Tree barks characterization envisioning an integrated use in a biorefinery

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Abstract

Tree barks are considerable waste streams in forest industries, and a potentially interesting resource for biorefineries. Barks of six relevant tree species, namely poplar (*Populus × canadensis*), black locust (*Robinia pseudoacacia*), red oak (*Quercus rubra*), willow (*Salix sp.*), Corsican pine (*Pinus nigra subsp. laricio*) and larch (*Larix decidua*) were anatomically and chemically characterized. Anatomical analysis illustrated the structural heterogeneity of the different barks with occurrence of various cell types and their arrangement in phloem, periderm and rhytidome. Summative chemical analysis showed a high extractives content (14-30 wt%) for all barks. Black locust bark presented a substantial suberin content (10 wt%). The lignin content was similar for most barks (22-29 wt%), except for Corsican pine bark (45 wt%). Analytical pyrolysis demonstrated that softwood bark lignin was mostly G type with a small amount of H units, whereas for hardwood species S, G and H units were present, with low S/G ratios (0.3-0.7). The cell wall structural polysaccharides were rather low. Total monosaccharides ranged from 35 to 44 wt%, with glucose being predominant for almost all barks, followed by xylose in hardwood and mannose in softwood barks. Identification of the lipophilic extractives highlighted the predominance of resin acids for softwood barks, and fatty acids and triterpenoids for all barks. Analysis of the polar bark extracts revealed large variations in the content of phenolics (265-579 mg gallic acid equivalent/g extract), flavonoids (177-391 mg catechin equivalent/g extract) and condensed tannins (88-670 mg catechin equivalent/g extract). Furthermore, the polar extracts presented a high antioxidant potential (500-1209 mg trolox equivalent/g extract), as determined by the FRAP assay. Additionally, a very strong antioxidant activity (AAI > 2), as evaluated by the DPPH assay, was observed for all barks. In summary, the results highlight the marked anatomical and chemical interspecies variability in barks, thus suggesting the need for tailored biorefining approaches.

Introduction

Given plant based biomass is generally viewed as resource for a more sustainable bio-economy, biorefinery concepts to unravel such lignocellulosic biomass receive considerable attention.[1-5] In a biorefinery, biomass is processed into various product streams, suitable for downstream conversion into fuels, chemicals and materials, while minimizing wastes and adopting a full resource use approach.[1, 6, 7]

A major factor affecting the sustainability and economic viability of a biorefinery is the type and characteristics of the biomass feedstock. Instead of dedicated crops like corn or sugarcane, the use of agricultural wastes or forest residues greatly improves the overall sustainability of the biorefinery.[8] Tree barks are a nice example of such forest residues. Bark comprises 13-21 wt% of a tree log on a dry-weight basis.[9] Prior to processing in wood industries, trees and logs are debarked, leaving vast amounts of bark as a low-value residue, which is mainly burned for energy recuperation or used as soil coverage in horticulture.[9-11] As such, tree barks combine abundance, availability and

low cost with intrinsically rich chemical, physical and bioactive characteristics, thus making them a compelling feedstock for higher valued purposes such as chemicals and materials in a biorefinery.

A pre-requisite of any biorefinery process is a profound knowledge of the anatomical, physical and chemical characteristics of its raw material. Anatomically, bark is defined as all the tissues outside the vascular cambium and it is formed by the activity of two meristems: the vascular cambium and phellogen.[12] It includes a secondary phloem comprising the conducting and non-conducting phloem, and a rhytidome, that encloses the inner and sequential periderms (with phelloderm, phellogen and phellem) interspersed with layers of dead phloem tissue.[13] Chemically, bark consists of carbohydrate polymers (cellulose, hemicelluloses and pectins), phenolic polymers (lignin and condensed tannins), polyesters of fatty acids and glycerol (suberin), extractives (e.g. fatty acids, terpenoids, tannins) and inorganics.[14, 15] Compared to wood, barks tend to have higher inorganics and, often ill-defined, extractives content. Adding to the complexity, the chemical composition of bark varies widely between different tree species or even within the same species (e.g. depending on tree age or location in the tree).[14] This variability may be looked upon as a negative aspect, especially regarding a steady and unchangeable feedstock stream for an industry, but it may also be seen as a virtually unlimited source of different compounds yielding a wider range of potential bio-based products.

A biorefinery strives for full utilization of the biomass components. In recent years, lignocellulose-based biorefineries received considerable attention both regarding the isolation and use of the (hemi)cellulosic [5, 16-19] and lignin [20-25] fractions. In bark specifically, the lignin and (hemi)cellulose fractions have been targeted in several strategies such as organosolv fractionation [26-28], hydrothermal or dilute acid pretreatment followed by enzymatic cellulose hydrolysis [29-31] and reductive catalytic fractionation [32-34]. In addition to lignin and (hemi)celluloses, barks can also contain another structural component, i.e. suberin, in their periderm. Suberin is a macromolecule of ester bonded fatty acids and glycerol and the main cell wall component of the phellem cells (also called cork cells).[35] In species with a well-developed phellem i.e. cork rich barks, the suberin content may be high and constitutes an interesting chemical resource.[36] Several species have been studied regarding their cork characteristics in view of a potential biorefinery integration, e.g. *Quercus cerris* [37], *Pseudotsuga menziesii* [38], *Betula pendula* [39], *Plathymenia reticulata*. [40], among others. Depolymerized suberin fragments constitute interesting building blocks for novel polyesters[41] or can be hydrodeoxygenated into biofuels[33, 42].

Apart from these structural components, barks contain a substantial amount of extractives (almost always higher than the respective wood).[43] In some cases, these extractives even constitute the largest fraction of the bark (e.g. 46 wt% for *Acacia dealbata*[44] and 56 wt% *Eucalyptus sideroxylon*[45]). This non-structural fraction contains secondary plant metabolites that protect the plant from various biological stresses (e.g. insects, pathogens)[46] and may therefore be an interesting source of bioactive and antioxidant components.[47-50] Furthermore, alluring material applications such as adhesives, foams and epoxy resins, have been demonstrated for the condensed tannin fraction found in bark extracts.[51-53]

Many species such as spruce, fir, pines, larch, douglas fir, oak, beech, ash, poplar and eucalyptus are exploited on a large scale for their wood, thus producing considerable amounts of bark. In Europe, an estimated 20 million m³ bark is produced annually.[54] While a few of those barks have received some attention, many are still lacking the extensive knowledge required to improve their use for higher valued purposes than energy production through direct burning.

This contribution studies the barks of six different species with regard to their anatomical and chemical composition. Poplar (*Populus × canadensis*)[55], black locust (*Robinia pseudoacacia*)[56], red oak (*Quercus rubra*)[57], willow (*Salix sp.*)[55], Corsican pine (*Pinus nigra subsp. laricio*)[58] and larch (*Larix decidua*)[59] were selected as they are all very relevant tree species in Europe and their handling in wood-processing industries produces large amounts of the

respective barks.[60, 61] *Quercus rubra* and *Robinia pseudoacacia* are native to North-America and considered invasive species in Europe, and are commercially exploited for their timber.[56, 57] *Robinia pseudoacacia* is especially valued for its highly durable wood, comparable to tropical hardwoods.[56, 62] Various poplar and willow species are fast-growing and are, next to timber, also considered for energy production.[55, 63] Corsican pine and larch are interesting conifer species for timber production.[58, 59] *Pinus nigra* is used for reforestation worldwide and the subspecies Corsican pine is valued for its straightness and high quality wood.[58, 64] *Larix decidua* is the only deciduous conifer species in Europe, and traditionally an important forestry species in alpine regions.[59]

For the bark of each of the aforementioned species, an anatomical characterization was carried out to gain insight in their structure and cellular features. Next, the summative chemical composition was determined for each bark, *i.e.* inorganics, extractives, (suberin,) lignin and holocellulose. Additionally, the carbohydrate composition of the polysaccharides fraction was ascertained. In order to gain more insight in the lignin composition of each bark, its H/G/S content was determined *via* fast pyrolysis coupled to GC/MS. Furthermore, the apolar bark extracts were identified by GC/MS after derivatization. The polar, ethanol/water extracts were analyzed with respect to their composition and reactivity. Their total phenolic, flavonoid and condensed tannin content were determined and their antioxidant properties were investigated.

Materials and methods

Sampling and fractionation

Barks of six different species were manually collected: poplar (*Populus × canadensis*), from around 50 year old trees, in Genk (Belgium), black locust (*Robinia pseudoacacia*), from around 20 year old trees, located in Heusden-Zolder (Belgium), provided by Biomassaplein, red oak (*Quercus rubra*), from around 60 year old trees, in Hasselt (Belgium), willow (*Salix sp.*), from around 30 year old trees, in Hasselt (Belgium), and Corsican pine (*Pinus nigra subsp. laricio*, and larch (*Larix decidua*), both located in Hamond-Achel (Belgium) and provided by the division ‘Forest, Nature and Landscape’ (KU Leuven). The remaining wood was manually removed from the bark samples. The samples were air-dried for several weeks at room temperature. The bark samples were knife-milled with a Retsch SM 2000 mill to pass a 6x6 mm screen, after which they were milled again to pass a 1x1 mm screen. The <1 mm fraction was sieved using various screen sizes, viz. 850 µm, 450 µm, 250 µm and 180 µm, to gain insight in the particle size distribution for the different bark samples. The sieved fractions were combined and mixed again, and the entire bark fraction <1 mm was used for subsequent analysis.

Anatomical Analysis

The anatomical studies were conducted on the bark samples of *Populus x canadensis*, *Robinia pseudoacacia*, *Quercus rubra*, *Salix sp.*, *Pinus nigra* subsp. *laricio* and *Larix decidua*. Bark samples were impregnated with DP1500 polyethylene glycol.[65] Transverse and longitudinal microscopic sections of approximately 17 µm thickness were prepared with a Leica SM 2400 microtome. The sections were stained with a double staining of chrysoidine/astra blue (lignin stains yellow with chrysoidine and cellulose stains blue by astra blue) and were mounted on Kaiser glycerin. After 24 h drying, the lamellas were submerged into xylene for 30 minutes, dehydrated on 96% and 100% alcohol and mounted on Eukitt. Sudan IV was used for detection of suberin (suberin stains brownish red with Sudan IV).

Individual specimens were also macerated in a 1:1 solution of 30% H₂O₂ (hydrogen peroxide) and CH₃COOH (glacial acetic acid) at 60°C for 48 h and stained with astra blue.

Light microscopic observations were made using Leica DM LA and photomicrographs were taken with a Nikon Microphot-FXA.

Bark terminology followed Angyalossy *et al.*[13]

Summative chemical analysis

Chemical summative analysis included determination of ash, extractives soluble in increasingly polar solvents (dichloromethane, ethanol and water), suberin, Klason and acid soluble lignin, and holocellulose, as well as the monomeric composition of polysaccharides. The procedures were adapted from Neiva *et al.*[66] and Miranda *et al.*[67].

Ash content was determined by combusting approximately 1 g bark overnight at 525°C and weighing the residue, following TAPPI standard T211 om-93. Extractives were determined in extraction thimbles by successive extractions with dichloromethane (6h), ethanol (16h) and water (16h) in a Soxhlet apparatus. The thimbles were oven-dried after each extraction and the extractives were quantified gravimetrically.

Suberin content was determined only for poplar, black locust, willow and red oak bark samples. The suberin in extractive-free barks was depolymerized via alkaline methanolysis. A 1.5 g extractive-free sample of each bark was refluxed with 100 ml of a 3% solution of NaOCH₃ in methanol during 3 h. The sample was filtrated and washed with methanol. The residue was refluxed with 100 ml methanol for 15 min and filtrated again. The combined filtrates were acidified to pH 6 with 2M H₂SO₄ and evaporated to dryness. The residues were suspended in 50 ml water and the methanolysis products were recovered with dichloromethane in three successive extractions, each with 50 ml dichloromethane. The combined extracts were dried over anhydrous Na₂SO₄. The dichloromethane was evaporated and the residue, containing the nonpolar monomer units of suberin (*e.g.* the long chain lipids) was quantified gravimetrically, and expressed relative to the oven-dry bark.[67]

Acid insoluble lignin (Klason lignin) and soluble lignin were determined on extractive-free (and suberin-free) material. Klason lignin was determined through acid hydrolysis, according to TAPPI standard T222 om-88. Sulphuric acid (72%, 3.0 ml) was added to 0.35 g of sample, at 30 °C for 1 h with occasional stirring after which it was diluted to a concentration of 3 wt% H₂SO₄ and further hydrolyzed for 1 h at 120 °C. After filtration in a G4 crucible, the residue was thoroughly washed with distilled water and oven dried. Klason lignin was determined as the mass of solid residue after correction for ash content (determined as described above). Soluble lignin was determined through UV spectrophotometry, measuring the absorbance of the hydrolysate, from the Klason lignin procedure, at 205 nm, according to TAPPI standard UM250 om-83. Total lignin content was calculated as the sum of Klason and soluble lignins.

The hydrolysis liquor resulting from the Klason lignin procedure was used to determine the polysaccharide composition. The neutral monosaccharides, glucuronic acid and galacturonic acid were determined by separation through a Dionex ICS-3000 High Pressure Ion Chromatographer, using an Aminotrap plus Carbopac SA10 column (4x250mm); a gradient of NaOH + CH₃COONa with a 1 mL/min flow at 25 °C was used as eluent. Acetic acid was determined in a Waters 600 and measured with a UV/Vis detector at 210 nm, with a Biorad Aminex 87H HPX (300 x 7.8 mm) column; 10 mM H₂SO₄ with a 0.6 mL/min flow at 30 °C was used as eluent.[68]

The holocellulose content was determined on the extractive-free (and suberin-free) samples using the sodium chlorite method, as described by Rowell *et al.*[14]

All the chemical analyses, except monosaccharide analysis via HPLC, were made in triplicate. Results are reported relative to the oven dry starting material.

Lipophilic extracts composition

Dichloromethane extracts were dried in aliquots under N₂ and then dried in a vacuum oven over phosphorus pentoxide. Around 1 mg of the extract was dissolved in pyridine (120 µl) and trimethylsilylated with bis(trimethylsilyl)trifluoroacetamide (80 µl) and oven heated at 60 °C during 30 min. Following the reaction time, the derivatized dichloromethane extracts were injected in an Agilent GC 7890A coupled to 5975C MSD 70 eV electron impact voltage and with a Zebron 5HT Inferno column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). The oven heating program started at 100 °C (held for 1 min), raised to 150 °C at a rate of 10°C/min (ramp 1), then increased to 200 °C at 5 °C/min (ramp2), followed to 300 °C at 4 °C/min (ramp 3), and lastly raised to 380 °C at 10 °C/min and held for 5 min (ramp4). The temperature of the injector was kept at 280 °C and the MS interface at 220 °C; helium was the carrier gas with a total flow of 1mL/min. The identification of the compounds (as TMS derivatives) were based on literature mass spectra, Wiley 6, NIST libraries data, and own interpretation of mass spectrometric fragmentation patterns.

Phenolic content and antioxidant activity of the bark extract

The extraction procedure and phenolic content analysis was adapted from Miranda *et al.*[45] The ground bark was extracted in an ethanol/water solution (50/50, v/v) for 60 min at 50 °C using an ultrasonic bath. The insoluble material was removed by filtration and the extract was stored at 4 °C. The solid residue was dried at 105°C and the extraction yield determined gravimetrically.

The phenolic content of the barks was determined as total phenols, flavonoids and tannins content. All the colorimetric assays were made in triplicate.

Total phenolics content was determined by the Folin–Ciocalteu method[69] using gallic acid as standard and expressed as milligrams of gallic acid equivalents (GAE)/g of dry bark or dry bark extract. Total flavonoids content was determined by an aluminium chloride colorimetric assay[70] and condensed tannins by the vanillin-H₂SO₄ method[71] using (+)-catechin as standard. The results were expressed as mg of catechin equivalents (CE)/g of dry bark or dry bark extract.

The analysis of antioxidant properties of the barks was determined by the DPPH method (free radical scavenging activity) and the FRAP method (ferric reducing antioxidant power).

The DPPH method was adapted from Miranda *et al.*[45] The antioxidant activity of the bark ethanol/water extract was determined using 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and expressed as (i) IC₅₀ (extract concentration required for 50% DPPH inhibition), (ii) antioxidant activity index (AAI = final DPPH concentration/IC₅₀)[72] and (iii) as Trolox equivalents (TE) or gallic acid equivalents (GAE) antioxidant capacity.

The FRAP assay was adapted from Benzie and Strain[73] and the results were expressed as mg of Trolox equivalents (TE) or gallic acid equivalents (GAE)/g of dry bark or dry bark extract.

Pyrolysis–GC/MS

The pyrolysis-GC/MS procedure was adapted from Marques *et al.*[74] For this procedure, extractive-free bark was used to avoid interference of extractives with the lignin analysis. The extractive-free barks were milled to a fine powder using a vibratory ball mill (Retsch MM200 mixer), dried under vacuum over phosphorus pentoxide and then characterized by Py-GC/MS. Approximately 100-120 µg of each bark was weighed in a quartz boat and pyrolysed at 550 °C for 1 min in a CDS Pyroprobe 5150 Pyrolyzer connected to an Agilent GC 7890B coupled to a mass detector system 5977B at 70 eV electron impact voltage. The fused-silica capillary column used was a ZB-1701 (60 m x 0.25 mm i.d. x 0.25 µm film thickness). The oven heating program started at 40 °C (held for 4 min), raised to 70 °C at a rate of 10 °C/min (ramp 1), then to 100 °C at 5 °C/min (ramp 2), raised to 265 °C at 3 °C/min and held for 3 min (ramp 3), and lastly to 270 °C at 5 °C/min and held for 9 min (ramp 4). The temperature of the injector was kept at 270 °C

and the GC/MS interface at 280 °C; and helium was the carrier gas with a total flow of 1mL/min. The compounds were identified using the Wiley-NIST libraries and literature.[75, 76] The *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units (lignin derived products) were identified and their pyrogram peak area was used to calculate their molar percentage, relative to the other lignin derived compounds, according to the following formula:

$$\text{Mol \% lignin - derived compound} = \frac{\text{Area compound} * \frac{1}{\text{MW compound}}}{\sum \left(\text{Area compound} * \frac{1}{\text{MW compound}} \right)} * 100$$

These molar percentages of individual lignin compounds were used to calculate the molar percentage of H, G and S units, and the S/G ratio.

Results and discussion

Anatomy

Figures S1-6 (Supplementary information) represent the bark structure of *Pinus nigra subsp. laricio* and *Larix decidua* (Gymnosperms) and *Populus x canadensis*, *Robinia pseudoacacia*, *Quercus rubra* and *Salix sp.* (Angiosperms) analyzed under light microscope. Anatomical differences were noted in tissues and layers of the barks, showing their structural heterogeneity within genus and species, as described in literature.[77] The tissues and their distribution pattern are indicated in each figure; in general the anatomical features are according to literature for the genera/species as already described *i.e.* for *Pinus nigra*[78] and other *Pinus sp.*[79-81]; *Larix decidua*[78]; *Populus tremula*[82], *P.alba* and *P.nigra*[77]; *Robinia pseudoacacia*[13, 77, 78]; *Quercus rubra*[78] and other *Quercus sp.*[79, 83-86] and *Salix sp.*[77, 87].

The rhytidome varied in thickness, texture and morphology. In all the studied species, the rhytidome is of scale bark type, since the sequent periderms are formed as overlapping layers, each cutting out a scale of tissue.[12] The external appearance of the barks was flaky-scale bark in *P. nigra*, fissured in *L. decidua*, longitudinally furrowed with short deep furrows in *Q. rubra*, deeply furrowed into grooves in *R. pseudoacacia* and *Populus*, and deeply fissured in *Salix sp.* The observations of bark under the stereoscope can be found in the supplementary information (Figs. S1.1 A-B, Figs. S2.1 A-B; Figs. S3.1 A; Figs. S4.1 A; Figs. S5.1 A, Figs. S6.1 A). In the rhytidome, the number of periderms varied *e.g.* 9 periderms in *Salix sp.* (Fig. S6.1 A1) and more than 10 in *R. pseudoacacia*. Phellem (cork) cells were observed in *R. pseudoacacia* (Fig. S4.1 B, C) and *L. decidua* (Fig. S2.1 C, D); especially *R. pseudoacacia* bark showed abundant phellem cells in each periderm. The barks of *P. nigra subsp. laricio*, *Q. rubra* and *Salix sp.* contained little or no cork. *P. nigra subsp. laricio* showed highly sclerified phellem cells (Fig. S1.1 C, E).

The conducting phloem, the mostly living tissue produced by the vascular cambium, includes the conducting cells, *i.e.* the sieve cells with Strasburger cells in Gymnosperms and sieve tube elements with companion cells in Angiosperms. These sieve cells were observed with different grouping and distribution within the phloem in *P. nigra subsp. laricio* (Figs. S1.1 E), in *L. decidua* (Figs. S2.1 E, G), in *P. canadensis* (Figs.S3.1 E, G), in *R. pseudoacacia* (Fig. S4.1 D), in *Q. rubra* (Fig. S5.2 C) and in *Salix sp.* (Fig. S6.1 C, E; Fig. S6.2 A). The conducting phloem also includes the axial parenchyma (storage tissue) and rays (storage/transversal conduction), both non-lignified and thin-walled, and the fibers (mechanical support) with lignified secondary cell walls. The occurrence and pattern of distribution of these tissues varied in the phloem of different genera, *e.g.* the axial parenchyma forms bands in *R. pseudoacacia* (Figs. S4.1 B); the fibers occur in tangential bands more or less regularly, alternating with bands containing the sieve tubes and axial parenchyma in *R. pseudoacacia* (Figs. S4.1 B), *Salix sp.* (Fig. S6.1C) or in tangential rows and grouped in and *P. canadensis* (Fig. S3.1 E, F). Fibers are thickened in *R. pseudoacacia* (Fig. S4.2 B) and are absent in *P. nigra* and *L. decidua*; the rays (storage/transversal conduction) varied from thin and uniseriate *i.e.* in *P. nigra*, *L. decidua*

(Fig. S2.2 A) and in *Salix* sp. (Fig. S6.2 A), to multiseriate *i.e.* in *Q. rubra* (Fig. S5.2 B, C) and *R. pseudoacacia*. Radially waving rays appeared in *L. decidua* (Fig. S2.1 C, E).

In the non-conducting phloem, the conducting tissue collapsed and the structure became disorganized with distortion and expansion of cells, *e.g.* expanded parenchyma cells occurred in *P. canadensis* (Figs. S3.2 A, B); in some cases sclereids (scl) and fibrosclereids (fscl), which are thick and heavily lignified cells walls of mechanical support, also appeared *i.e.* in *P. canadensis* (Figs. S3.1C; 3.2 C) and in *Q. rubra* as abundant groups and clusters (Figs. S5.1 E-G; S5.2 A, C) and in *L. decidua* (Figs. S2.1 E; S2.2 A, B) in form of isolated and round cells, when observed in transverse view.

Crystals, presumably of calcium oxalate and mostly present as prismatic crystals, were common in all the barks and found in sclereids (*i.e.* in *Q. rubra*, Figs. S5.2 A, E) and parenchyma cells (*i.e.* in *Q. rubra* Fig. S5.2B, in *R. pseudoacacia* Figs. S4.2 A, B, C or in *Salix* sp. Figs. S6.2 A, B, C, also occurring along the fibers groups); cubical crystals were found in *L. decidua* (Fig. 2.2 A); mineral inclusions were also observed in ray cells, *i.e.* in *Salix* sp. (Fig. S6.2 A).

Axial and radial resin ducts, provided with a distinct epithelium, appeared in *P. nigra x laricio* (Fig. S1.1 F) and in *L. decidua* (Figs. S2.1 F; 2.2 A). Phenolic compounds were observed by dark color staining in axial and ray parenchyma cells of phloem and rhytidome (*i.e.* in *P. nigra x laricio* Figs. S1.2 C, in *P. canadensis* Fig. S3.1 F, in *Q. rubra* Fig. S5.1 C or in *Salix* sp. Figs. S6.1 B, D, S6.2 A) in the sclereids (*i.e.* in *P. canadensis*, Figs. S3.1 C) and in phellem (*i.e.* in *P. nigra subsp. laricio* Figs. S1.1 C, E and in *Q. rubra* Figs. S5.1 C, D).

Chemical analysis

A summative chemical composition was determined for the barks of the six different species using the entire <1 mm fraction for analysis, to ensure that the complete bark was analyzed and therefore no bias through sieving could occur.[67] The granulometric distribution within this <1 mm fraction is shown in Fig. S7 (Supplementary information), illustrating that willow bark produces a large share of fines upon milling (55 wt% <180 µm), while red oak bark milling results in predominantly coarser particles (41 wt% >425 µm).

The chemical summative analysis encompasses the determination of ash, extractives, suberin, lignin, and holocellulose. The results are shown in table 1. For comparison, some studies on the chemical composition of barks of these species (or genera) have already been conducted elsewhere: poplar (*Populus tremuloides*)[88], red oak[89, 90], black locust[89], larch (*Larix leptolepis*)[91], willow[92, 93], pine (*Pinus sylvestris*)[67].

Table 1: Chemical composition and monomeric carbohydrate composition of six different barks. For all the analyses, the entire <1 mm fraction was used. For larch and Corsican pine, the suberin content was not determined (-). All values are expressed relative to the oven dry, raw material (wt%).

	Poplar	Red oak	Black locust	Willow	Corsican pine	Larch
Ash	3.24 ± 0.21	8.89 ± 0.05	7.27 ± 0.02	10.25 ± 0.03	1.68 ± 0.03	1.06 ± 0.01
Extractives	21.78 ± 0.37	24.22 ± 0.4	16.27 ± 0.63	29.09 ± 0.47	13.91 ± 0.42	30.05 ± 1.86
Dichloromethane	3.01 ± 0.19	7.43 ± 0.44	3.58 ± 0.04	3.29 ± 0.58	3.97 ± 0.05	2.76 ± 0.04
Ethanol	13.73 ± 0.3	12.67 ± 0.75	7.18 ± 0.34	19.89 ± 0.06	5.45 ± 0.31	19.13 ± 0.35
Water	5.05 ± 0.47	4.11 ± 0.36	5.50 ± 0.30	5.91 ± 0.42	4.49 ± 0.65	8.16 ± 2.04
Suberin	3.03 ± 0.08	3.92 ± 0.32	10.34 ± 0.86	1.82 ± 0.03	-	-
Lignin	28.98 ± 0.15	21.87 ± 0.37	23.36 ± 0.10	21.89 ± 0.27	45.07 ± 1.18	28.81 ± 0.13
Klason	27.93 ± 0.14	19.33 ± 0.17	21.28 ± 0.17	20.09 ± 0.22	44.49 ± 1.20	28.38 ± 0.11
Acid soluble	1.06 ± 0.07	2.54 ± 0.24	2.08 ± 0.07	1.8 ± 0.07	0.58 ± 0.04	0.43 ± 0.03

Holocellulose	34.58 ± 0.4	44.45 ± 0.55	37.31 ± 0.05	37.4 ± 0.67	42.87 ± 1.59	41.96 ± 1.13
Polysaccharides						
Arabinose	1.85	1.70	2.94	1.57	2.93	1.82
Galactose	1.05	0.81	1.11	1.17	1.97	1.45
Glucose	19.23	13.47	17.43	16.66	18.63	22.49
Xylose	8.09	14.08	4.86	5.05	3.54	3.32
Mannose	1.14	0.27	0.96	0.75	4.79	4.52
Rhamnose	0.38	0.28	0.42	0.33	0.23	0.21
Galacturonic acid	1.87	0.90	1.49	1.15	1.17	1.17
Acetic acid	0.14	0.21	0.10	0.11	0.61	0.41

The ash content of the barks varied considerably between species. In some reports, the ash content is overestimated, due to contamination with extraneous material from the handling and debarking process.[68] However, since all the barks were obtained directly from the trees, it is acceptable to assume that these values correspond to the true content and are not distorted by any extraneous contaminants.

The softwood species larch and Corsican pine have the lowest ash content (1.1 and 1.7 wt% respectively), which corroborates earlier findings that softwood barks tend to have lower ash content when compared to hardwood barks.[90, 94] Very high ash contents were observed for black locust, red oak and willow barks (7-10 wt%), whereas poplar bark displayed an intermediate ash content (3.2 wt%). Barks are known to contain higher amounts of minerals than their respective woods, with some species presenting crystals (*e.g.* calcium oxalate) as a potential defense mechanism.[46, 95] Indeed, the high amount of ash in black locust, red oak and willow may be explained by the abundant presence of mineral crystals as observed by anatomical analysis, *i.e.* in red oak (Fig. S5.2 A, B, C, E), black locust (Fig. S4.2 A, B, C) and willow (Fig. S6.2A, B, C). The high ash content of these barks will make them poor raw materials for energy purposes by direct burning, or for production of pellets and briquettes.

The total content of extractives was determined *via* successive extractions with increasingly polar solvents, *i.e.* dichloromethane, ethanol and water. In most species, barks tend to be especially enriched in extractives when compared to their respective woods, making them interesting materials for extracting these fractions through simple processes and at mild conditions.[49]

The highest amount of extractives was found in larch and willow barks (30.1 and 29.1 wt%) for which the ethanol extraction yielded roughly two-thirds of the total extractives. Black locust bark has the highest amount of apolar extractives (7.4 wt%), *i.e.* extracted by dichloromethane, while in the other barks these dichloromethane extracts comprise roughly 2.5 - 4 wt%. Corsican pine and black locust barks have the lowest total amount of extractives (13.9 and 16.3 wt%). For black locust and red oak barks, the total amount of extractives (16.3 and 24.2 wt%) was higher than previously reported by Sillero *et al.* (12.7 and 12.1 wt%).[89] This illustrates the intra-species variations that can occur with different growing conditions or age.[14]

Suberin content was determined on the pre-extracted samples *via* alkaline methanolysis, followed by dichloromethane/water extraction to obtain the apolar compounds. The suberin content was highest for black locust (10.3 wt%), which is in good agreement with earlier reports.[32, 89] Indeed, the anatomical analysis highlighted the large amount of phellem cells in the periderms of black locust (Fig. S4.1 B, C), thereby confirming the chemical results. Suberin content in barks is related to the proportion of cork in the periderms since only the phellem cells contain suberin.[35] In fact, the suberin content in cork tissue is particularly high *e.g.* 42.8 wt% in cork oak [96] and 36.2 wt% in birch [39] corks. Poplar, red oak, and willow barks contain substantially less suberin (3.0, 3.9 and 1.8 wt%, respectively) in accordance with the anatomical observations showing that these barks possess thin periderms with a small amount of phellem cells. The suberin content was not determined for Corsican pine and larch barks, as

pinces and larches contain a very small proportion of phellem and therefore little to no suberin.[67, 80, 91]. Our anatomical observations showed that the periderm of larch included 5 or more phellem cell layers in the periderm (Fig. S1.1 C, D) as recently highlighted.[97] Corsican pine bark showed a small number of phellem cells in the rhytidome, but they were thick-walled and sclerified (Fig. S1.1 C, E),

Lignin content was determined on the suberin-free (or extractive-free in case of Corsican pine and larch) samples *via* acid hydrolysis of the carbohydrates (Klason lignin) and with the acid soluble lignin analyzed *via* UV-Vis spectroscopy. The lignin content of most barks ranged from 21 wt% to 29 wt%, with Corsican pine bark presenting an exceptionally high lignin content. The softwood barks presented a higher lignin content than most of the hardwood barks (with the exception of poplar bark) and lower acid soluble lignin content, which is in line with the observations for wood.[20, 98] Corsican pine presented by far the highest lignin content (45.1 wt%). However, this high lignin content should be interpreted with caution, as also non-lignin components could precipitate after acid hydrolysis, *e.g.* condensed tannins that were not removed during the successive soxhlet extractions, thereby resulting in a false high lignin content.[14] As mentioned before, Corsican pine bark showed the lowest amount of extractives (especially ethanol and water extractives), which might also point towards the presence of high molecular weight condensed tannins that were not extracted with ethanol or water in a soxhlet setup.

Holocellulose content was measured by delignification of the suberin-free (or extractive-free) samples with sodium chlorite. The holocellulose content ranged from 35 to 45 wt%, with red oak bark having the highest holocellulose content (44.5 wt%).

The monosaccharides were quantified by liquid chromatography of the hydrolysis liquor resulting from the Klason procedure. The monomeric composition of polysaccharides, *viz.* neutral sugars, uronic acids and acetates, is shown in table 1. Larch bark has the highest glucose content (22.5 wt%), indicating a higher cellulose content. Red oak bark, on the other hand, has the lowest glucose content (13.5 wt%) but also the highest content of non-glucose carbohydrates, indicating a higher hemicellulose content. The hardwood barks have the highest xylose content (highest in red oak, 14.1 wt%), in agreement with glucuronoxylan being the major hardwood hemicellulose type.[14] The softwood barks, larch and Corsican pine, contain a substantial amount of mannose (4.5 and 4.8 wt%), in contrast to the hardwood barks (< 1.1 wt%), in line with the hemicellulose composition of softwoods containing mainly galactoglucomannan.[14] The lack of significant galacturonic acid indicates low pectin content of for all barks.[14]

Comparing the polysaccharides content, *i.e.* determined by summing up the monosaccharides, with the holocellulose content, *i.e.* determined by delignification, shows a consistently higher value for the holocellulose content. This is due to incomplete delignification for barks during the sodium chlorite procedure used for holocellulose determination, therefore making the polysaccharide quantification through monosaccharides determination the most viable and correct one.[9] As chemical compositional analyses have been tailored for wood, they are sometimes less adequate to describe non-wood lignocellulosic materials such as barks.

Lignin composition

The lignin composition of the different bark samples was analyzed by pyrolysis in extractive-free samples to avoid interference of phenolic extractives. The lignin-derived compounds were quantified and presented on a molar basis (Table 2). The lignin-derived compounds accounted for 17.5 - 25.8% of the total chromatogram peak area for the different barks, with willow showing the lowest and poplar the highest relative proportion of lignin-derived compounds.

In line with the expectations [99], the pyrolysis of softwood barks of larch and Corsican pine released phenolic compounds derived predominantly from G units (88-91 mol% of all lignin compounds), with guaiacol, 4-methylguaiacol and 4-vinylguaiacol being the predominant ones, also presenting lower amounts of H units and

showing absence of S units, as typical of softwoods. The hardwood barks showed a predominance of G units (53-69 mol%) in relation to S units (20-38 mol%) and also presenting lower amount of H units (5-15 mol%). The low S/G ratio implies a more condensed lignin structure for hardwood barks when compared to typical values for woods that tend to have S/G ratios above one.[100] This variation of relative abundances of S and G units between wood and bark has been previously reported for several species.[74, 101]

The relative abundance of H units is quite high for most species (10-15 mol%), with exception for the red oak (5 mol%). The true H unit content, derived from *p*-coumaryl alcohol, is rarely above 5 mol% but has been often overestimated, mostly due to *p*-coumarate and *p*-hydroxybenzoate units that upon pyrolysis undergo decarboxylation, thus being released and accounted for as H units.[102] This is especially true for poplar and willow species, which are known to have *p*-hydroxybenzoate groups acetylated to their lignin structure[102], possibly explaining the large share of H units in the respective barks. Another possibility is the occurrence of demethoxylation reactions of S and G units to H units which might occur at temperatures above 400-450 °C.[103] Nevertheless, for some barks, such high H contents are valid and confirmed by NMR techniques (e.g. 22 mol% H content by pyrolysis for *Picea abies* bark corresponded to 14 mol% by 2D-HSQC-NMR).[44]

Bark lignins can be fairly similar to their respective wood (as for *E. globulus*) with a similar overall content, aromatic units distribution and inter-unit linkages types[44] or, on the contrary, present highly differentiating chemical characteristics, whether by having higher overall lignin content (as it happens here for Corsican pine, table 1), presenting specific monomers not seen in wood lignins (e.g. hydroxystilbenes glucosides in *Picea abies*[104]) or having high content in *p*-hydroxycinnamates (as in *Acacia melanoxylon* bark[44]).

From a biorefinery point of view, the S/G ratio of lignin is a major factor influencing its valorization.[105] A higher S content tends to correspond to a higher fraction of inter-unit ether bonds (β -O-4), which are readily cleaved during lignin depolymerization.[20, 106] A higher G content, as reported for these hardwood barks, tends to yield fewer monomers upon depolymerization.[20, 106] As for the high H unit abundance of some barks, it might indicate a high *p*-benzoate or *p*-coumarate content, which could make them an interesting source of these compounds that are also present in grass lignins.[20]

Table 2: Identities and relative molar abundances of lignin derived phenolic compounds obtained via Py-GC/MS of the different, extractive-free barks. Also the H, G and S molar percentages and the S/G ratio are displayed. For more details on the calculations, the reader is referred to the section 'Materials and methods'.

Compound	Poplar	Red oak	Black locust	Willow	Corsican pine	Larch
phenol	7.1	3.0	5.4	10.4	6.7	5.1
guaiacol	9.8	6.5	7.8	8.0	12.1	11.2
4-methylphenol	3.2	1.7	5.4	4.6	5.0	3.6
4-methylguaiacol	10.3	5.1	1.6	5.8	14.9	13.1
4-ethylphenol	0.7	0.4	2.5	0.4	0.8	0.8
4-ethylguaiacol	2.0	1.1	1.3	0.9	2.7	2.2
4-vinyl guaiacol	16.3	13.8	14.9	15.9	19.5	18.3
eugenol	2.1	2.0	1.7	1.5	2.5	2.7
4-propylguaiacol	0.6	0.5	0.4	0.3	0.7	0.7
syringol	3.0	4.7	4.3	4.0	-	-
cis-isoeugenol	1.6	1.4	1.8	1.5	1.9	2.3
trans-isoeugenol	6.8	5.3	5.4	6.0	7.6	8.7
4- methylsyringol	3.3	4.2	4.1	4.1	-	-
vanillin	4.4	4.0	3.9	3.2	3.8	5.1
(4-hydroxy-3-methoxyphenyl)propyne	3.1	3.8	3.2	4.0	2.8	3.0
homovanillin	0.9	1.5	0.1	0.2	2.2	2.5

4-ethylsyringol	0.5	0.6	0.7	0.8	-	-
acetoguaiacone	3.0	2.5	1.8	2.1	3.1	3.1
4-vinylsyringol	3.7	6.5	6.7	7.0	-	-
guaiaacylacetone	1.3	1.1	1.8	0.8	1.4	1.3
4-allylsyringol	0.9	1.9	1.5	1.3	-	-
propiovanillone	0.9	0.4	0.6	0.4	2.4	2.0
coniferyl alcohol	1.0	1.7	0.6	0.2	1.2	1.8
cis-4-propenylsyringol	0.7	1.2	1.5	1.2	-	-
4-propinylsyringol	0.4	1.2	1.0	0.9	-	-
trans-4-propenylsyringol	2.7	5.5	5.2	5.0	-	-
dihydroconiferyl alcohol	0.1	1.3	-	-	2.2	3.1
syringaldehyde	1.4	4.1	2.7	2.1	-	-
cis-coniferyl alcohol	0.7	0.7	0.7	0.2	0.3	1.0
homosyringaldehyde	-	0.6	-	-	-	-
acetosyringone	1.4	2.1	2.2	1.8	-	-
trans-coniferyl alcohol	1.0	1.4	3.0	-	0.4	2.0
trans-coniferaldehyde	3.4	3.2	2.0	2.7	5.6	6.5
syringylacetone	0.6	1.1	1.1	1.1	-	-
propiosyringone	0.2	0.4	0.3	0.1	-	-
syringyl vinyl ketone	0.1	0.3	0.1	0.1	-	-
sinapyl alcohol	-	0.3	-	0.1	-	-
trans-sinapaldehyde	1.0	3.1	2.4	1.3	-	-
H (mol%)	10.9	5.1	13.3	15.3	12.5	9.5
G (mol%)	69.1	57.2	52.7	53.5	87.5	90.5
S (mol%)	19.9	37.7	34.0	31.1	-	-
S/G ratio	0.3	0.7	0.6	0.6	-	-

Lipophilic extractives

The composition of the lipophilic dichloromethane (DCM) extracts of the various barks were analyzed by GC/MS after trimethylsilylation. Although DCM extracts are not the major group of extractives (representing between 9.2% up to 30.7% of the total extractives, Table 1) they have a rich chemical diversity and often include compounds with interesting bioactivities and properties. Because of the low volatility of most compounds present in the ethanol and water extracts, such GC/MS analysis was not carried out for these extracts but a chemical determination of phenolic families was made instead (see next section).

An overview of the identified compound classes is presented in table 3. For the detailed table containing the individual identified molecules, the reader is referred to table S1 (Supplementary information). For comparison with data presented here, the reader is also referred to other studies on the lipophilic bark extracts of the same species or genus: different *Salix* species[107], *Populus tremuloides*[108], *Quercus faginea*[109], *Pinus* species[110, 111], *Larix decidua*[50].

From the results in table 3, it is clear that the aliphatic monoacids present a large share of the DCM extractives. For the hardwood barks, it is the most abundant class, whereas for softwood barks, it is the second-most abundant class of compounds. Aliphatic monoacids are highly prevalent in the bark of red oak, in which they comprise 56.6% of the total DCM extractives, identifiable by GC/MS. A previous study also demonstrated a high content of alkanolic acids in the DCM extracts of the bark of *Quercus faginea*. [109] Furthermore, red oak also has the highest fraction of DCM extracts (7.4 wt%) on a dry bark basis (vide supra), making it a potential source of extractable fatty acids. One aliphatic monoacid, 9,12-octadecadienoic acid, was specifically abundant in red oak, making up 29.4% of the DCM extractives. In fact, unsaturated C18 acids (C18:1, C18:2 and C18:3) make up a large share in the DCM extracts of all the hardwood barks (12.5 - 40%), but they were significantly less abundant in the softwood DCM extracts (< 4%). In

the softwood barks, saturated aliphatic acids were more common. For instance, a large share of docosanoic acid (C22:0, 13.9%) and eicosanoic acid (C20:0, 6.4%) was observed in the DCM extracts of Corsican pine.

Aliphatic alcohols were much less abundant than the aliphatic monoacids. The DCM extract of poplar bark, however, still presents a high share of these aliphatic alcohols (12.6%). The diacids and hydroxyacids, both presenting multiple functional groups, were found chiefly in the hardwood barks, especially in black locust bark (10.9%). Also monoacylglycerols were found predominantly in hardwood barks, with poplar having the largest share of them.

Alkyl hydroxycinnamates (coumarates, ferulates and caffeates) were encountered mainly in poplar and black locust, 5.4 and 9.2% of DCM extractives, respectively. Alkyl coumarates and ferulates were found in poplar, whereas alkyl caffeates and ferulates were detected in black locust bark. Such long-chain alkyl hydroxycinnamates are interesting compounds in view of their anticancer activity.[112]

Diterpenoids (C₂₀ terpenoids) were the most abundant class in the softwood barks of Corsican pine and larch, representing respectively 35.9 and 48.7% of the identifiable DCM extractives. Within this class of molecules, pimaric acid, isopimaric acid, abietic acid and dehydro abietic acid, which are all resin acids, were dominant. Hardwood barks did not contain any diterpenoids. Triterpenoids (C₃₀ terpenoids) were observed for all barks (4.8 - 14.9%), as many of these molecules are common phytosterols. The phytosterol β -sitosterol was the most common triterpenoid and was found in all barks. Indeed, triterpenoids are an important fraction of the lipophilic extracts, as previous studies on *Eucalyptus globulus* bark and stump bark demonstrated.[113, 114] Sesquiterpenoids (C₁₅ terpenoids) were only found in the bark of red oak, where they make up 9.1% of DCM extractives. Such sesquiterpenoids were also detected in the bark of *Eucalyptus globulus*. [113]

Carbohydrate (derivatives) were mainly encountered in the DCM extracts of willow bark (20.0%). In most cases, these molecules could not be conclusively identified by GC/MS. However, it does highlight the high content of free carbohydrates or carbohydrate linked to other monomeric moieties (e.g. phenolics) in the bark of willow.

Flavonoids were detected almost exclusively in the lipophilic extract of poplar bark, with one specific flavonoid compound detected in a very large quantity (15.5%). Although we were unable to conclusively identify this compound, we tentatively suggest 7-O-methyl taxifolin. The MS pattern of its trimethylsilylated analogue is depicted in Fig. S10. Sakuranetin, which is structurally similar to 7-O-methyl taxifolin, was found in a small amount in poplar bark. This compound is the non-glycosylated analogue of sakuranin, which has been detected and reported previously in a study on poplar bark.[108]

Table 3: Composition of dichloromethane extracts of the different barks. The identified compounds were grouped and their combined peak area was expressed as % of the total chromatographic peak area in the total ion current (TIC), as obtained by GC/MS. The complete identification of all the individual compounds is presented in table S1.

	Poplar	Red oak	Black locust	Willow	Corsican pine	Larch
Aliphatic monoacid	33.8	56.6	47.9	38.7	30.6	16.6
Aliphatic alcohol	12.6	1.1	4.9	1.7	1.6	4.8
Hydroxyacid	1.5	0.7	5.3	0.9		
Diacid	1.0	2.4	5.6	1.9	0.3	0.2
Monoacylglycerol	5.3	1.9	2.0	1.8	0.5	1.3
Alkyl hydroxycinnamate	5.4		9.2	0.2	1.2	2.9
Sesquiterpenoid		9.1				
Diterpenoid					35.9	48.7
Triterpenoid	8.2	6.7	14.9	7.6	8.2	4.8
Carbohydrate (derivative)	0.3			20.0		
Flavonoid	15.9	0.3				

Phenolic	4.6	1.1	0.7	1.2	1.8	0.2
Other	5.1	7.2	1.3	6.5	2.0	1.2
Sum identified	93.7	87.2	91.8	80.2	82.1	80.6

Phenolic content and antioxidant activity of bark polar extracts

An ethanol/water (1/1) extraction at 50°C with sonication was carried out on the different bark samples with the extraction yields shown in table 4. This single extraction step removed most of the polar extractives (as determined via successive soxhlet extractions, table 1). These ethanol/water extracts were then analyzed for their total phenolic, flavonoid and tannin content, as well as for their antioxidant capacity, as shown in table 4.

The total phenolic content is highest in red oak and larch bark extracts, *viz.* 579 and 573 mg GAE/g extract, respectively. Corsican pine and black locust bark extracts have the lowest phenolic content (265.2 and 286.5 mg GAE/g extract). Flavonoid content is highest in larch and willow (390.9 and 310 mg CE/g extract) and lowest in black locust (177.3 mg CE/g extract). Condensed tannin content varies strongly between bark species with larch presenting the highest values (669.9 mg CE/g extract) and black locust the lowest (88.4 mg CE/g extract). Red oak and Corsican pine barks have similar condensed tannin content despite red oak having twice as many phenolics, meaning that the majority of phenolics in red oak do not originate from condensed tannins. Indeed, oak barks tend to contain substantial amounts of hydrolysable tannins, explaining their high phenolic but lower condensed tannin content.[115]

Despite being important to know and understand the characteristics of the extracts obtained, from a biorefinery point of view it is also important to observe their content on dry bark basis, therefore taking into account not only the extracts composition but also their extraction yield. Although different extraction processes and conditions might alter the extraction yield and product composition, the values obtained here can serve as a point of reference.

On a dry bark basis, larch has the highest total phenolic, flavonoids and condensed tannins of the six barks studied. Besides having the high extraction yield (26.1 wt%), the extracts obtained are also enriched in phenolic molecules. The abundance of condensed tannins present in this bark (168 mg CE/g bark) shows an interesting potential to be sourced for condensed tannins.[116] Willow presented the second highest total phenolic content on a dry bark basis (102.6 mg GAE/g bark), which could be linked to anatomical observation of phenolic deposits in the cell lumens of the rhytidome of willow bark (Fig. S6.1 B D; Fig. S6.2A). Comparing the results of red oak and black locust bark with a previous report on the same bark species, the differences are striking. The extraction yields (17.3 and 9.5 wt%, respectively) are much higher than those presented by Sillero *et al.* (3.2 and 3.1 wt%), and the total phenolic content of these extracts was higher in this study (579 and 286.5 mg GAE/g extract) compared to those previously reported (276.5 and 178.1 mg GAE/g extract).[89] In this study, the total amount of phenolics extracted, on a dry bark basis, is thus over 11 times higher for red oak and almost 5 times higher for black locust compared to Sillero *et al.*, highlighting the formidable intra-species differences that will likely be influenced by tree age and health condition, and edaphoclimatic environment among other factors.[14, 89] Comparing the results obtained in this study with previous results of barks from different tree species (*Eucalyptus globulus*, *Picea abies*, *Acacia melanoxylon* and *Acacia dealbata*) shows that the total phenolics and condensed tannins on dry bark basis largely fall within the same range, and the inter-species variability is high.[49]

The antioxidant activity of the ethanol/water bark extracts was screened using the FRAP assay [73] and the DPPH inhibition assay.[72] The FRAP antioxidant activity for all barks fell within a 500-1200 mg TE/g extract interval, with red oak showing the highest and black locust the lowest value. By using trolox (a synthetic antioxidant analog to vitamin E) as standard, it was verified that the extracts possess very good capacity to counter a metal ion oxidation,

with the lowest results showing half the trolox antioxidant activity, while red oak extract gave even higher antioxidant activity than this known standard. The values reported here are higher than those reported by Sillero *et al.* for six different barks species (146.1 - 640.3 mg TE/g extract).[89] As a point of reference, Neiva *et al.* reported antioxidant activities within the range of 300 - 1300 mg TE/g extract for their ethanol and water extracts of four different barks.[49]

Free radical scavenging capacity was analyzed *via* the DPPH inhibition assay.[72] The results are expressed as IC₅₀ values, which represents the concentration of extract solution needed to achieve 50% DPPH inhibition. Since this IC₅₀ is dependent on the concentration of the DPPH solution used in the test, the antioxidant activity index (AAI) was calculated as well.[72] According to the definitions used by Scherer *et al.*,[72] all bark extracts displayed very strong antioxidant activity (AAI > 2). Red oak and larch have the highest AAI (5.5 and 5.2, respectively) whereas black locust has the lowest AAI (2.2). The results are comparable to the AAI obtained for eucalyptus and spruce barks (both 3.7) [66, 68], but are still lower than known antioxidant compounds such as trolox (6.9) and gallic acid (23.4). The AAI of trolox and gallic acid, measured here, are in good agreement with those previously reported by Luís *et al.* (6.62 and 22.77 respectively) and Neiva *et al.* (6.7 and 23.6), indicating the robustness of the analytical procedure.[49, 66, 117]

Table 4: Chemical analysis (phenolics, flavonoids and condensed tannins) and antioxidant activities (FRAP and DPPH) of the ethanol/water extracts of different bark species. Extraction conditions: 1 g bark, 10 mL ethanol/water (1:1), 1 h, sonication at 50°C. Gallic acid, catechin and Trolox were used as standard in the different assays. Results are therefore expressed in GAE (gallic acid equivalent), CE (catechin equivalent) and TE (trolox equivalent). *IC₅₀ = extract concentration required for 50% DPPH inhibition; Trolox IC₅₀ = 3.4 mg/mL and gallic acid IC₅₀ = 1.0 mg/mL. **AAI (antioxidant activity index) = final DPPH concentration/IC₅₀; Trolox AAI = 6.9 and gallic acid AAI = 23.4.

	Poplar	Red oak	Black locust	Willow	Corsican pine	Larch
Extraction yield (wt%)	16.8	17.3	9.5	23.8	11.1	26.1
Phenolics						
mg GAE / g extract	369.9 ± 2.9	579 ± 20	286.5 ± 3.2	477.9 ± 19.8	265.2 ± 3.1	573 ± 16.1
mg GAE / g bark	56 ± 0.4	91.9 ± 3.2	25.3 ± 0.3	102.6 ± 4.2	27.9 ± 0.3	143.7 ± 4
Flavonoids						
mg CE / g extract	294.8 ± 20.7	238.9 ± 4.9	177.3 ± 1.4	310 ± 7.4	253.6 ± 3.6	390.9 ± 12.6
mg CE / g bark	44.6 ± 3.1	37.9 ± 0.8	15.6 ± 0.1	66.6 ± 1.6	26.7 ± 0.4	98 ± 3.2
Tannins						
mg CE / g extract	140.1 ± 1.6	354.8 ± 5	88.4 ± 0.5	608.4 ± 4.2	363.2 ± 3	669.9 ± 13.4
mg CE / g bark	21.2 ± 0.2	56.3 ± 0.8	7.8 ± 0	130.7 ± 0.9	38.2 ± 0.3	168 ± 3.4
FRAP						
mg TE / g extract	626.8 ± 11	1208.6 ± 18.8	500.5 ± 11.3	921.2 ± 23.5	677.1 ± 1.4	840.5 ± 37.9
mg TE / g bark	94.9 ± 1.7	191.9 ± 3	44.2 ± 1	197.8 ± 5.1	71.3 ± 0.1	210.8 ± 9.5
mg GAE / g extract	141.3 ± 2.5	272 ± 4.2	112.8 ± 2.5	207.3 ± 5.3	152.7 ± 0.3	189.2 ± 8.5
mg GAE / g bark	21.4 ± 0.4	43.2 ± 0.7	10 ± 0.2	44.5 ± 1.1	16.1 ± 0	47.4 ± 2.1
DPPH						
IC ₅₀ (mg/L)*	8.7	4.2	10.4	6.5	7.3	4.5
AAI**	2.7	5.5	2.2	3.6	3.2	5.2

Valorization potential

A large variety of compounds can be found in barks - with a profound diversity between different species - as illustrated in the preceding sections, thereby leading to a potential chemical valorization of barks. Several valorization opportunities and processing flows can be envisioned based on the specific chemical and structural

features of the targeted barks. Some of them are briefly discussed here, although a thorough evaluation of these opportunities falls outside the scope of this paper.

The chemical composition of these barks revealed a high content of extractives (14-30 wt%) which constitutes a prime valorization step. In the lipophilic extractives that can be extracted using apolar solvents, like DCM or light naphtha, resin acids are potentially interesting as they can be extracted in relatively large quantity from larch and Corsican pine bark and could provide an alternative to the tall oil produced as by-product from coniferous wood in the Kraft process.[118] In poplar bark, the tentatively identified monoflavonoid (7-O-methyl taxifolin) that was observed in a large proportion, might have potentially interesting bioactive properties. Also the alkyl hydroxycinnamates found in poplar and black locust bark might be used in view of their anticancer activity.[112]

The polar extractives, extracted via a single ethanol/water extraction, also possess interesting properties. As their yield is much higher (up to 26 wt% for larch), valorization may be easier. For instance, the extracts of red oak, willow and larch are especially rich in phenolic compounds, potentially having antimicrobial properties.[49, 119] Alternatively, these phenolic moieties also impart excellent antioxidant activities - even comparable to trolox - to these extracts. One could thus envision them being used as antioxidant in applications such as lubricants or rubbers.

Suberin content was low in most of these barks, although for black locust the proportion (10.3 wt%, table 1) might advise the study of a chemical valorization of the fatty acids and alcohols *e.g.* after an alkaline depolymerization. In all cases the barks after fractionation of extractives are a lignocellulosic material that may undergo further thermal or chemical processing.

Conclusion

The barks of six relevant tree species in Europe were anatomically and chemically characterized in view of their potential use in a biorefinery. Anatomical characterization showed the fundamental cell types and their arrangement in phloem, periderm and rhytidome, and the distinction among them was quite clear. Barks with highly lignified cells were found in *R. pseudoacacia*, *P. canadensis* and *Salix sp.* (*e.g.* fibers in tangential bands), *Q. rubra* (*e.g.* abundant clusters of sclereids) or *L. decidua* (*e.g.* fibrosclereids). In contrast, fibers were absent in *P. nigra*. *R. pseudoacacia* bark contained a conspicuous amount of phellem cells in the periderms. Abundant crystalliferous parenchyma was reported for *Salix sp.*, *Q. rubra* and *R. pseudoacacia*. Phenolic deposits were common in all barks in phloem parenchyma cells and sclereids and especially in the rhytidome (*e.g.* *Salix sp.* or *P. nigra*). Resin ducts were observed in both gymnosperms.

Chemical characterization highlighted the differences in composition between the different bark species. All barks present a high extractive content (14 to 30 wt%) with larch and willow bark being the richest in extractives. Black locust bark contains a substantial amount of suberin, in line with its substantial proportion of suberized phellem cells in the periderms, making it an interesting resource for suberin valorization. The lignin content ranges from 22 to 29 wt% for all barks, except for Corsican pine (45 wt%). Pyrolysis results showed that hardwood bark lignin is much richer in G than in S units, with low S/G ratios. This implies a more condensed lignin structure in hardwood barks compared to their woods, pointing to a lower susceptibility towards depolymerization. The content of H units was high in the bark lignins (5-15%). The barks had a low holocellulose content (34.6 to 44.5 wt%) and the main hemicellulosic monosaccharides were xylose in hardwood and mannose in softwood barks. The low polysaccharide content suggests that a possible biorefinery process should focus primarily on the extractives and lignin fractions for these barks.

The lipophilic extracts contained predominantly fatty acids in hardwood barks, and resin acids in softwood barks. Next to these compounds, triterpenoids also constituted an important class of the lipophilic extractives in all barks.

The polar extracts of the barks presented a high inter-species variability with larch bark showing the highest phenolics, flavonoids and condensed tannins content, suggesting its use as a resource for the targeted extraction and valorization of these compounds. All bark extracts exhibited strong antioxidant activities, especially red oak, larch and willow bark extracts, making them interesting additives for industrial antioxidant applications.

Overall, the results demonstrate the strong anatomical and chemical variability between barks of different species. This suggests that biorefining processes will need to be tailored for the valorization of specific bark streams and that the product outcome in a biorefinery will be strongly dependent on the choice of the bark feedstock.

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