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Cellular structure and chemical composition of cork from *Plathymenia reticulata* occurring in the Brazilian Cerrado

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ABSTRACT

Herein is described for the first time the chemical composition and cellular structure of *Plathymenia reticulata* cork from the *cerrado* in Brazil. The cork constitutes an outer layer around the stem with deep longitudinal fissures. The cells are mostly hexagonal prisms arranged in radial rows with a prism height and cell-wall thickness of 20.6 μm and 1.2 μm (earlycork) and 12.2 μm and 1.4 μm (latecork). The chemical composition is: 0.8% ash, 12.7% extractives, 24.7% suberin, 34.5% lignin and polysaccharides 20.9% (glucose 10.2%, xylose 3.7%, arabinose 2.6%, galactose 1.6%, mannose 1.6%, acetyl groups 0.5%, uronic acids 0.7%). The lipophilic extractives include mainly saturated fatty acids (31.6% of the extractives), terpenoids/terpenes (28.0%), mainly lupeol (11.3%), β-amyrin (4.9%) and taraxerone (5.9%). The sterol β-sitosterol (4.8%) was also identified in interesting amounts (4.8%). Suberin monomers include mainly saturated ω-hydroxyacids (40.7% of the total compounds), saturated α,ω-diacids (21.4%), as well as substituted α,ω-diacids (8.7%), alkanoic acids (8.4%), substituted ω-hydroxyacids (8.0%). The differences in relation to *Quercus suber* cork are discussed. The characteristics of *P. reticulata* cork allows considering usages similar to those of *Q. suber*. Further the extraction of sterols and triterpenoid compounds may be included in a valorisation framework for *P. reticulata* cork.

1. Introduction

The barks of some tree species contain a substantial proportion of cork in their periderms that enhances their protective role e.g. as an insulator against high temperatures or fire, a barrier to water loss or against biotic attacks. Cork has a particular set of properties, including low density, hydrophobicity, low thermal conductivity, fire resistance, elastic behavior, chemical and biological inertia, that are the result of its cellular and chemical characteristics (Pereira, 2015). Cork is most known as the material obtained from *Quercus suber* (cork oak) from a sustainable process based on periodical extractions, and used for the production of wine stoppers as well as of insulation and surfacing products (Pereira, 2007). Cork is an industrial raw-material of considerable economic importance for the main production regions and therefore other potential sources of cork i.e. other tree species are under investigation.

In this context, several tree species from the *cerrado* regions that have thick corky barks may be considered as potential cork providers. The *cerrado* is a floristically diverse savanna that covers more than 2 million km^2 of Central Brazil and parts of Bolivia and Paraguay (Pennington et al., 2009), and is highlighted as a global hotspot for biodiversity conservation with more than 10,000 plant species, of which 44% are endemic (Simon et al., 2009). *Cerrado* has a pronounced dry season and supports a unique array of drought- and fire-adapted plant species. It was estimated that more than 20 species in *cerrado* could supply cork, e.g. *Kielmeyera coriacea* (Rios et al., 2011) or *Erythroxylum tortuosum* (Alonso and Machado, 2008).

Plathymenia reticulata Benth. (Fabaceae) is one of the species with a corky bark. It is a neotropical tree native to South America and one of the interesting species from *cerrado*, commonly known as *vinhático* due to the wine-red stem color (Silva, 2005), and occurring in the Atlantic forest (rainforest) and in *cerrado* (characterized by savanna vegetation). Although the trees can reach 15–30 m and over 70 cm stem diameter, they are smaller in the *cerrado* e.g. 6–12 m and 30–50 cm diameter, and have a thick bark (Lorenzi, 2002). *P. reticulata* is a source of high quality wood used frequently

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in high-end carpentry as is also useful in restoring deforested areas, contributing to an economically and sustainable economy (Della Torre et al., 2011). Although the bark features of *P. reticulata* were evaluated for taxonomic purposes (Costa et al., 1997), there are no references to the cork cellular structure, chemical composition or even a possible industrial application.

The properties of cork result from its combined structural and chemical features, and any consideration of a potential use has to rely on this specific knowledge (Pereira, 2015). Cork has been extensively studied in the cork oak (*Quercus suber)*, and its structure, chemistry and properties were reviewed in a reference book (Pereira, 2007). Cork in other barks was also studied for a few species e.g. *Quercus cerris* (Şen et al., 2010, 2011), *Quercus variabilis* (Miranda et al., 2013a), *Betula pendula* (Miranda et al., 2013b; Pinto et al., 2009), *Kielmeyera coriacea* (Rios et al., 2011, 2014), *Pseudotsuga menziesii* (Graça and Pereira, 2000; Ferreira et al., 2015a,b; Cardoso et al., 2016).

In this study, the cork of *Plathymenia reticulata* was analyzed in a chemical composition and cellular perspective, emphasizing the summative composition, the monomeric composition of suberin, and of lipophilic extractives, as well as the geometry and dimensions of the cork cells, and their three-dimensional arrangement. This is the first time that *P. reticulata* cork is characterized. The underlying objective of considering its adequacy for cork applications is the prospect of increasing the potential revenues for the *cerrado* region.

2. Material and methods

2.1. Samples

Bark samples were collected at 1.30 m of tree height from six *Plathymenia reticulata* trees, in the Alvação estate, Coração de Jesus, Minas Gerais, Brazil (16°41′07″ S, 44° 21′54″W). The area is a *sensu stricto cerrado*, with a climate of type Aw according to Köppen, with well-defined dry and rainy seasons. The trees were 17–23 years old, with 12–17 cm stem diameter and 4–6 m height.

The cork and phloem portions of the bark were separated manually, and the cork layer was kept for the anatomical and chemical studies.

2.2. Optical microscopy

The bark samples were impregnated with DP 1500 polyethylene glycol. Transversal, tangential and radial sections of approximately 17 μm thickness were prepared with a Leica SM 2400 microtome using Tesafilm 106/4106 adhesive for sample retrieval (Quilhó et al., 1999). The sections were stained with a triple staining of chrysodine/acridine red and astra blue and mounted on Eukitt. Light microscopic observations were made using Leica DM LA and photomicrographs were taken with a Nikon Microphot-FXA.

2.3. Scanning electron microscopy

Small cubes of cork with approximately 5 mm of edge were cut with a sharp razor blade. The cubes were mounted on stubs (ProSciTech, Australia) and sputter coated (Polaron E 5100 E, USA) with gold palladium for 3 min at 20 mA with their faces oriented so that the observation surface corresponded to transverse, radial and tangential sections. The surfaces were observed with a scanning electron microscope (SEM) Hitachi S-2400 at magnifications ranging from 50 to 1000, and the images were recorded in digital format.

The cell measurements were made on the images using image analysis software (Leica Qwin Plus) on approximately 900 cells (Şen et al., 2011). On the tangential sections, the number of edges of each cell was counted and the average cell area was calculated. On the transverse sections ring width was measured and the following measurements were made separately on earlycork and latecork cells: number of edges of each cell, radial and tangential dimensions of the lumen of each cell, radial width of the growth ring.

The radial and tangential cell wall thickness was calculated as (cell dimension−lumen dimension)/2.

The distribution function of the number of edges of each cell was calculated for the tangential and the non-tangential (radial and transverse) sections as:

$$fi = \frac{Ni}{\sum Ni}$$

where N_i represents the number of cells with i edges. The dispersion of the function in relation to the mean was calculated as

$$u^2 = \sum (i-6)^2 fi$$

Considering the average dimensions of the cellular units, it can be calculated how much of the cork volume is occupied by the solid. The individual cell is taken as a hexagonal prism, and the solid volume V_s as the difference between total volume V and the empty (lumen) volume V_0, as given by:

$$V = 3\sqrt{3}/2 l^2 h$$

$$V_o = 3\sqrt{3}/2(l\text{-}e/\sqrt{3})^2(h\text{-}e)$$

$$V_s = 3\sqrt{3}/2 l^2 h - 3\sqrt{3}/2(l\text{-}e/\sqrt{3})^2(h\text{-}e)$$

with l as the base edge, h the prism height and e the wall thickness (Pereira 2007). The solid fraction in the cork is calculated in percent volume.

2.4. Chemical analyses

The separated cork was ground in a Retsch SK hammer mill, sieved and the 40–60 mesh fractions were kept for analysis. Chemical summative analyses included determination of ash, extractives soluble in dichloromethane, ethanol and water, suberin, klason and acid soluble lignin, and the monomeric composition of polysaccharides, including determination of neutral sugars, uronic acids and acetates.

Ash content was determined according to TAPPI Standard T 15 os-58 using 2.0 g of material that were incinerated at 525 °C overnight and the residues weighed.

The extraction was performed in a Soxtec extractor during 1.5 h with each solvent, successively with dichloromethane, ethanol and water; the solvents were recovered and the extractives content determined from the mass of the solid residue after drying at 105 °C, and reported as a percentage of the original samples.

Suberin content was determined in the extractive-free material by use of methanolysis for depolymerisation (Pereira, 1988). A 1.5 g sample of extractive-free material was refluxed with 100 ml of a 3% methanolic solution of $NaOCH_3$ in CH_3OH during 3 h. The sample was filtrated and washed with methanol. The filtrate and the residue were refluxed with 100 ml CH_3OH for 15 min and filtrated again. The combined filtrates were acidified to pH 6 with 2 M H_2SO_4 and evaporated to dryness. The residues were suspended in 50 ml water and the products recovered with dichloromethane in three successive extractions, each with 50 ml of dichloromethane. The combined extracts were dried over anhydrous Na_2SO_4, and the solvent was evaporated to dryness. The suberin extracts, that include the fatty acid and fatty alcohol monomers of suberin, were quantified gravimetrically, and the results expressed in percent of the initial dry mass.