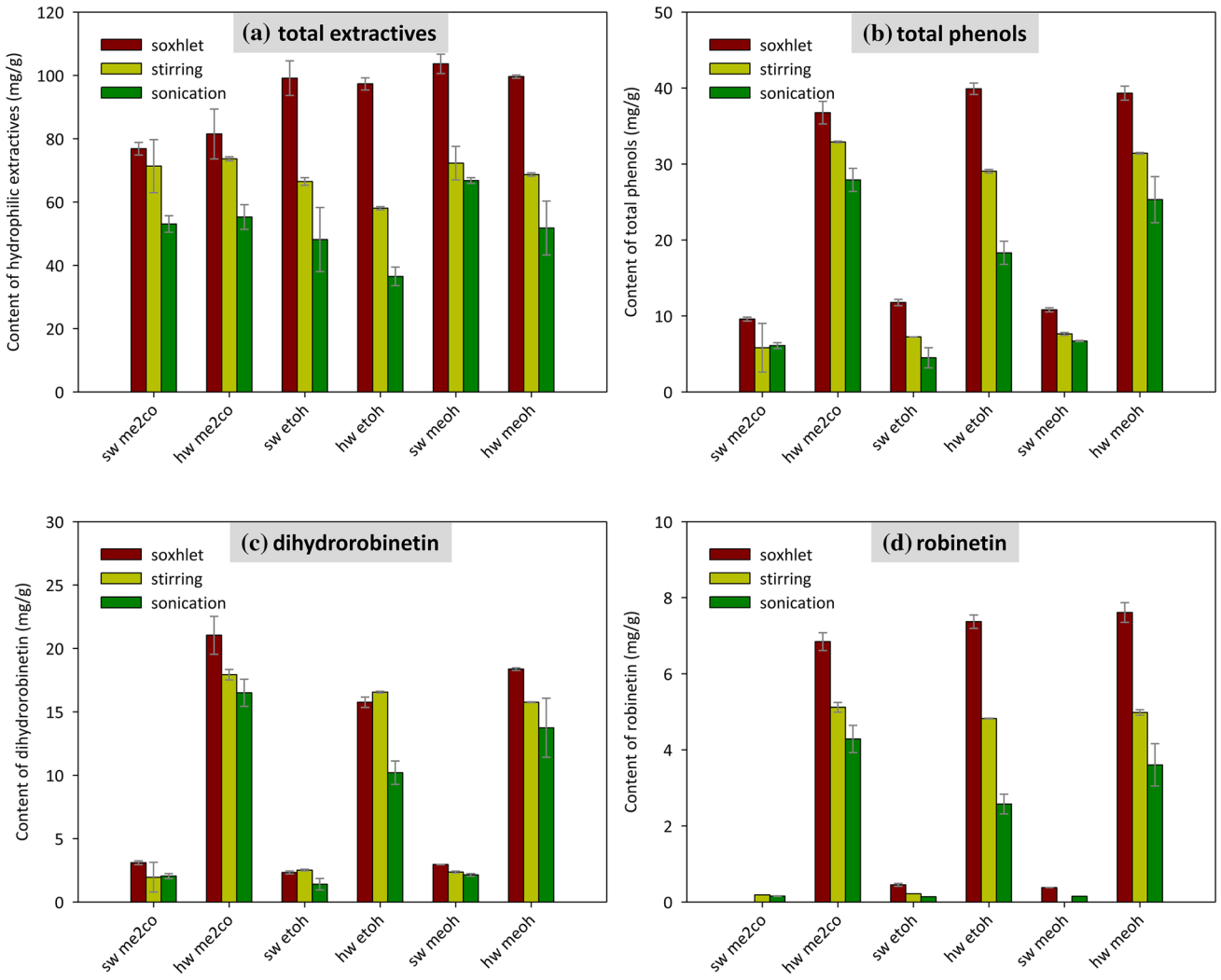

Fig. 4 Content of, **a** total extractives, **b** total phenols, **c** dihydrorobinetin, and **d** robinetin in acetone (sw me2co, hw me2co), ethanol (sw etoh, hw etoh) and methanol (sw meoh, hw meoh) wood extracts of black locust (*Robinia pseudoacacia* L.). sw, sapwood; hw, heartwood

Table 2 Content of extractives in sapwood (sw) and heartwood (hw) extracts of black locust (*Robinia pseudoacacia* L.)

Wood sample	Total extractives (mg/g dw)		Total phenols (mg GAE/g dw)		Dihydrorobinetin (mg/g dw)		Robinetin (mg/g dw)	
	avg.	SD	avg.	SD	avg.	SD	avg.	SD
sw	73.1[a]	1.84	7.8[a]	2.51	2.3[a]	0.591	0.27[a]	0.133
hw	69.1[a]	2.08	31.2[b]	6.87	16.2[b]	3.04	5.2[b]	1.68

[a–b]different letters within the same column indicate statistically significant differences at a 95% confidence level (LSD test). sw, sapwood; hw, heartwood

syringenin, dimeric prorobinetinidins, as well as resveratrol, has been confirmed by various analytical tools in hydrophilic extracts of wood of black locust (Destandau et al. 2016; Hosseinihashemi et al. 2013; Meszaros et al. 2007; Sanz et al. 2011; Scheidemann and Wetzel 1997; Sergent et al. 2014). Moreover, starch, simple sugars (e.g., sucrose, glucose and fructose), as well as stigmasterol and soluble proteins, have also been reported for extracts of black locust (De Filippis and Magel 2012; Magel et al. 1994). The high amounts of total extractives in sapwood can be explained by the presence of soluble sugars, proteins and other compounds involved in the primary metabolism of living cells in functional sapwood. The chemical identities and quantities of compounds occurring in wood extracts of black

locust have recently been comprehensively evaluated by gas chromatography, thermogravimetry, thermally assisted hydrolysis and methylation-gas chromatography and high performance liquid chromatography, all of them coupled with mass spectrometry (GC–MS, TG/MS, THM-GC/MS, HPLC/MS) (Destandau et al. 2016; Meszaros et al. 2007; Sanz et al. 2011).

For the purpose of the present investigation, dihydrorobinetin (DHR) and robinetin (Rob) were analyzed as target compounds by HPLC. As presented in Fig. 3, the applied HPLC protocol ensured good separation of the two targeted compounds. As shown in Table 2, extraction of hw yielded on average 69.1 mg/g of total extractives, 31.2 mg/g of total phenols, 16.2 mg/g of DHR and 5.25 mg/g of Rob. Heartwood contained more than 4 times larger amounts of phenolic extractives than sw samples (Table 2). The results in relation to the quantities of DHR and Rob in wood of black locust are in good agreement with the reports of other research groups (Bostyn et al. 2018; Sanz et al. 2011). Furthermore, very similar quantities of DHR and Rob in black locust heartwood were reported by Bostyn et al. (2018), i.e. 16 mg/g and 3.8 mg/g, meanwhile the reports by Sanz et al. (2011, 2012) mention slightly higher values, viz. 32.27 mg/g of DHR and 7.87 of Rob.

DHR and Rob were confirmed as the characteristic compounds of black locust extracts (Magel et al. 1994; Smith et al. 1989). Soxhlet extraction ensured the highest yields of DHR and Rob (LSD test). The extraction of wood in an ultrasound bath yielded the smallest amount of polyphenols (Table 3). However, the concentrations of DHR gained with sonication amounted to 73.4% of DHR obtained with 6 hours Soxhlet extraction (Table 3). In spite of sonication giving the smallest extraction yields, this extraction method actually gave relatively high amounts of polyphenols in relation to the relatively short time of the extraction process, viz. 1 h (Fig. 4b–d). It has been reported that ultrasound increases the permeability of the cell wall, causing cell lysis, thus enabling effective extraction (Marinas et al. 2014). In addition, green impacts of using ultrasound assisted extraction have been presented due to the decreased extraction time and reduced solvent and energy consumption and CO_2 emissions. (Chemat et al. 2017). Extraction in an ultrasonic bath has already been recognized as an efficient method of extraction of non-structural wood components (Hofmann et al. 2015; Sivakumar et al. 2017). However, possible impact of ultrasound on the chemical composition and eventual degradation of wood extractives needs to receive more research attention (Meullemiestre et al. 2016).

Extraction of black locust heartwood (hw) with acetone yielded significantly larger amounts of DHR (ANOVA $_{DHR}$; $p < 0.050$) than extraction with methanol or ethanol (Fig. 4). The amounts of total extractives, total phenols and Rob leached with the three solvents were not significantly different (ANOVA $_{Ext, TP, Rob}$; $p > 0.671$). There were no significant differences in the concentration of total extractives in the acetone, ethanol and methanol extracts of black locust sapwood (sw) (ANOVA $_{Ext, TP, DHR, Rob}$; $p > 0.320$). This investigation showed that an acetone/water mixture (9:1, v/v) was the most suitable solvent for the extraction of DHR from the wood of black locust. In addition to methanol and ethanol, acetone or aqueous acetone has already been used for the extraction of phenolic compounds from black locust wood (Bostyn et al. 2018; Dunisch et al. 2010; Fan et al. 2010; Magel et al. 1994).

In view of the results of chemical analysis, several advantages of conventional Soxhlet extraction should be highlighted. Firstly, the wood sample was repeatedly exposed to a fresh portion of solvent during the extraction process. Additionally, no filtration is required after extraction, there are relatively low costs of extraction due to the basic equipment and it is a simple methodology that requires little training (Luque de Castro and Priego-Capote 2010). It should also be mentioned that the Soxhlet apparatus allows the extraction of a higher sample mass than some of the more advanced lab scale extraction techniques (e.g., microwave-assisted extraction, supercritical fluid extraction) (Luque de Castro and Priego-Capote 2010). On the other hand, Soxhlet extraction has certain drawbacks, viz. relatively large volumes of solvents are used and it is time consuming (Thurbide and Hughes 2000). Extraction of black locust samples has frequently been carried out with maceration, whereby the solubility of compounds can be increased by applying heat or agitation. Bostyn et al. (2018) obtained the highest quantities of DHR and

Table 3 Content of extractives in heartwood (hw) extracts of black locust (*Robinia pseudoacacia* L.) yielded with three extraction techniques

Extraction technique	Total extractives (mg/g dw)		Total phenols (mg GAE/g dw)		Dihydrorobinetin (mg/g dw)		Robinetin (mg/g dw)	
	avg.	SD	avg.	SD	avg.	SD	avg.	SD
Soxhlet	92.8[c]	9.54	38.7[c]	1.72	18.4[b]	2.46	7.27[c]	0.392
Stirring	66.8[b]	7.15	31.1[b]	1.75	16.7[b]	1.00	4.97[b]	0.148
Sonication	47.9[a]	9.95	23.8[a]	4.74	13.5[a]	3.08	3.49[a]	0.834

[a–c]different letters within the same column indicate statistically significant differences at a 95% confidence level (LSD test)

Rob from black locust heartwood by stirring at 27.5 °C and with 177 g/l of wood/solvent ratio (w/v). A literature review on wood extractives of black locust showed that Soxhlet extraction is still one of the most frequently used extraction techniques on a laboratory scale (Fan et al. 2010; Meszaros et al. 2007; Smith et al. 1989).

Since Soxhlet extraction is considered time consuming, with wood extraction lasting up to 24 h, the number of extraction cycles needed for efficient extraction of black locust heartwood (hw) was also tested. The results are presented in Fig. 5. The average time of one extraction cycle was 13 min and 15 s. Figure 5 shows that more than 90% of quantified extractives were leached out of the wood sample in less than 2 h, i.e., with 8 extraction cycles. Furthermore, 50–60% of extractives were leached in the first 30 min. The initial phase of extraction can therefore be described as a period of constant and relatively fast extraction. After 4 hours (18 cycles) of Soxhlet extraction, the concentration of the obtained extract became constant (Fig. 5). It can therefore be concluded that the optimal Soxhlet extraction time for black locust wood is 4–5 h. A five-hour Soxhlet extraction actually leads to almost complete recovery of the black locust extractives. It should be stressed that shortening the extraction time can drastically reduce the cost of the extraction process, as well as its hazardous effects.

The present analysis also showed significant correlations (regression, $p < 0.050$) between the results of gravimetry or spectrophotometry and the contents of both DHR and Rob (Figs. 6 and 7). Similar findings have also been reported for wood of other tree species. This could have practical use in a quick assessment of the chemical properties of wood (Karppanen et al. 2007).

In view of the results of this investigation, it can be concluded that Soxhlet extraction is still the best option among the technologies considered in this study. It is true that modern and automatized extraction systems, for example, Thermo's ASE or Buchi's SpeedExtractor, are appropriate alternatives (Pietarinen et al. 2006; Vek et al. 2014). These modern systems for accelerated extraction of material are frequently praised due to the shorter extraction time and lower solvent consumption. However, it has been demonstrated that extraction of wood in a Soxhlet apparatus gives similar amounts of hydrophilic extractives to speed extraction (Vek et al. 2018). Conventional extraction of wood with maceration, sonication or Soxhlet extraction is therefore a reliable alternative to advanced but expensive extraction systems.

4 Conclusion

The present investigation showed that extraction of black locust wood with Soxhlet apparatus, sonication and maceration gave different amounts of total extractives. The highest concentrations of phenolic extractives were measured in extracts obtained by Soxhlet extraction, irrespective of the solvents used. More than 90% of quantified extractives were leached in the Soxhlet apparatus in less than 2 h, i.e., with 8 extraction cycles. In relation to the short extraction time, extraction in an ultrasonic bath yielded relatively high

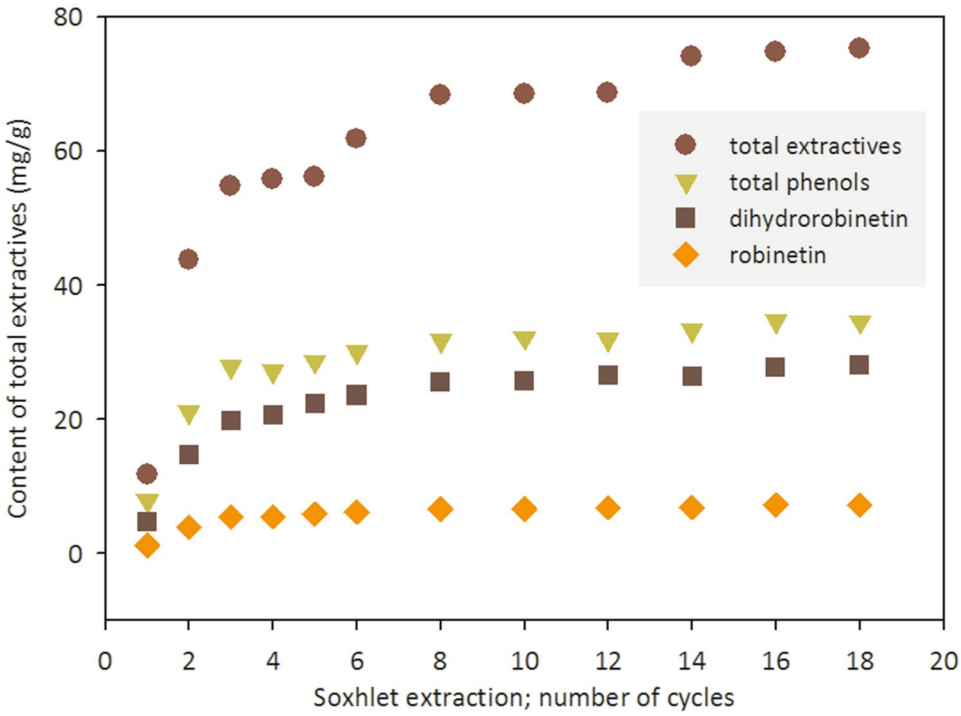

Fig. 5 Content of total extractives, total phenols, dihydrorobinetin, and robinetin in heartwood of black locust (*Robinia pseudoacacia* L.). Extraction yields regarding the relation to the number of extraction cycles

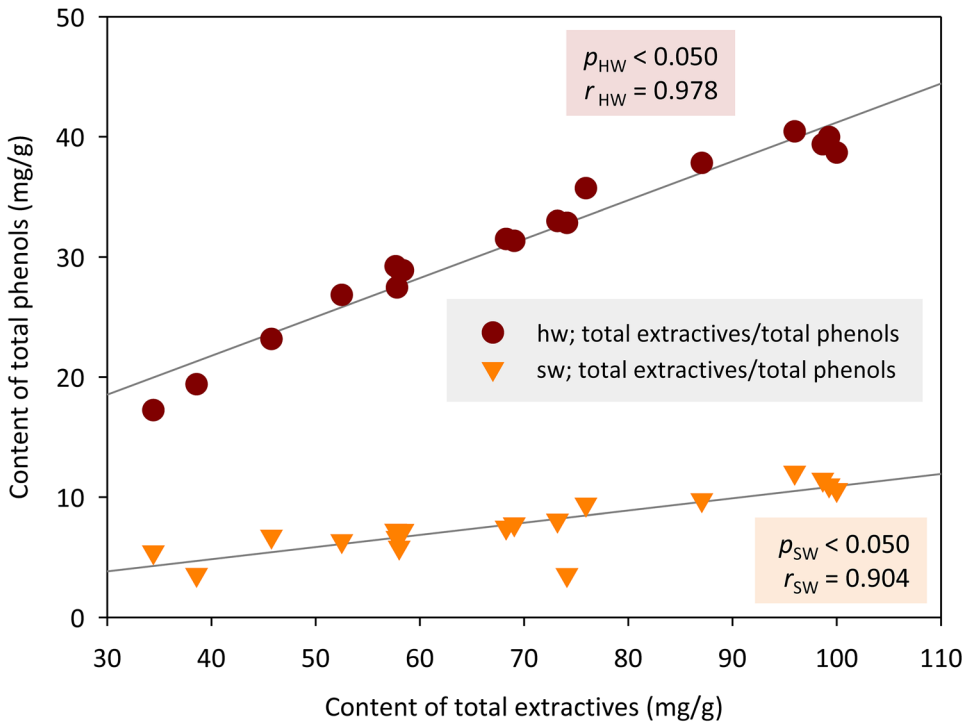

Fig. 6 Correlations between the content of total extractives and total phenols in sapwood and heartwood of black locust (*Robinia pseudoacacia* L.)

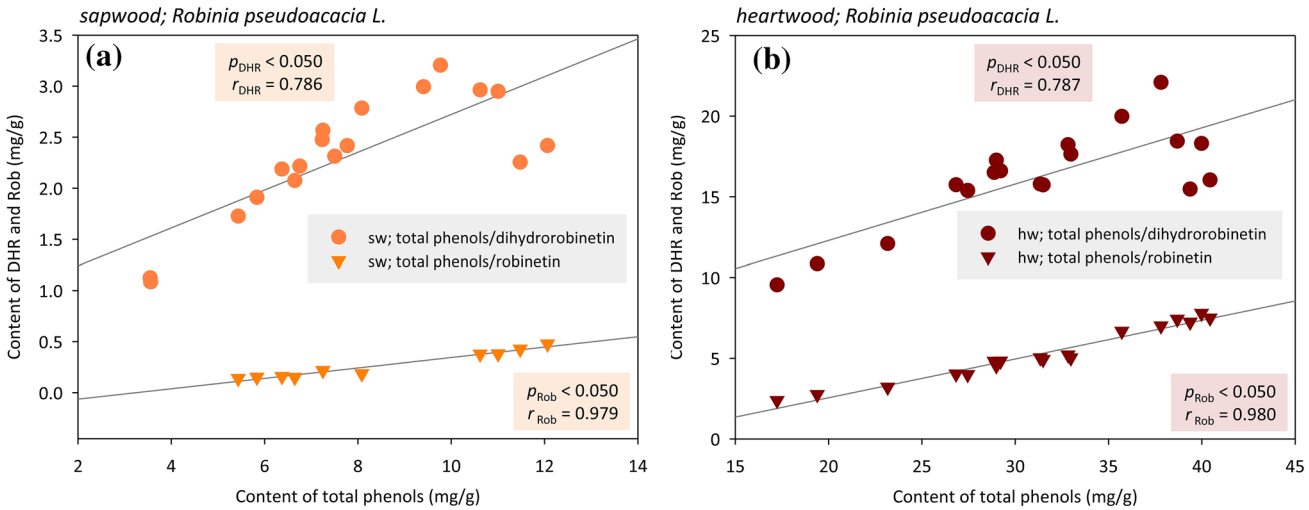

Fig. 7 Correlations between the content of total phenols and content of dihydrorobinetin and robinetin, measured in **a** sapwood (sw) and **b** heartwood of black locust (*Robinia pseudoacacia* L.)

amounts of total extractives. Soxhlet extraction with acetone gave the highest concentrations of dihydrorobinetin and robinetin in wood extracts of black locust. Dihydrorobinetin was confirmed to be the characteristic and dominant low molecular phenolic compound in wood of black locust. The concentration of robinetin was significantly lower than that of dihydrorobinetin. Heartwood extracts contained larger amounts total phenols, dihydrorobinetin and robinetin than did sapwood samples of black locust. Significant correlations between the results of gravimetry or spectrophotometry and the contents of the two individual phenolic compounds were found. The present study showed that Soxhlet extraction and the use of acetone with the addition of water is the optimal bench scale extraction system. When total extraction is desired, it is recommended that black locust wood should be extracted with 18 extraction cycles, which means 4 hours of extraction in a Soxhlet apparatus. After all, knowledge of the potential of woody biomass as the source for production

of green chemicals related to different extraction methods represents an important part of a biorefinery concept.

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