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Characteristics of hot water extracts from the bark of cultivated willow (*Salix* sp.)

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ABSTRACT. Willow bark is a rich source of heterogeneous polyphenolic compounds and a potential feedstock for biorefinery processes aiming at chemicals and fiber production. Here mild hot water treatment of willow hybrid Karin was studied to find a practical means of isolating its non-cell-wall components for their utilization in a willow biorefinery proposed to aid valorization of the willow biomass. A short aqueous treatment of the bark at 80 °C liberated the extract in >20% yield under unpressurized conditions. The extract was characterized using mainly gas chromatography–mass spectrometry and one- and two-dimensional NMR techniques. Authentic analytes were applied to confirm the identification and quantification of the main components that were picein, (+)-catechin, triandrin, glucose, and fructose. Fructose was converted into 5-hydroxymethylfurfural (HMF) in an acidic treatment which led to its condensation with the phenolic components and formation of a recalcitrant precipitate that should be avoided.

Keywords. Picein, Triandrin, (+)-Catechin, Fructose, Willow bark, 5-hydroxymethylfurfural.

Synopsis. Valuable aromatics picein, (+)-catechin and triandrin were extracted simply by hot water from willow bark.

INTRODUCTION

Actions have been taken increasingly around the world to move away from today's fossil carbon based economy towards a second generation bioeconomy based on sustainable use of lignocellulosic biomass. Besides its positive impact on climate, the transformation attracts the chemical, forest and pharmaceutical industries by offering several platforms for production of high-valued chemicals and materials.[1-3] In addition to the utilization of natural coppices, highly productive cultivated crops with short rotation times might be adopted for sustainable biomass production.[4] In this regard, willow (*Salix* sp.) plantations have been considered for liquid fuel production in addition to their use for combined heat and power generation.[5] In comparison with the rich uses of whole wood or its xylem fraction, bark has seldom been considered to have any significant value besides its use as a solid fuel. However, both hardwood and softwood bark has long been recognized to contain a plethora of various substances.[6] Many of these are bioactive compounds that could be potentially utilized in treatment of a wide spectrum of diseases[7,8]. The bark often contains significant amounts of water soluble substances. For example, up to 50% tannin can be extracted from spruce bark simply using hot water as the solvent.[9,10]

Compared to willow xylem, willow bark has almost tenfold higher ash and extractives content and its fibers are much longer and stiffer.[11] These observations led us to propose a willow biorefinery concept in which the bark is used for the isolation of sclerenchyma fibers and extracts whereas the debarked wood is converted into sugars or their products, such as ethanol, and a solid lignin residue.[11] The proposed deconstruction scheme for willow biomass could potentially provide significant added value for the energy crop through production of high-quality fibers, liquid biofuels, sulfur-free lignin, and specific extractives.

Pressurized hot water treatment has been considered as a green extraction method for biomass.[12] The elevated temperature and pressure affect van der Waals and H-bonding interactions on interfaces, which enhances the penetration of the solvent into the biomass.[13,14] Hot water processing

of various lignocellulosic materials may result in removal of extractives, deacetylation and degradation of hemicelluloses, and some structural changes in lignin and cellulose depending on the conditions applied. Intensive research has been carried out on high-temperature water treatment of pulpwood (prehydrolysis) for isolation of hemicelluloses and their subsequent conversion e.g. into oxygen barrier films, edible food coatings or biofuels.[15,16] Steam explosion, that applies hydrolytic conditions at high temperature, has been regarded as having high potential as a pretreatment method to increase the accessibility and reactivity of lignocellulose towards its enzymatic saccharification.

Willow bark extracts have been widely studied due to their medicinal impacts and role in plant-herbivore interactions. A variety of secondary metabolites, such as salicin, triandrin, (+)-catechin, picein and salicortin, as well as polymeric tannins, have been reported in Salicaceae; however, the quantities of these compounds vary widely depending on the willow species, age of the plant, growing season, and also the solvent used for the extraction.[17–19] Salicin, a principal component of *Salix* bark, is well-known for its ability to treat lower back pain, fever, and mild headache, as well as serving as the fundamental prodrug for various salicylate derivatives.[20–22] Triandrin has been considered for the symptom-based treatment of rheumatic conditions.[23] Picein and catechin are used as antioxidants in the food industry.[24,25] The diversity of extractable compounds that exists in the willow bark provides a potentially rich source of high-value products that is at worthy of further investigation.

Although articles have been published on the isolation of phenolic extracts of willow bark, this paper demonstrates that the principle products can be recovered through a mild hot water treatment of the bark and that these extracts undergo deleterious chemical conversion when subjected to harsher hydrolytic conditions.

MATERIALS AND METHODS

Plant Material and Hot Water Extraction. Four-year-old trees of willow hybrid 'Karin' were harvested and willow bark was separated manually with scalpel and stored at 20 °C.[11] Deionized water (20 mL) and bark (2 g) were added in a Monowave 300 single-mode 25 mL borosilicate glass microwave reactor (Anton Paar GmbH, Graz, Austria). A magnetic stirrer operating at 600 rpm was utilized to mix the solution during the reaction. Time-to-maximum temperature was set to 1.5 min with 850 W maximum output power used. The isolation of water-soluble extractives was carried out at temperatures of 60, 80 and 100 °C with different reaction times in the range of 5–150 min. The water-soluble fraction was oven-dried at 40 °C overnight after filtering through a crucible (pore size of 10-16 μm). The extraction yield was determined based on the proportion of pure extractives as the average of three different runs.

Acid Treatment of Hot Water Extracts. Freeze dried hot water extract (HWE, 2 mg) was placed into a test tube filled with 20 mL distilled water, and slowly mixed with 697 μL of 72% H_2SO_4 to bring liquid acid concentration to 4% similar to NREL/TP-510-42623. Subsequently, the sample was treated in an autoclave at 121 °C for 2 h. After cooling at room temperature, the hydrolysed sample was diluted and passed through a 0.2 μm filter to isolate the insoluble material formed during the treatment.

High Performance Anion Exchange Chromatography (HPAEC). Monosaccharides in the willow bark extracts and hydrolysates were quantified by high-performance anion-exchange chromatography with pulse amperometric detection (HPAEC-PAD) in a Dionex ICS-3000 system (Sunnyvale (CA), USA) equipped with CarboPacPA20 column Pure water was used as the mobile phase and the analyses was carried out under room temperature at a flow rate of 0.38 mL min^{-1}.

High Performance Liquid Chromatography (HPLC). HPLC was applied for the hydrolysates using a Dionex UltiMate 3000 HPLC (Dionex, Sunnyvale, CA, USA) equipped with an ultraviolet (UV) diode-array detector and Hyper REZ XP Carbohydrate Ca^{2+} column (8μm, 300

x 7.0 mm, Thermo Scientific, Waltham, MA, USA). Hydroxymethylfurfural (HMF) in the hydrolysate was detected by UV absorption at 280 nm. The analytical system was calibrated for HMF concentration range of 10 to 150 mg L^{-1}. A 0.0025 mol L^{-1} H_2SO_4 solution was used as the eluent at a flow rate of 0.8 mL min^{-1}. The column temperature was set to 70 °C.

Gas Chromatography (GC) and Gas Chromatography–Mass Spectrometry (GC-MS). Water extracts (0.5 mg) were frozen and freeze-dried prior to use. Afterwards, 1 mL of internal standard (0.1 mg mL^{-1} xylitol solution in methanol:water 9:1, v/v) was added and then evaporated under a nitrogen gas flow. The dried samples were then trimethylsilylated overnight in 150 µL pyridine with 150 µL hexamethyldisilazane (HMDS) and 70 µL trimethylchlorosilane (TMCS), then transferred to GC vials. The samples were analyzed by gas chromatography (PerkinElmer AutoSystemXL) with flame-ionization detection (FID) using an HP-1 column (25 m*0.2 mm, 0.11 µm film) with a carrier gas of 0.8 mL min^{-1} H_2. The injector was kept at 250 °C with split ratio of 1:30. The column temperature was ramped from 100 °C (8 min) to 170 °C (0 min) at 4 °C min^{-1}, and then to 290 °C at 12 °C min^{-1} with a hold time of 5 min. The GC parameters used for quantitative determination of the main components in the HWE are summarized in the Supporting Information (**Table S1**). GC-MS analysis of the samples was performed with an Agilent G1530A gas chromatograph equipped with an HP-5 column connected to Hewlett-Packard 5973-N Mass Selective Detector (California, USA). The column temperature was ramped from 80 °C after hold of 1 min to 320 °C at 8 °C min^{-1} followed by a hold of 15 min.

Nuclear Magnetic Resonance (NMR) spectroscopy. The solution-state NMR experiments were performed in D_2O at 22 °C on a Bruker Avance III spectrometer operating at 400.13 and 100.61 MHz for ^{1}H and ^{13}C, respectively. NMR data was processed using TopSpin 3.0 software. The ^{1}H chemical shifts were referenced to water at 4.75 ppm at 22 °C. The NMR spectral parameters used for the HWE and purity identification of picein **1**, catechin **2**, and triandrin **3** are summarized in the Supplementary Information (**Table S2**). The solid-state ^{13}C-CP/MAS spectra

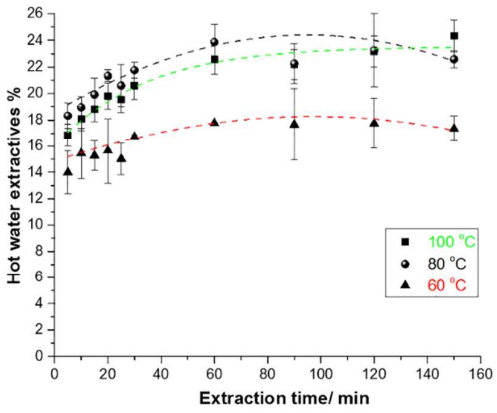
were acquired on a Bruker AVANCE III spectrometer operating at 100.61 MHz using a 4.0 mm ZrO_2 rotor. The following parameters were used: a relaxation delay of 5 s, a spectral width of 306 ppm, 40K transients of 2K data points, a contact time of 1 ms, and a spinning rate of 8 kHz.

Ultraviolet Resonance Raman spectroscopy (UVRR). UVRR spectra of the acid precipitates of the hot water extracts were obtained with a Renishaw 1000 UV Raman spectrometer coupled with an Innova 90C FreD frequency-doubled argon ion laser (Coherent) and a Leica DMLM microscope. Spectra were collected using a 40 X deep UV objective (OFR) and an excitation wavelength of 244 nm. The detector was a UV coated CCD camera with a diffraction grating of 3600 grooves/mm. Samples were rotated during the measurements in order to avoid changes in the sample due to the focused UV light on the sample and to obtain averaged spectra. The spectra were acquired for 60 s at 200–2400 cm^{-1} at a resolution of 7 cm^{-1}.

Time-gated Raman spectroscopy, Infrared spectroscopy and Ultraviolet–visible (UV-Vis) spectroscopy. All the spectral parameters and collected results are summarized in the Supplementary Information (**Figure S1**).

RESULTS AND DISCUSSION

Extraction yield. Figure 1 illustrates the relationship between the hot water extract (HWE) yield and the duration of the hot water treatment at three different temperatures. Although the yield was relatively high already after 5 min, it increased with time until 60 min and then remained relatively



constant during longer treatments. Raising the temperature from 60 to 80 °C led to a significant increase in the yield. A further increase in the temperature to 100 °C possibly lowered the yield, which might have been attributable to partial degradation and/or precipitation of the extracts at the higher temperature. Notably, in only 30 minutes at 80 °C, ~21.5% of the original bark mass was solubilized, a level similar to that reported for acetone extraction of powdered inner bark of the same Karin willow.[11]

Figure 1. Effects of temperature and time on the hot water extract (HWE) yield from willow bark.

Chemical composition of hot water extract

GC-MS and GC. HWE were derivatized for increased volatility using hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS), which converted the free hydroxyl groups to TMS ethers [O-Si$(CH_3)_3$]. The main aromatic compounds, *i.e.,* picein, catechin and triandrin (**Figure 2**), were identified by GC-MS at retention times of 23.5, 25.1, and 25.8 min, respectively (**Figure 3**). The identity of the aforementioned compounds was confirmed by comparison with the retention times and mass spectra of the authentic compounds (see Supporting Information, **Figure S2**). The mass spectra of the TMS derivatives of picein and triandrin are plotted in **Figure 4**.

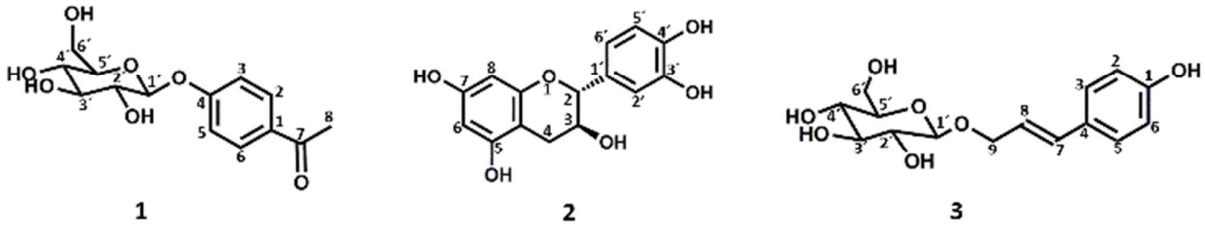


Figure 2. The main aromatic components of hot water extractives: Picein **1**, (+)-catechin **2**, and triandrin **3**, along with several sugars.

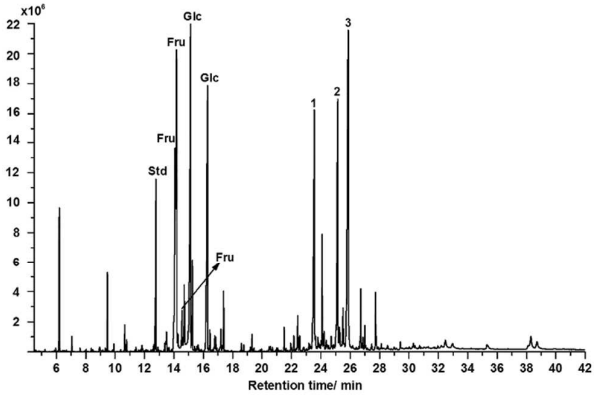


Figure 3. GC-MS total-ion chromatogram of HWE showing major peaks for: Picein **1**, catechin **2**, and triandrin **3**, along with glucose (**Glc**), fructose (**Fru**), and internal standard (xylitol, **Std**).

The mass spectrum of the TMS derivative of picein had a molecular ion of m/z 586 and the expected fragmentation. A characteristic m/z 361 fragment for trimethylsilylated hexopyranosides was present in the mass spectra of both picein and triandrin. The mass spectrum of the TMS derivative of triandrin had a molecular ion at m/z 672. The ion at m/z 205 was typical of the trimethylsilylated *p*-coumaryl alcohol substructure. Characteristic fragments of the TMS derivative of catechin match those observed in previous studies (see Supporting Information, **Figure S3**).[25,26]

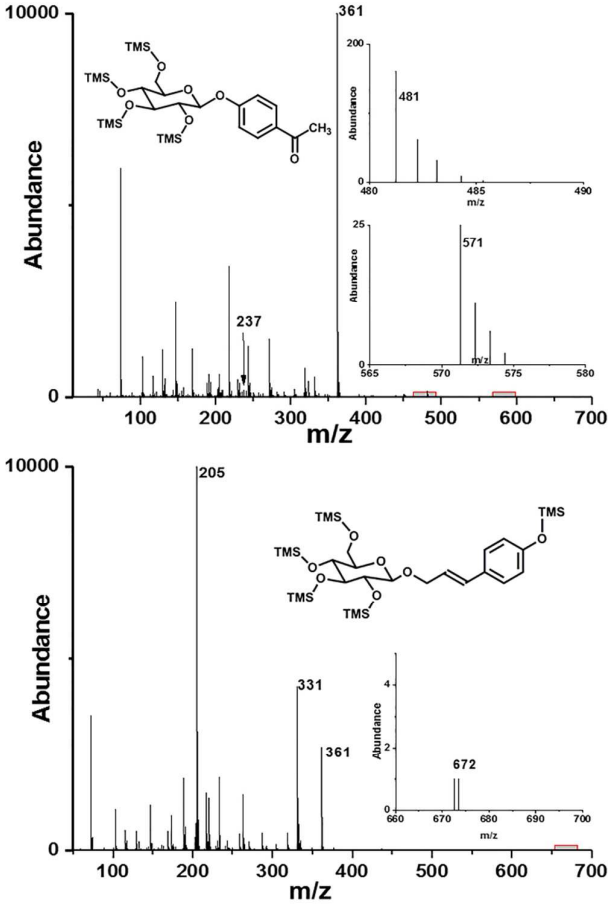


Figure 4. Mass spectra of trimethylsilylated **picein 1** (top) and **triandrin 3** (bottom); the insert images are 32x magnified.

Xylitol was used as an internal standard (**Std**) for quantitative analysis of the extracted aromatic compounds and sugars by GC/FID. The extracted water-soluble compounds included mostly glucose (**Glc**) and fructose (**Fru**) in addition to the three previously noted aromatic compounds (see

Supporting Information**, Figure S4**), which were identified earlier with GC using a different column and detectors.[27]

NMR and IR spectroscopy. Successful structural verification of the main components of HWE was further illustrated through the usual array of NMR spectra: one-dimensional ^{1}H and ^{13}C, 2D ^{1}H–^{1}H COSY, and 2D ^{1}H–^{13}C HSQC NMR spectroscopy. The HSQC spectrum of HWE (**Figure 5**) confirms that the extracts contain sugars (60-105 ppm) as well as aromatic (110-150 ppm)

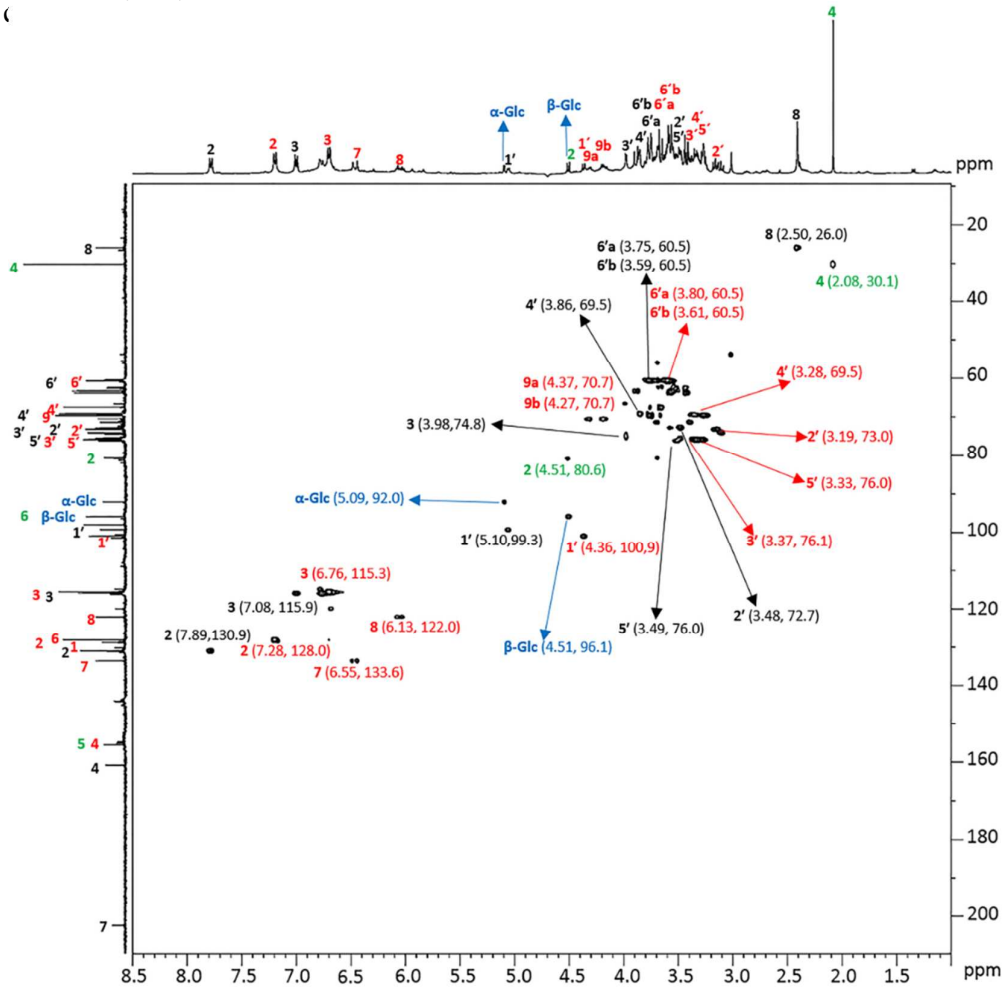


Figure 5. 2D ^{1}H–^{13}C HSQC NMR spectrum of the HWE in D_2O: Picein 1 (black); Catechin 2 (green); Triandrin 3 (red); α-Glc, β-Glc (blue).

observed chemical shifts and full NMR interpretation are summarized respectively in **Table 1** and the Supporting Information.[28–31] Major signals in the HSQC spectrum matched those published for

picein **1**, catechin **2** and triandrin **3** as well as the synthesized authentic compounds (see Supporting Information**, Table S2** and **Figures S5-S16**). For extended ^{1}H, ^{13}C, and COSY spectra of HWE in D_2O, see Supporting Information, **Figures S17-S19**).

1,3,5-Trioxane was used as an internal standard for quantitative 1D ^{1}H NMR spectroscopy of HWE. The NMR and GC-MS data together established that picein **1**, catechin **2** and triandrin **3** were the main aromatic components of the willow bark HWE (**Table 2** and **Table S1** from Supporting information), as previously reported in the bark of various willow varieties.[17,21,32] The IR spectrum of HWE (see Supporting Information, **Figure S20**) confirmed the presence of large quantities of aromatic components characterized by the strong absorption at 1605 cm^{-1}.[33]

Table 1. ^{1}H and ^{13}C NMR chemical shifts[a] of picein **1**, catechin **2** and triandrin **3**.

	Picein **1**	Catechin **2**	Triandrin **3**
H-2	7.89 d (*J*=8.90)	4.51 d (*J*=7.89)	7.28 d (*J*=8.64)
H-3	7.08 d (*J*=8.94)	4.18 d	6.76 d (*J*=8.56)
H-4	-	2.08 d	-
H-5	7.08 d (*J*=8.94)	-	6.76 d (*J*=8.56)
H-6	7.89 d (*J*=8.90)	-	7.28 d (*J*=8.64)
H-7	-	-	6.55 d (*J*=16.11)
H-8	2.50 s	-	6.13 ddd (*J*=16.11)
H-9a	-	-	4.37 dd
H-9b	-	-	4.27 dd
H-1'	5.10 d (*J*=3.86)	-	4.36 d (*J*=6.19)
H-2'	3.48 dd	-	3.19 dd
H-3'	3.98 dd	-	3.37 dd
H-4'	3.86 dd	-	3.28 dd
H-5'	3.49 ddd	-	3.33 ddd
H-6'a	3.75 dd	-	3.80 dd
H-6'b	3.59 dd	-	3.61 dd
C-1	133.6	-	130.1
C-2	130.9	80.6	128.0
C-3	115.9	70.4	115.3
C-4	160.7	30.1	155.4
C-5	115.9	155.4	115.3
C-6	130.9	95.8	128.0
C-7	202.4	155.4	133.6
C-8	26.0	95.8	122.0
C-9	-	-	70.7
C-1'	99.3	-	100.9
C-2'	72.7	-	73.0
C-3'	74.8	-	76.1
C-4'	69.5	-	69.5
C-5'	76.0	-	76.0
C-6'	60.5	-	60.5

[a] Chemical shifts are expressed in ppm, and coupling constants (expressed in Hz) are in parentheses
- Ambiguous or undefined values due to overlapping signals

Table 2. Mass balance of the hot water extract (HWE) from willow bark. The extraction yield was 21.5%.

Component	mg/g HWE
Picein	286
Triandrin	248
Catechin	105
Ash	59
Monosugars [1]	265
Total detected	963
Others [2]	37

[1] Determined by HPAEC and 1D ^{1}H NMR spectroscopy

[2] By difference, unassigned compounds detected by GC and NMR spectroscopy.

Chemical conversions under acid treatment.

Monomeric sugar composition and HMF yield. The main monomeric sugars of HWE were glucose and fructose with traces of galactose (**Figure 6**). The acid treatment of the extract more than doubled the concentration of glucose through hydrolysis of glucosides, such as those in picein **1** and triandrin **3**. Arabinose, rhamnose and galactose, which are characteristic monomeric sugars derived from pectin,[34,35] also increased in content after the acid treatment of HWE; fructose was nearly absent (the content dropped from 12.3 mg/g to 0.33 mg/g), presumably due to its facile conversion to hydroxymethylfurfural (HMF) under the acidic conditions.[36] However, little HMF was detected in the hydrolysate (0.76 mg/g detected by HPLC) for reasons identified below.

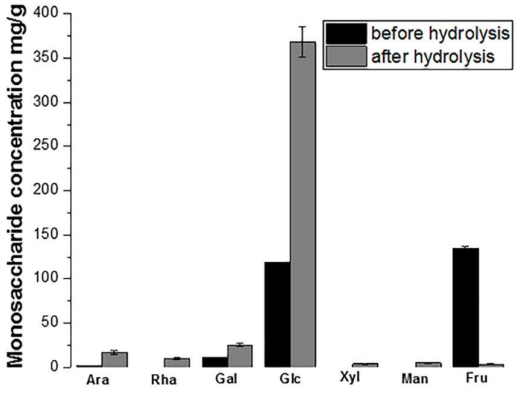


Figure 6. Monosaccharide contents of HWE before and after its treatment with acid. Arabinose (**Ara**), Rhamnose (**Rha**), Galactose (**Gal**), Glucose (**Glc**), Xylose (**Xyl**), Mannose (**Man**), Fructose (**Fru**).

Precipitate composition and yield. The acid treatment of HWE formed a black precipitate with a yield of 376 mg/g. The precipitate formation was accompanied with the aforementioned 'disappearance' of fructose/HMF and a decrease in UV absorption of the dissolved material (see Supporting Information, **Figure S21**). The acid treatment shifted the UV absorption maximum from 265 nm to 280 nm corresponding to a drop in the content of aromatic substances and an increase in the content of HMF.[37]

IR spectroscopy of the precipitate revealed a strong absorption at 1605 cm^{-1} that is characteristic of aromatic structures (**Figure 7a**).[33] An ultraviolet resonance Raman (UVRR) spectrum of the precipitate possessed a major band at 1604 cm^{-1} that can be attributed to the symmetric aromatic ring stretching (**Figure 7b**).[38] Time-gated Raman spectroscopy revealed major emission bands at 1556, 1597, and 1628 cm^{-1} (**Figure 7c**). The 1597 cm^{-1} band represents the aromatic C=C ring breathing in aromatics with various types of substitution whereas the emission at 1628 cm^{-1} is typical of conjugated aromatic aldehydes and ketones.[39] The additional Raman peak at 1556 cm^{-1} is unusual for lignocellulose and reveals a high degree of conjugation in an organic structure, with roughly 7 photon flux units conjugated together.[40] Such a high degree of conjugation might have a connection with the black color of the precipitate. The ^{13}C-CP/MAS spectrum of the precipitate had signals in the aliphatic carbon region (0-90 ppm), in the unsaturated carbon region, including aromatic and furan structures (90-160 ppm), and the carbonyl region (160-220 ppm) (**Figure 7d**). In the spectrum, high signal intensities for the aromatic structures were observed at 109, 131 and 156 ppm, characteristic furan ring resonances were found at 118 and 146 ppm, and a carbonyl signal at 211 ppm.[41,42]

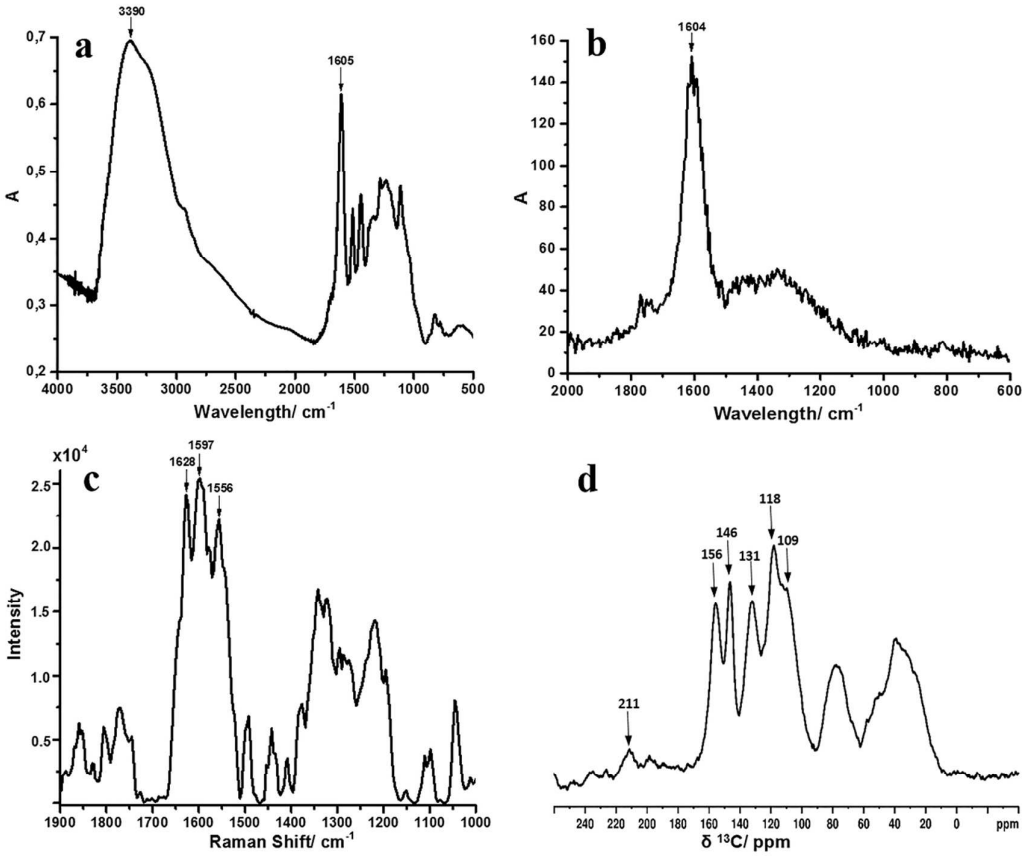


Figure 7. Characterization of the precipitate: **(a)** Infrared spectrum; **(b)** UVRR spectrum; **(c)** Time-gated Raman spectrum; **(d)** Solid-state ^{13}C CP/MAS spectrum.

In all, the gathered analytical data strongly suggests that under acidic conditions the fructose present in HWE forms HMF which then condenses with the phenols to form a solid precipitate. Although the conversion was demonstrated here with acid treatment of the extract, similar conversion might take place, e.g., during steam explosion of biomass that combines acid conditions with high temperature.

Conclusion

Mild hot water treatment seems to be a practical and cheap way of isolating non-cell-wall substances from willow bark in high yield under very short time. The extract from hybrid Karin, studied in this work, is rich in certain aromatic compounds, such as picein, catechin, and triandrin, all of which could find specific uses as pure components, that could probably

be isolated and purified based on differences in their solubility and adsorption behavior.[43,44] Fructose, also present in the extract, makes it unstable under harsher acidic conditions through formation of hydroxymethylfurfural that spontaneously condenses with the phenolic substances to form highly insoluble polymeric material. Further efforts are still needed to understand how to avoid, control, or utilize these secondary reactions. The hot water extracted bark could be subjected to additional treatments to separate the high-quality fibers that are present in willow inner bark and to realize the willow biorefinery scheme as proposed earlier.[11]

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org/.

Quantification of picein 1, catechin 2, and triandrin 3 in HWE by GC and ^{1}H NMR spectroscopy; NMR spectroscopy of authentic picein 1, catechin 2, and triandrin 3 and parameters for the identification of HWE compounds; Parameters and measurement set-up for portable Time-Gate

Raman spectrometer; Mass spectra of authentic trimethylsilylated compounds and trimethylsilylated catechin from HWE; Gas chromatogram of a mixture of the trimethysilyl derivatives of reference glycosides; Full NMR interpretation for identification of HWE compounds; Parameters and results obtained from Infrared (IR) spectroscopy; Parameters and results obtained from Ultraviolet–visible (UV-Vis) spectroscopy.

REFERENCES

(1) Vennestrøm, P. N. R.; Osmundsen, C. M.; Christensen, C. H.; Taarning, E. Beyond Petrochemicals: The Renewable Chemicals Industry. *Angew. Chem., Int. Ed.* **2011**, *50*, 10502–10509.

(2) Nikolau, B. J.; Perera, M. A. D. N.; Brachova, L.; Shanks, B. Platform biochemicals for a biorenewable chemical industry. *Plant J.* **2008**, *54*, 536–545.

(3) Sanders, J.; Scott, E.; Weusthuis, R.; Mooibroek, H. Bio-Refinery as the Bio-Inspired Process to Bulk Chemicals. *Macromol. Biosci.* **2007**, *7*, 105–117.

(4) Volk, T. A.; Abrahamson, L. P.; Nowak, C. A.; Smart, L. B.; Tharakan, P. J.; White, E. H. The development of short-rotation willow in the northeastern United States for bioenergy and bioproducts, agroforestry and phytoremediation. *Biomass Bioenergy* **2006**, *30*, 715–727.

(5) Krzyżaniak, M.; Stolarski, M. J.; Szczukowski, S.; Tworkowski, J.; Bieniek, A.; Mleczek, M. Willow biomass obtained from different soils as a feedstock for energy. *Ind. Crops Prod.* **2015**, *75*, 114–121.

(6) Feng, S.; Cheng, S.; Yuan, Z.; Leitch, M.; Xu, C. Valorization of bark for chemicals and materials: A review. *Renewable Sustainable Energy Rev.* **2013**, *26*, 560–578.

(7) Domingues, R. M. A.; Sousa, G. D. A.; Silva, C. M.; Freire, C. S. R.; Silvestre, A. J. D.; Neto, C. P. High value triterpenic compounds from the outer barks of several *Eucalyptus* species cultivated in Brazil and in Portugal. *Ind. Crops Prod.* **2011**, *33,* 158–164.

(8) Jablonsky, M.; Nosalova, J.; Sladkova, A.; Haz, A.; Kreps, F.; Valka, J.; Miertus, S.; Frecer, V.; Ondrejovic, M.; Sima, J.; Surina, I. Valorization of softwood bark through extraction of utilizable chemicals. A review. *Biotechnol. Adv.* **2017**, *35,* 726–750.

(9) Kemppainen, K.; Siika-aho, M.; Pattathil, S.; Giovando, S.; Kruus, K. Spruce bark as an industrial source of condensed tannins and non-cellulosic sugars. *Ind. Crops Prod.* **2014**, *52,* 158–168.

(10) Sauro, B.; Ivana, K.; Ron, J.; Ingo, M.; Bodo, S.; Frédéric, P. Characterization of condensed tannins and carbohydrates in hot water bark extracts of European softwood species. *Phytochemistry* **2015**, *120,* 53–61.

(11) Dou, J.; Galvis, L.; Holopainen-Mantila, U.; Reza, M.; Tamminen, T.; Vuorinen, T. Morphology and Overall Chemical Characterization of Willow (*Salix* sp.) Inner Bark and Wood: Toward Controlled Deconstruction of Willow Biomass. *ACS Sustainable Chem. Eng.* **2016**, *4*, 3871–3876.

(12) Teo, C. C.; Tan, S. N.; Yong, J. W. H.; Hew, C. S.; Ong, E. S. Pressurized hot water extraction (PHWE). *J. Chromatogr. A* **2010**, *1217*, 2484–2494.

(13) Kilpeläinen, P.; Kitunen, V.; Hemming, J.; Pranovich, A.; Ilvesniemi, H.; Willför, S. Pressurized hot water flow-through extraction of birch sawdust-Effects of sawdust density and sawdust size. *Nord. Pulp Pap. Res. J.* **2014**, *29*, 547–556.

(14) Bergeron, C.; Gafner, S.; Clausen, E.; Carrier, D. J. Comparison of the Chemical Composition of Extracts from *Scutellaria lateriflora* Using Accelerated Solvent Extraction and Supercritical Fluid Extraction versus Standard Hot Water or 70% Ethanol Extraction. *J. Agric. Food Chem.* **2005**, *53*, 3076–3080.

(15) Hansen, N. M. L.; Plackett, D. Sustainable Films and Coatings from Hemicelluloses: A Review. *Biomacromolecules* **2008***, 9,*1493–1505.

(16) Gírio, F. M.; Fonseca, C.; Carvalheiro, F.; Duarte, L. C.; Marques, S.; Bogel-Łukasik, R. Hemicelluloses for fuel ethanol: A review. *Bioresour. Technol.* **2010***, 101,* 4775–4800.

(17) Minakhmetov, R. A.; Onuchak, L. A.; Kurkin, V. A.; Zapesochnaya, G. G.; Medvedeva, S. A. Determination of Triandrin and Salicin in *Salix viminalis* L. by Reversed-Phase High-Performance Liquid Chromatography. *J. Anal. Chem.* **2002***, 57,* 401–405.

(18) Heiska, S.; Tikkanen, O. -P.; Rousi, M.; Julkunen-Tiitto, R. Bark salicylates and condensed tannins reduce vole browsing amongst cultivated dark-leaved willows (*Salix myrsinifolia*). *Chemoecology* **2007***, 17,* 245–253.

(19) Kammerer, B.; Kahlich, R.; Biegert, C.; Gleiter, C. H.; Heide, L. HPLC-MS/MS Analysis of Willow Bark Extracts Contained in Pharmaceutical Preparations. *Phytochem. Anal.* **2005***, 16,* 470–478.

(20) Mahdi, J. G. Medicinal potential of willow: A chemical perspective of aspirin discovery. *J. Saudi Chem. Soc.* **2010***, 14,* 317–322.

(21) Agnolet, S.; Wiese, S.; Verpoorte, R.; Staerk, D. Comprehensive analysis of commercial willow bark extracts by new technology platform: Combined use of metabolomics, high-performance liquid chromatography-solid-phase extraction-nuclear magnetic resonance spectroscopy and high-resolution radical scavenging assay. *J. Chromatogr. A* **2012***, 1262,* 130–137.

(22) Chrubasik, S.; Eisenberg, E.; Balan, E.; Weinberger, T.; Luzzati, R.; Conradt, C. Treatment of Low Back Pain Exacerbations with Willow Bark Extract: A Randomized Double-Blind Study. *Am. J. Med.* **2000***, 109,* 9–14.

(23) Kurkin, V. A. Phenylpropanoids as the biologically active compounds of the medicinal plants and phytopharmaceuticals. *Adv. Biol. Chem.* **2013***, 3,* 26–28.

(24) Chen, D.; Fan, J.; Wang, P.; Zhu, L.; Jin, Y.; Peng, Y.; Du, S. Isolation, identification and antioxidative capacity of water-soluble phenylpropanoid compounds from *Rhodiola crenulata*. *Food Chem.* **2012***, 134,* 2126–2133.

(25) Luthria, D. L.; Jones, A. D.; Donovan, J. L.; Waterhouse, A. L. GC-MS Determination of Catechin and Epicatechin Levels in Human Plasma. *J. High Resolut. Chromatogr.* **1997***, 20,* 621–623.

(26) Rohloff, J. Analysis of Phenolic and Cyclic Compounds in Plants Using Derivatization Techniques in Combination with GC-MS-Based Metabolite Profiling. *Molecules* **2015***, 20,* 3431–3462.

(27) Julkunen-Tiitto, R. Phenolic Constituents in the Leaves of Northern Willows: Methods for the
Analysis of Certain Phenolics. *J. Agric. Food Chem.* **1985**, *33*, 213–217.

(28) Schroeder, C.; Lutterbach, R.; Stöckigt, J. Preparative Biosynthesis of Natural Glucosides and Fluorogenic Substrates for β-Glucosidases followed by *in vivo* ^{13}C NMR with High Density Plant Cell Cultures. *Tetrahedron* **1996***, 52,* 925–934.

(29) Wu, Z.; Ouyang, M.; Wang, S. Two new phenolic water-soluble constituents from branch bark of Davidia involucrata. *Nat. Prod. Res.* **2008***, 22,* 483–488.

(30) Seto, R.; Nakamura, H.; Nanjo, F.; Hara, Y. Preparation of Epimers of Tea Catechins by Heat Treatment. *Biosci., Biotechnol., Biochem.* **1997***, 61,* 1434–1439.

(31) Takano, T.; Tobimatsu, Y.; Hosoya, T.; Hattori, T.; Ohnishi, J.; Takano, M.; Kamitakahara, H.; Nakatsubo, F. Studies on the Dehydrogenative Polymerizations of Monolignol β-Glycosides. Part 1. Syntheses of Monolignol β-glycosides, *(E)*-Isoconiferin, *(E)*-Isosyringin, and *(E)*-Triandrin. *J. Wood Chem. Technol.* **2006**, *26*, 215–229.

(32) Pobłocka-Olech, L.; Nederkassel, A. -M. V.; Heyden, Y. V.; Krauze-Baranowska, M.; Glód, D.; Bączek, T. Chromatographic analysis of salicylic compounds in different species of the genus *Salix*. *J. Sep. Sci.* **2007***, 30,* 2958–2966.

(33) Sun, R.; Salisbury, D.; Tomkinson, J. Isolation and characterization of hot water-soluble lipophilic extractives from wheat straw. Part II. Spectroscopic and thermal characterization. *Wood Fiber Sci.* **2003**, *35*, 469–477.

(34) Viëtor, R. J.; Renard, C. M. G. C.; Goldberg, R.; Catesson, A. -M. Cell-wall polysaccharides in growing poplar bark tissue. *Int. J. Biol. Macromol.* **1995***, 17,* 341–344.

(35) Fang, X.; Yin, X.; Yuan, G.; Chen, X. Chemical and biological characterization of polysaccharides from the bark of *Avicennia marina*. *Eur. Food Res. Technol.* **2015***, 241,*17–25.

(36) Yang, F.; Liu, Q.; Bai, X.; Du, Y. Conversion of biomass into 5-hydroxymethylfurfural using solid acid catalyst. *Bioresour. Technol.* **2011***, 102,* 3424–3429.

(37) Chi, C.; Zhang, Z.; Chang, H.; Jameel, H. Determination of Furfural and Hydroxymethylfurfural Formed From Biomass Under Acidic Conditions. *J. Wood Chem. Technol.* **2009***, 29,* 265–276.

(38) Jääskeläinen, A. -S.; Toikka, K.; Lähdetie, A.; Liitiä, T.; Vuorinen, T. Reactions of aromatic structures in brightness reversion of fully-bleached eucalyptus kraft pulps. *Holzforschung* **2009**, *63*, 278–281.

(39) Hoang, T. M. C.; van Eck, E. R. H.; Bula, W. P.; Gardeniers, J. G. E.; Lefferts, L.; Seshan, K. Humin based by-products from biomass processing as a potential carbonaceous source for synthesis gas production. *Green Chem.* **2015**, *17*, 959–972.

(40) Castiglioni, C.; Tommasini, M.; Zerbi, G. Raman spectroscopy of polyconjugated molecules and materials: confinement effect in one and two dimensions. *Philos. Trans. R. Soc., A* **2004**, *362,* 2425–2459.

(41) Zandvoort, I. V.; Wang, Y.; Rasrendra, C. B.; van Eck, E. R. H.; Bruijnincx, P . C. A.; Heeres, H. J.; Weckhuysen, B. M. Formation, Molecular Structure, and Morphology of Humins in Biomass Conversion: Influence of Feedstock and Processing Conditions. *ChemSusChem* **2013**, *6*, 1745–1758.

(42) Zandvoort, I. V.; Koers, E. J.; Weingarth, M.; Bruijnincx, P. C. A.; Baldus, M.; Weckhuysen, B. M. Structural characterization of ^{13}C-enriched humins and alkali-treated ^{13}C humins by 2D solid-state NMR. *Green Chem.* **2015**, *17*, 4383–4392.

(43) Soto, M. L.; Moure, A.; Domínguez, H.; Parajó, J. C. Recovery, concentration and purification of phenolic compounds by adsorption: A review. *J. Food Eng.* **2011**, *105*, 1–27.

(44) Azmir, J.; Zaidul, I. S. M.; Rahman, M. M.; Sharif, K. M.; Mohamed, A.; Sahena, F.; Jahurul, M. H. A. Techniques for extraction of bioactive compounds from plant materials: A review. *J. Food Eng.* **2013**, *117*, 426–436.