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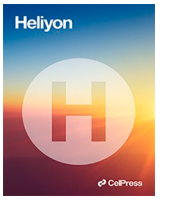

Cork cellular and chemical features underlying bark environmental protection in the miombo species *Parinari curatellifolia*

Abílio Santos Malengue [a,b], Isabel Miranda [a], Rita Simões [a], Ana Lourenço [a], Jorge Gominho [a], Helena Pereira [a,*]

[a] Centro de Estudos Florestais, Laboratório Associado Terra, Instituto Superior de Agronomia, Universidade de Lisboa, Portugal
[b] Faculdade de Ciências Agrarias da Universidade José Eduardo dos Santos, Huambo, Angola

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ABSTRACT

Parinari curatellifolia is an important evergreen tree from the Miombo woodland of south-central and eastern Africa. The bark is corky, suggesting an increased protection against the ecosystem high temperatures and drought conditions as well as against wild fires. The cork in the bark rhytidome of *P. curatellifolia* was analyzed here for the first time with a focus on chemical and cellular features. *P. curatellifolia* cork has the cellular characteristics of cork tissues, with typical honeycomb structure in the tangential section and a brick-wall layer in the transverse and radial sections, without intercellular voids. Chemically *P. curatellifolia* cork has 8.4 % extractives, 33.9 % suberin, 31.9 % lignin and 25.2 % polysaccharides of the cork. The hemicelluloses are mostly xylans, with a substantial proportion of arabinose and galactose. Suberin showed a proportion of long chain lipids to glycerol (LCLip:Gly, mass ratio) of 8.5, and the long chain monomeric composition included a similar proportion of α,ω-diacids and ω-hydroxy acids (35.4 % and 31.5 % of long chain monomers) with a substantial proportion of monoacids (19.4 % of long chain monomers). Lignin is a guaiacyl-syringyl lignin with S/G of 0.32 and H:G:S of 1:14.1:4.5. The rhytidome composition and the cellular and chemical features of its cork are in line with environment-targeted protective features namely as a transpiration and insulation barrier, and as an increased fire protection.

1. Introduction

Tree barks are complex cellular tissues at structural and chemical levels that result from the activity of two meristems, the cambium and the phellogen, which generate phloem and periderm, respectively, surrounding stem and branches. Bark support important functions e.g. in tree physiology regarding the photosynthates downward flow from the canopy, and the control of radial gas and water transport, as well as in protection against biotic and abiotic attacks arising from the surrounding environment. Bark also contributes to the mechanical support of the tree.

Bark periderms, and especially cork-rich periderms, are barriers to the environment and therefore play a major role in protection of the trees. The periderm contains cork cells, or phellem, which is some species are produced in very substantial amounts by a long-lived

* Corresponding author.
E-mail address: hpereira@isa.ulisboa.pt (H. Pereira).

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phellogen e.g. in *Quercus suber* [1]. More frequently, the phellogen is short-lived and several phellogens differentiate during the tree's life, therefore leading to the presence of sequential periderms that are separated by layers of non-functional phloem. Together they constitute the system known in plant anatomy as rhytidome (Fig. 1). In some species, these peridems contain substantial cork layers e.g. in *Quercus cerris* and *Pseudotsuga menziesii* [2,3]. The presence of cork enhances the protective role of tree barks, namely by providing increased insulation capacity against high temperatures or fire, and by setting a barrier to water loss or against biotic attacks due to a specific set of properties, that result from the cork cellular and chemical characteristics [4].

The cork from the cork oak (*Quercus suber)* has been extensively studied due to its commercial importance, as reviewed by Pereira [5]. In recent years, more information has been gathered on corks from other species from temperate and tropical regions, aiming both at a potential use of the cork material as well as at understanding the enhanced protection given by cork in adverse tree growth conditions [6].

This is the case of the miombo ecosystem, the most extensive tropical seasonal woodland and dry forest formation in Africa [7]. Miombo covers about 2.7 million km^2 (about 10 % of the African continent) in seven countries from Angola in the west to Tanzania in the east, and from southern Congo down to the northern edge of South Africa [8]. The ecosystem is characterized by a continuous herbaceous cover, which mostly consists of heliophilous C4 grasses and sedges with a clear seasonality associated to water stress [9]. Miombo has a pronounced dry season, and the climate semi-aridity is the main edaphic determinant [10]. The region has a mean annual temperature range of 15–20 °C, annual rainfall of 55–1200 mm and length of dry season of 3–7 months [7]. Fire has a critical impact in determining tree cover, and may convert miombo woodland to grassland, even if most mature trees and woody plants are fire resistant [10,11]. In this context, trees may adapt by developing thick corky barks that may offer increased protection towards environmental conditions. A similar situation occurs in the Brazilian cerrado that supports several drought- and fire-adapted plant species that develop cork-rich barks [6].

Parinari curatellifolia (Chrysobalanaceae), with the common name of uchia, is one of the miombo species with a corky bark (Fig. 2A and B), growing in tropical Africa from Senegal to Kenya with the highest concentration in Zimbabwe and the lowveld region of South Africa [12]. It is an evergreen tree growing up to 20 m with a distinct mushroom shape and a rough and corky bark [13]. Very little is known on the growth and physiology of this species, and nothing was yet reported on the characteristics of its wood or bark. *P. curatellifolia* is at present used in traditional medicine for the treatment or easing of symptoms of many diseases or ailments, for instance cancer, pneumonia, fever, malaria, typhoid, hypertension, microbial infections, pain, anti-inflammation, and toothaches [14,15]. The fruits are very rich in vitamin C, protein and calcium, with a sweet and strong pineapple smell, and are used as food, particularly during times of potential household food insecurity, and also to produce an alcoholic drink [12,13]. The tree is also used by rural communities for fuelwood [16].

This study focus on the cork fraction within the bark rhytidome of *Parinari curatellifolia.* The cork was carefully separated, and analyzed in relation to its chemical composition, including the monomeric analysis of the cell wall structural components, and to its cellular features, namely the cell structural arrangement and geometry. Beyond being a first time report of the cork features of *P. curatellifolia*, this study aims at discussing the features that potentiate the species protective adaptation to the miombo ecosystem. The results will also enlarge the overall knowledge on corks of different species, giving an insight on their chemical and structural variability in tree barks.

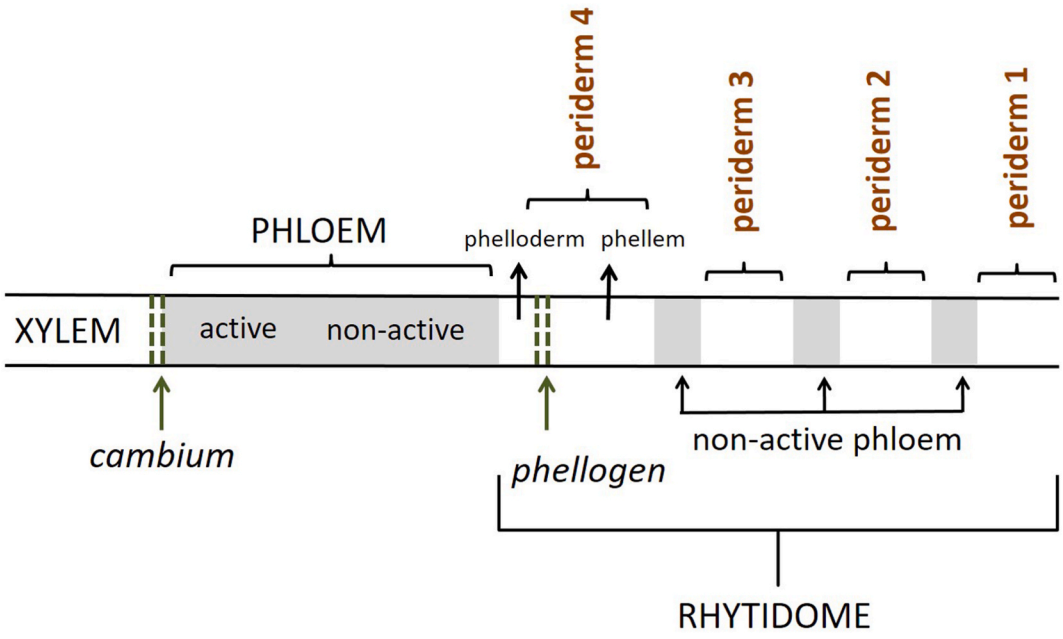

Fig. 1. Schematic representation of a tree bark with a cork-rich rhytidome, showing the different successive periderm layers.

Fig. 2. Photographs of one mature *Parinari curatellifolia* tree in the miombo of Angola (A) and of its stem showing the external appearance of the bark (B).

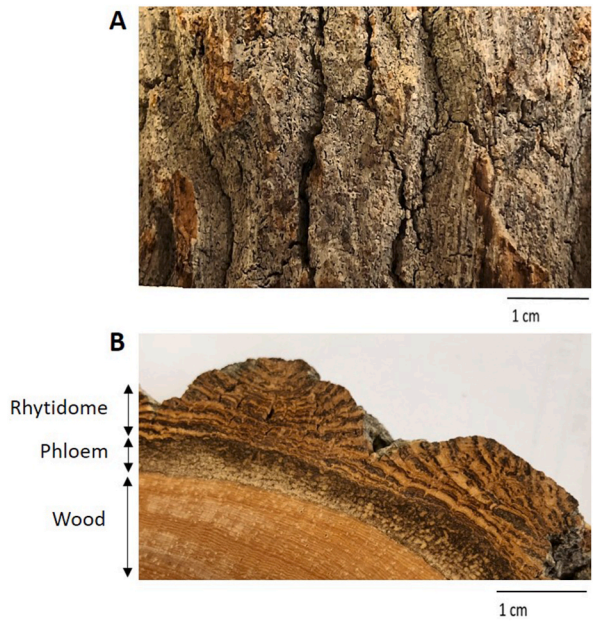

Fig. 3. Macroscopic aspect of the bark of *Parinari curatellifolia*: A) external appearance of the rhytidome showing longitudinal fractures; B) cross-section of a stem disc, showing the wood, phloem and the rhytidome with successive periderms with the cork layers highlighted by a lighter brown colour.

2. Material and methods

2.1. Samples

Bark samples were collected from stem discs taken at approximately 1.30 m of tree height from three *Parinari curatellifolia* trees, growing in the community of Cachindongo, Province of Huambo, Angola (13°33.216′S, 15°36.956′E, altitude 1582 m). The area is a miombo ecosystem, with a mean annual temperature of 20 °C and annual rainfall ranging from 1200 to 1600 mm. The trees were on average 13 years old, with 12.83 cm stem diameter at breast height (1.30 m above ground).

The bark, constituted by the phloem and the rhytidome, was separated manually from the wood, and the rhytidome was subsequently cut out from the phloem. The rhytidome showed longitudinal fractures on the external surface. In cross-section, the rhytidome was clearly visually distinguished from the underlying phloem and showed conspicuous cork layers of a light brown color separated by darker colored phloem layers (Fig. 3).

Rhytidome samples were prepared by cutting small pieces approximately as 1 cm^3 cubes for observation of transverse, tangential and radial sections of the cork layers by scanning electron microscopy (SEM).

For chemical analysis, rhytidome pieces were triturated, and the granules of cork and phloem separated by density and visual observation. The procedure involved a coarse trituration with a knife mill to a particle size of approximately 2 mm, a separation of the lower density cork particles by air blowing, and a visual observation with the help of a magnified glass to sort out the cork granules; the granules still containing cork and phloem portions were further triturated and the procedure repeated to sort out the pure cork granules. The pure cork granules obtained from each tree were combined and stored for chemical analysis.

2.2. Scanning electron microscopy

The small samples of rhytidome were cut with a sharp bisturi blade, and observed in scanning electron microscopy (SEM) Hitachi TM 3030 Plus Tabletop at 5 kV acceleration, 30 Pa vacuum, Mix observation mode, without sample surface metallization, at magnifications ranging from 50 to 1000 in sections oriented to correspond to transverse, radial and tangential sections. The number of edges of each cell was counted for 500 cells in each section, and the distribution function of the number of edges of each cell was calculated as well as the dispersion of the function in relation to the mean. The average cell area corresponding to the average cell prism base was calculated on the tangential sections based on the image area and the number of cells. The cell prism height was measured on the non-tangential sections in the radial direction from prism cell base to cell base. A total of 500 cells were measured for each section. The cell wall thickness was measured in highly magnified photographs. The individual cork cell volume was calculated by taking the individual cell as a prism and using the mean values for base area and prism height; this value was used to calculate the average number of cells per unit volume. A detailed description of cork cell biometric calculations is given in Ref. [5].

2.3. Chemical analyses

The separated cork particles were ground in a Retsch SK knife mill, sieved, and the 40–60 mesh fractions were kept for analysis. Chemical summative analyses included determination of ash, soluble extractives 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.

The ash content was determined after incineration at 525 °C [17]. The soluble extractible compounds were determined in a Soxhlet apparatus with a solvent sequence of dichloromethane, ethanol and water during 6 h, 16 h and 16 h (adapted from Ref. [18].

Suberin content was determined in the extractive-free material by use of methanolysis with sodium methoxide in absolute methanol [19]. The suberin extracts after pH adjustment to 6 were concentrated, suspended in water and extracted with dichloromethane in successive liquid-liquid extractions. The dichloromethane extracts were kept for determination of the long chain monomeric composition of suberin, and the water phase for quantification of glycerol. The suberin content of cork was determined as the solid mass loss after methanolysis and expressed in percent of the initial dry mass.

Klason and acid-soluble lignin, and carbohydrates contents were determined on the extracted and desuberinised materials by acid hydrolysis with 72 % sulphuric acid [20,21].

The polysaccharides content was determined by quantifying the total monosaccharide monomers released by the acid hydrolysis used for lignin determination using a Dionex ICS-3000 system in HPIC-PAD and an Aminotrap plus Carbopac PA10 column (250 × 4 mm) for monosaccharides and uronic acids, and by HIPCE-UV using a Waters 600 system with a Biorad Aminex 87H column (300 × 7.8 mm) for acetic acid. In the conditions used, mannose was eluted with xylose.

2.4. Suberin monomeric composition

The monomeric composition of suberin was determined in aliquots from the dichloromethane extracts from the suberin depolymerization. The samples were evaporated under N_2, derivatized by trimethysilylation to trimethylsilylate all the hydroxyl and carboxyl groups into trimethylsilyl (TMS) ethers and esters, respectively, and immediately analyzed by GC-MS Agilent 5973 MSD with the following conditions: Zebron 7HG-G015-02 column (Phenomenex, Torrance, CA, USA) (30 m, 0.25 mm; ID, 0.1 μm film thickness), flow 1 mL/min, injector 380 °C, oven temperature program: 50 °C (held 1 min), rate 10 °C/min up to 150 °C, rate of 5 °C/min up to 200 °C, 4 °C/min up to 300 °C, and rate of 10 °C/min up to 380 °C (held 5 min). The MS source was kept at 220 °C and the electron

impact mass spectra (EIMS) taken at 70 eV of energy. The area of peaks in the total ion chromatograms of the GC–MS analysis was integrated and their relative proportions expressed as percentage for semi-quantitative analysis. Compounds were identified as TMS derivatives by comparing their mass spectra with a GC–MS spectral library (Wiley, NIST), and by comparing their fragmentation profiles with published data.

The glycerol released by suberin depolymerization was quantified in the aqueous layer obtained from the liquid–liquid separation of the solubilised compounds by suberin depolymerization using high-performance liquid chromatography (HPLC) with a Dionex ICS-3000 system equipped with an electrochemical detector (Sunnyvale, CA, USA), with Aminotrap plus CarboPac SA10 anionexchange columns (Thermo Scientific, Waltham, MA, USA) and a mobile phase of an aqueous 2-nM sodium hydroxide (NaOH) solution at a flow rate of 1.0 mL/min at 25 °C.

2.5. Lignin monomeric composition

The extractive-free and desuberinised cork samples were milled in a Retsch MM200 for 10 min, and dried under vacuum at 35 °C overnight. An amount of 0.10 mg was weighted and pyrolysed at 550 °C (for 1 min) in a platinum coil Pyroprobe connected to a CDS 5150 valved interface linked to the GC-MS with a fused-silica capillary column (ZB-1701: 60 m × 0.25 mm i.d. X 0.25 μm film

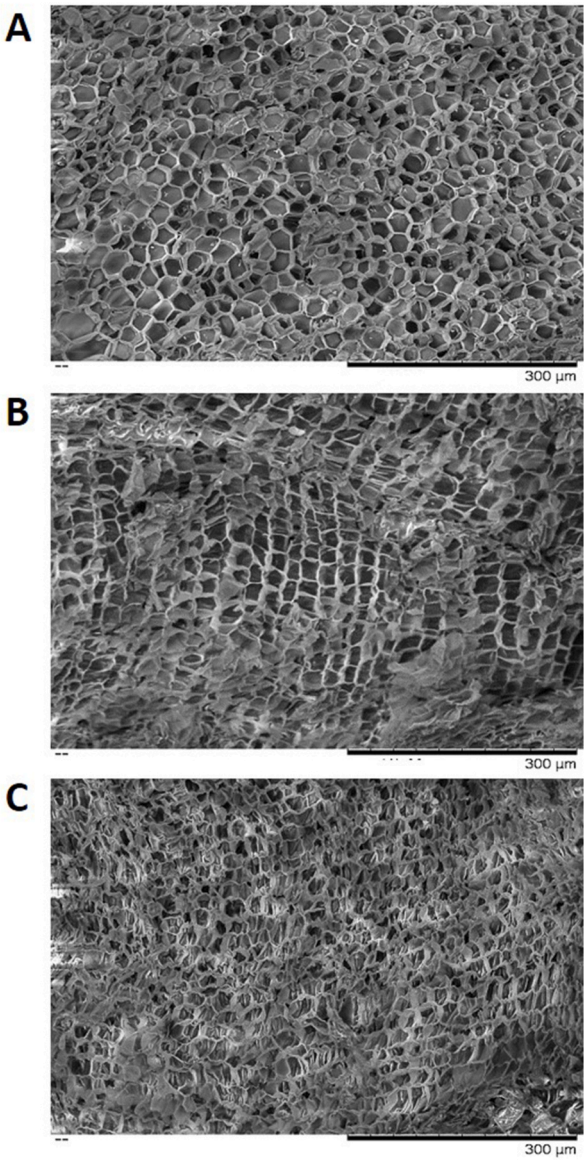

Fig. 4. Scanning electron microscopic photographs of the cork in the rhytidome of *Parinari curatellifolia* in the tangential (A), transverse (B) and radial (C) sections.

thickness) working under the following chromatographic conditions with helium flow of 1 mL/min: 40 °C (4 min), increased to 100 °C at 20 °C/min, and to 270 °C at 6 °C/min (held for 5 min); injector at 270 °C and MS interface at 280 °C; electron ionization energy 70 eV. The compounds were identified comparing their mass spectra with Wiley, NIST2014 database and literature. The percentage area of each compound identified was calculated using the total area of the chromatogram. The peaks were assigned to their initial compound origin (i.e. polysaccharides and lignin) and to the lignin precursors (*p*-hydroxyphenol, guaiacyl and syringyl units). The monomeric composition of lignin was calculated based on the total area of peaks identified as originated from H, G, and S units. The ratio S/G and the relation H:G:S were calculated from these values.

2.6. FTIR spectroscopy

One aliquot of the *P. curatellifolia* cork sample was ground in a ball mill and dried overnight at 40 °C in a vacuum oven. The pellet was prepared with 200 mg of KBr and 1.50 mg of the sample powder and molded into a 13 mm-diameter disc with a hydraulic press (10-ton load). The FTIR-spectra were acquired in a PerkinElmer Spectrum 1000 with 4 cm^{-1} resolution, and 32 scans were taken.

3. Results

3.1. Bark and rhytidome structure

The bark of *Parinari curatellifolia* had an external corky appearance with deep fractures that run lengthways along the stem (Fig. 3A). The stem cross-section showed a clearly distinct bark with a thick layer of phloem that is externally covered by a substantial rhytidome consisting of multiple periderms. The periderms were characterized by conspicuous successive layers of cork with a light brown color and a thickness of about 1 mm, that were interspersed by darker colored phloem layers (Fig. 3B). In the observed tree cross-sections, the rhytidome contained an average of six periderms which suggests a phellogen longevity of approximately 2–3 years given the tree age estimated by the number of wood rings.

3.2. Cell structure, topology and dimensions

The structural features of the cork in the bark of *Parinari curatellifolia* were observed by scanning electron microscopy in tangential, transverse and radial sections (Figs. 4–6). In all the sections, the cells make up a two-dimensional network of edges and vertices,

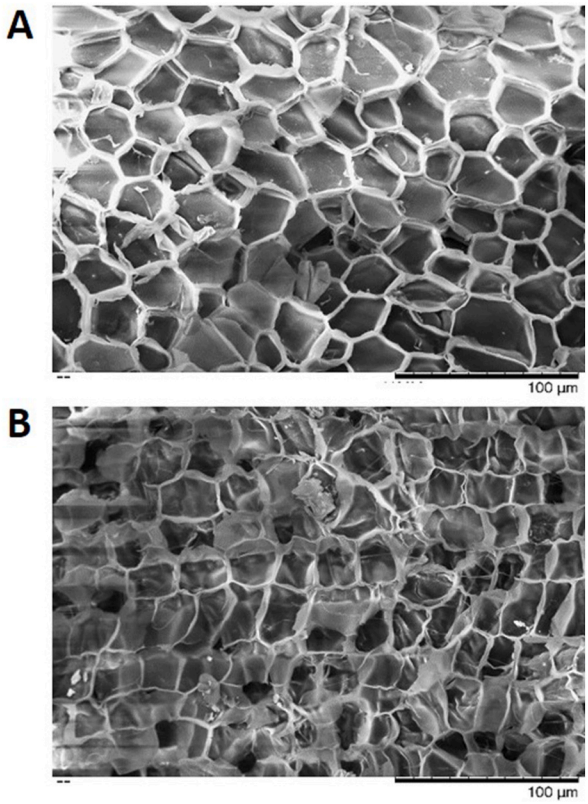

Fig. 5. Cellular dimensional diversity and organization of the cork in the rhytidome of *Parinari curatellifolia*: A) tangential section; B) radial section.

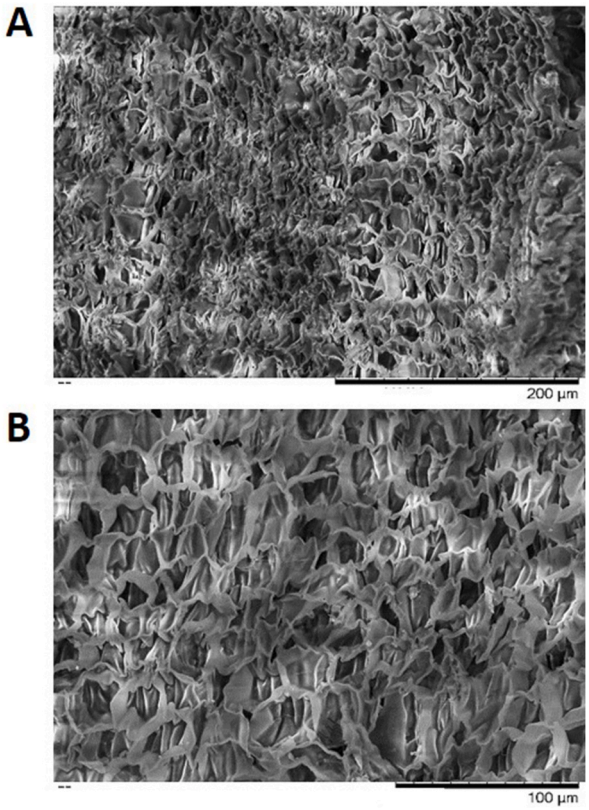

Fig. 6. Cellular corrugation and collapse of the cork in the rhytidome of *Parinari curatellifolia* in the radial section: A) heterogeneity of cell corrugation; B) cell wall undulation and distortion of the cell rows radial arrangement.

arranged without gaps or intercellular voids. In the tangential section, the cork cells appear as polygons with a honeycomb arrangement (Fig. 4 A). About half of the cells have six sides, with five-and seven-sided polygons making up most of the other cases (Table 1). The radial and transverse sections show a similar cellular structure, with approximately rectangular cells that are aligned in parallel rows with a brick wall appearance (Fig. 4 B–C). Their topological description shows similarity to that of the tangential section (Table 1), with an average number of cell sides of six and five (Table 1) but with a higher proportion of 5-sided cells distribution in the non-tangential sections (36.7 % vs. 23.4 %).

The cork cells are therefore rectangular prisms, stacked base to base, and aligned in radial rows. The cell dimensions i.e. the mean prism dimensions are summarized in Table 2. On average, the prism height of cork cells was 27.7 µm, the prism base area 527 μm^2, corresponding to a base edge of 15.6 µm (assuming a hexagonal shape) and an approximate cell wall thickness of 1.0–1.1 µm measured in the tangential section. Overall there was a large variation in cell dimensions and topology, as exemplified in Fig. 5A for the tangential section and Fig. 5B for the radial section.

The cells showed frequent corrugations, especially of the lateral prism walls, that decreased substantially the cell dimension in the radial direction, in some cases nearing cell collapse, accompanied by distortion of the radial cellular rows. Fig. 6 exemplifies these aspects of cell corrugation heterogeneity (Fig. 6A) and distortion of the cell radial rows (Fig. 6B). No lenticular channels were observed in the studied samples.

Table 1
Distribution and dispersion of the number of sides in the cork cells in the rhytidome of *Parinari curatefolia* in the transverse, radial and tangential sections.

Number of edges	Tangential section	Transverse section	Radial section
4	0.058	0.157	0.086
5	0.234	0.386	0.348
6	0.496	0.421	0.493
7	0.212	0.074	0.072
μ_2	0.678	0.774	0.764

Table 2
Dimensions of the cork cells in the rhytidome of *Parinari curatellifolia;* for comparison, the range of cell dimensions of *Quercus suber* reproduction cork is also given [5].

	Parinari curatellifolia	*Quercus suber*
Prism height, μm	27.7	30–40
Prism base edge, μm	15.6	13–15
Average base area, cm^2	5.27×10^{-6}	4–6 x 10^{-6}
Total cell volume, cm^3	1.46×10^{-8}	1–3 x 10^{-8}
Number of cells per cm^3	6.85×10^{7}	4–7 x 10^{7}

3.3. Cork chemical composition

The summative chemical composition of the cork fraction of *Parinari curatellifolia* bark rhytidome is shown in Table 3, including the monomeric composition of polysaccharides regarding neutral monosaccharides and acetyl groups. Ash content was low and extractives amounted to 8.4 % of the cork, of which 38 % are compounds soluble in dichloromethane and 62 % are polar compounds solubilised by ethanol and water. As regards the structural cell wall components, the cork contained a considerable amount of suberin and lignin of 33.9 % and 31.9 % of the cork, respectively, while polysaccharides accounted for 25.2 %. The composition of the cork polysaccharides was dominated by glucose (with 54.7 % of the total) and xylose (31.9 %); arabinose and galactose amounted to 7.6 % and 3.7 % of total monosaccharides, respectively, while acetyl groups represented 0.5 %; no uronic acids were detected.

The FTIR spectrum of the extractive-free cork from the bark rhytidome of *P. curatellifolia* is shown in Fig. 7, where for easier comparison the spectrum of *Q. suber* cork is also included. The cork spectra of both species are overall similar but showing some small differences. The spectra are dominated by the absorbance bands of suberin, with two peaks at approximately 2925 and 2851 cm^{-1} corresponding to the asymmetric and symmetric vibration, respectively, of C–H in the olephinic chains, and a peak at 1737 cm^{-1} corresponding to the carbonyl C═O in aliphatic esters. The band at 1161 cm^{-1} can also be attributed to the contribution of C–O–C of ester groups in suberin. At 1636 $cm^{-1}$and 724 cm^{-1} occurs the absorbance of C═C in midchain of suberin.

The peaks at approximately 1510 cm^{-1}, 1466 cm^{-1} and 1267 cm^{-1} are typical for lignin. The peaks at 1094 cm^{-1} and 1035 cm^{-1} correspond to the C–O bond and are characteristic of polysaccharides.

3.4. Suberin composition

The fragments released upon suberin depolymerization from *P. curatellifolia* cork include the lipid monomers soluble in dichloromethane and the water soluble glycerol that was quantified in the aqueous fraction of the methanolysis reaction solution. The compounds solubilised by methanolysis, in mass % of cork, grouped by chemical families, are presented in Table 4. The major component of suberin depolymerisates are α,ω-diacids and ω-hydroxyacids that together accounted for about 64 % of all monomers released (22.8 % of cork) followed by carboxylic acids (6.5 % of cork). Glycerol amounts to 9 % of all monomers released (3.5 % of cork).

The monomeric composition of suberin of *Parinari curatellifolia* cork, based in peak areas of the GC-MS ion chromatograms of the organic phase of the acidified methanolysates, are summarized in Table 5. This composition includes the long chain aliphatic

Table 3
Chemical composition (% of dry mass) and monomeric composition of polysaccharides (% of total monomeric units) of cork in the bark rhytidome *Parinari curatellifolia* trees, as mean and standard deviation of three trees.

Chemical composition	% of dry mass
Ash	0.63 ± 0.11
Total extractives	8.41 ± 0.90
DCM extractives	3.19 ± 1.00
EtOH extractives	1.26 ± 0.05
H_2O extractives	3.95 ± 0.06
Suberin	33.87 ± 3.10
Total lignin	31.93 ± 1.30
Klason lignin	31.36 ± 1.20
Soluble lignin	0.56 ± 0.05
Polysaccharides*	25.18 ± 1.30
Polysaccharides composition, % of total monosaccharides	
Glucose	54.70 ± 1.70
Rhamnose	0.43 ± 0.05
Galactose	3.69 ± 0.49
Xylose	31.88 ± 0.74
Arabinose	7.58 ± 0.05
Acetyl groups	0.44 ± 0.02

*calculated by difference.

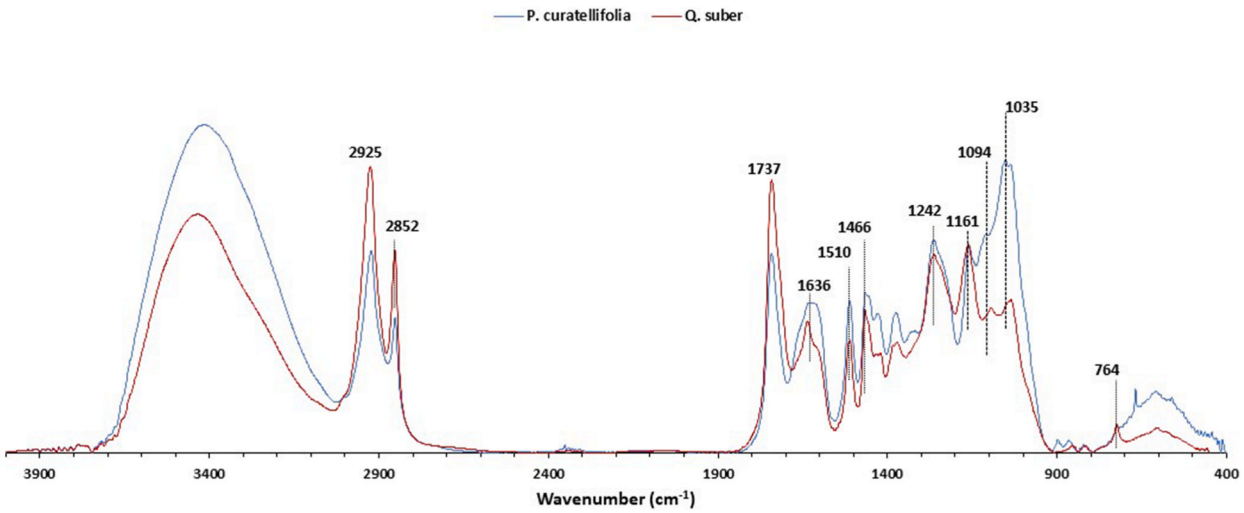

Fig. 7. FTIR spectra of extractive-free cork from the bark rhytidome of *Parinari curatellifolia* and of extractive-free cork from *Quercus suber*.

Table 4
Suberin composition (in mass percent of cork) including glycerol, long chain aliphatic compounds and aromatics solubilised by methanolysis, of cork in the bark rhytidome of *Parinari curatellifolia* trees, as mean and standard deviation of three trees.

Chemical family	mass % of cork
Glycerol	3.50 ± 0.49
Alkanoic acids	6.53 ± 0.21
ω-Hydroxyalkanoic acids	10.91 ± 1.77
α, ω-Alkanoic diacids	11.95 ± 1.24
Alkanols	1.32 ± 0.24
Glycerol derivatives	0.50 ± 0.04
Aromatic compounds	0.72 ± 0.07

compounds and aromatics that were soluble in the organic phase; therefore, it does not include the glycerol content that is shown in Table 4. The main components are α,ω-diacids accounting to 35.4 % of total monomers (16.8 % saturated α,ω-diacids and 18.6 % unsaturated and substituted α,ω-diacids), followed by ω-hydroxyacids that amount to 32.1 % of total monomers (12.3 % saturated hydroxyacids and 19.2 % substituted hydroxyacids) while alkanoic acids corresponded to 19.4 % of the monomers. In smaller amounts, alkanols (4.4 %), glycerides derivatives (1.5 %) and aromatics (2.4 %) were also identified.

Octadec-9-enedioic acid was the major α,ω-diacid (16.4 % of all compounds). The ω-hydroxyacids were mostly midchain-functionalised with hydroxyl groups, mainly 18-hydroxy-9-octadecenoic acid (19.1 % of all compounds); the major non-functionalised hydroxyacids were 16-hydroxyhexadecanoic acid (4.6 %) and 22-hydroxydocosanoic acid (5.0 %). Among alkanoic acids the most abundant was octadecanoic acid (10.9 %), and also docosanoic and tetracosanoic acids (4.2 and 3.6 % of all compounds respectively). Ferulic acid was present (2.3 %) together with minor amounts of other aromatic molecules.

3.5. Monomeric composition of lignin

The lignin composition determined based on the pyrolysed compounds of the extractive- and suberin-free cork of *P. curatellifolia* bark rhytidome is given in Table 6. The detailed composition regarding the lignin-assigned compounds is given in Table ASup, as supplementary material. Lignin is rich in guaiacyl units (71.7 % of the lignin), including also syringyl units (23.2 %) and a low proportion of *p*-hydroxyphenyl units (5.1 %). The S/G ratio was 0.32 and the H:G:S relation 1:14.1:4.5. The main lignin compounds that were identified in the pyrogram (Table ASup) were 4-vinylguaiacol, guaiacol, *trans*-isoeugenol, syringol, 4-methylguaiacol, 4-ethylguaiacol, 4-vinylsyringol, vanillin, syringylacetone, and *trans*-coniferaldehyde.

4. Discussion

The cork in the rhytidome of *Parinari curatellifolia* bark was analyzed here for the first time with focus on a chemical and cellular perspective. In the following discussion, comparison of *P. curatellifolia* cork features with those of corks from other species is made, especially with *Quercus suber* cork. Overall the features are similar to those already reported, and the differences that were found

Table 5
Suberin composition of the cork fraction of *Parinari curatellifolia* bark rhytidome, in % of the chromatographic peak areas of the compounds detected by GC-MS. Mean of three trees and standard deviation.

Chemical class and compounds	%
Alkanols	**4.37 ± 0.46**
1-Octadecanol	0.02 ± 0.01
1,22-Docosanediol	0.91 ± 0.27
1-Tetracosanol	3.44 ± 0.19
Alkanoic acids	**19.40 ± 2.15**
Octadecanoic acid	10.87 ± 1.76
2-Hydroxytetradecanoic acid, methyl ester	0.76 ± 0.04
Docosanoic acid	4.15 ± 0.35
Tetracosanoic acid	3.63 ± 0.09
ω – Hydroxyacids saturated (as methly ester)	**12.29 ± 0.87**
16-Hydroxyhexadecanoic acid	4.57 ± 0.26
20-Hydroxyeicosanoic acid	0.19 ± 0.01
22-Hydroxydocosanoic acid	5.02 ± 1.09
24-Hydroxytetracosanoic acid	2.52 ± 0.23
ω – Hydroxyacids substitued (as methyl ester)	**19.17 ± 1.87**
18-Hydroxy-9-octadecenoic acid	19.13 ± 1.98
9,10,18-Trihydroxyoctadecanoic acid	0.64 ± 0.12
α, ω – Diacids saturated (as dimethyl ester)	**16.75 ± 1.03**
Octanedioic acid	0.08 ± 0.01
Nonanedioic acid	0.04 ± 0.00
Hexadecanedioic acid	9.38 ± 0.49
Octadecanedioic acid	0.51 ± 0.02
Eicosanedioic acid	0.08 ± 0.01
Docosanedioic acid	4.84 ± 1.03
Triacontanedioic acid	1.81 ± 0.46
α, ω – Diacids substituted (as dimethyl ester)	**18.62 ± 2.25**
Octadec-9-enedioic acid	16.44 ± 2.10
9,10-Dihydroxyoctadecanedioic acid	2.17 ± 0.21
Phenolics	**2.41 ± 0.39**
Vanillin	0.05 ± 0.00
3,4-Dihydroxybenzoic acid	0.02 ± 0.01
Ferulic acid + isomer	2.28 ± 0.40
Alkyl ferulate esters	0.06 ± 0.01
Glycerol derivatives	**1.49 ± 0.06**
Glycerol-2-docosanoic acid	1.49 ± 0.06
Unidentified	**4.94**

Table 6
Monomeric composition of lignin (S, G and H units) as determined by analytical pyrolysis in the extractive- and suberin-free cork in the bark rhytidome of *Parinari curatellifolia* bark, in % of the chromatographic peak areas of the lignin-assigned compounds detected by GC-MS. Mean of three trees and standard deviation.

Monomeric composition % of lignin	
Syringyl units	23.2 ± 0.7
Guaiacyl units	71.7 ± 0.2
p-Hydroxyphenyl units	5.1 ± 0.4
Ratios	
S/G	0.32 ± 0.01
H:G:S	1:14.1:4.5

mostly relate to the natural species variability that has been reported [6].

4.1. Cellular features

Parinari curatellifolia bark has a short-lived phellogen and therefore a rhytidome is formed with the cork layers of the successive periderms interspersed with phloem (Fig. 3). The specific insulation properties of cork certainly play a protective role in this bark, namely regarding high temperatures and drought. This follows the schematic representation shown in Fig. 1, and is similar to what happens in most cork-rich barks that form rhytidomes e.g. *Quercus cerris* or *Pseudotsuga menziesii*, while a long-lived periderm leading

to a single layer of cork surrounding the stem is less frequent e.g. *Quercus suber* and *Quercus variabilis* [6].

Overall the cellular arrangement of *Parinari curatellifolia* cork shows a typical 3D-structure (Fig. 4) with radially aligned cells in parallel rows with a compact arrangement without intercellular voids, clearly similar to that of *Q. suber* cork, and resulting from its biological formation process [1,22].

This cellular structure of cork, and its topology mostly with 6-sided cells in each section (Table 1), has been reported for the corks from several species that have been studied: *Quercus suber* [22], *Quercus cerris* [2], *Quercus variabilis* [23], *Pseudotsuga menziesii* [3], *Kielmeyera coriacea* [24] and *Plathymenia reticulata* [25]. The average cell dimensions of *Parinari curatellifolia* cork regarding cell prism height and base edge, and cell wall thickness (Table 2) are also in the range of those already reported, mostly on their lower dimensional range.

The radial arrangement was somewhat disturbed in some regions with the radial rows of cells showing some curvature, while cell wall undulations were intense in some cases (Fig. 6). This is the result of the compression forces that the new cork layers undergo when they are pressed against the hard lignocellulosic phloem layer during cell formation and expansion in the radial direction from inside to the outside (Fig. 3). A similar occurrence appears in the rhytidome of *Quercus cerris* and *Pseudotsuga menziesii*, while in the latter substantial portions of the cork were collapsed [2,3].

4.2. Chemical composition

The distinctive chemical feature of cork is the presence of suberin as a structural cell wall component, a polymer of ester linked long chain acids with glycerol that is the chemical signature of cork [4]. Lignin is the second most important structural cork cell wall component but this component is not specific to cork and is present in most secondary cellular plant tissues. Together these macromolecular components of cork participate in determining its properties, for which their ratio is an important feature [4]. Consequently, the characteristics of cork of *P. curatellifolia* will relate mostly to the combination and ratio of these two polymers.

The cork of *P. curatellifolia* bark is chemically dominated by the presence of suberin and lignin in almost the same proportion (33.9 % and 31.9 % respectively), leading to a suberin-to-lignin ratio of 1.0. The suberin and lignin contents of *P. curatellifolia* cork are within the range found in *Q. suber* cork, but their ratio is lower than the mean value of 2.0 for *Q. suber*, suggesting that suberization occurred to a lesser extent than in the cork cells of *Q. suber* [26]. This cork suberin content is well within the range found in cork of other species, e.g., *Quercus variabilis* (39.2 %, suberin:lignin ratio of 1.4 [23]), *Quercus cerris* (28.5 %, suberin:lignin ratio of 1.0 [27]), *Pseudotsuga menziesii* (36.2 %, suberin:lignin ratio of 2.2 [28]), and *Betula pendula* (32.2 %, suberin:lignin ratio of 2.5 [29]). However, the cork cells of *P. curatellifolia* have much higher suberin than cork from species from the Brazilian cerrado *Kielmeyera coriacea* (16.1–30.3 %, suberin:lignin ratio of 0.4 [24]) and *Plathymenia reticulata* (24.7 %, suberin:lignin ratio of 0.7 [25]). Overall, the suberization level of the cork cell walls from *P. curatelifolia* resembles more that of *Q. cerris* cork.

Polysaccharides represent a smaller fraction of the cork cell wall structural components (25.2 %, Table 3). Hemicelluloses are mostly xylans, with a substantial amount of arabinose and galactose, including acetyl substitutions, but without the presence of uronic acids. The estimation of cellulose and hemicelluloses content based on the carbohydrate composition and the assumption that all the glucose is from cellulose, results in 13.8 % and 11.4 % respectively. This composition is similar to that reported for other corks e.g. *Quercus suber* (glucose, xylose and arabinose 41–51 %, 32–34 % and 6–13 % of neutral sugars respectively [19]), *Quercus variabilis* (51.7 % glucose, 27.9 % xylose, 10.5 % arabinose [30]), *Quercus cerris* (49.7 % glucose, 27.9 % xylose, 11.5 % arabinose [27]), *Betula pendula* (41.7 % glucose, 19.1 % xylose, 26.3 % arabinose [29]), *Plathymenia reticulata*, (49 % glucose, 18 % xylose [25]), and *Pseudotsuga menziesii* (55.4 % glucose, 13.3 % xylose, 10.9 % arabinose [28]).

The cork of *Parinati curatellifolia* has less extractives (8.4 %, Table 3) than most of the other corks analyzed, e.g. 14–17 % for *Q. suber* [26], 9.6 % for *Q. variabilis* [23], 16.7 % for *Q. cerris* [27], 32.2 % for *Betula pendula* [29], 15–23 % for *Kielmeyera coriacea* [24], 12.7 % for *Plathymenia reticulata* [25], and 29.2 % for *Pseudotsuga menziesii* [28].

4.3. Suberin composition

The suberin composition of *Parinati curatellifolia* cork (Table 4) is in line with the polyester-linked macromolecule of glycerol to long chain lipid monomers that is characteristic of cork cell walls [5]. The FTIR spectrum (Fig. 7) shows the typical peaks that are the fingerprint of suberin, mainly the 2925 cm^{-1} and 2852 cm^{-1} peaks (of the CH_2 in the long-chain aliphatic chains), and the 1737 cm^{-1} peak of C═O in the ester linkages. These are typical suberin markers that are found in corks of other species e.g. in *Quercus variabilis* [23], *Quercus cerris* [31] and *Pseudotsuga menziesii* [32].

Glycerol content corresponded to 3.5 % of cork and long chain acids and glycerides to 29.9 % of cork (Table 4). The relative proportion of long chain lipids and glycerol (LCLip:Gly), is reported as an important parameter for the material's properties since it relates to the spatial development of the macromolecule [26]. The LCLip:Gly was found to be 8.5 (mass ratio). This is in the lower range of values reported for this ratio for *Quercus suber* cork of 8.2–14.5 [26] or above the 5.7 calculated from Ref. [33].

The suberin composition (Tables 4 in mass % of cork, and Table 5 in mass % of the organic soluble suberinic compounds) shows a similar proportion of α,ω-diacids and ω-hydroxy acids (12.0 % and 10.9 % of cork respectively, or 35.4 % and 31.5 % of long chain monomers) with also a substantial proportion of monoacids (6.5 % of cork or 19.4 % of long chain monomers). These results are in line with the suberin composition of other species as regards the presence of the main chemical families, although their proportion varies largely with species [6]. For instance, in *Q. suber* cork, suberin has 36.8 % α,ω-diacids and 26.3 % ω-hydroxyacids, and only 1.1 % of carboxylic acids [34], in *Q. variabilis* suberin has ω-hydroxyalkanoic acids (55.5 %) and α,ω-alkanoic diacids (34.4 %) with a low content of alkanoic acids of 3.5 % [30], and in *Q. cerris* suberin has ω-hydroxyalkanoic acids (85.9 %) and α,ω-alkanoic diacids (7.4 %)

with only 1.8 % of alkanoic acids [27]. The cork of *Plathymenia reticulata* is also mainly constituted by ω-hydroxyalkanoic acids (48.7 %) and α,ω-alkanoic diacids (30.1 %) but with a relevant alkanoic acid proportion of 10.5 % [25].

The proportion of the chemical families is different in corks of other species regarding the ratio of diacids and hydroxyacids, or of the monoacid proportion. For instance, in *B. pendula*, ω-hydroxyalkanoic acids constitute 70.6 % and α,ω-alkanoic diacids 23.9 % of all the compounds, with only 0.2 % alkanoic acids [29], in *Pseudotsuga menziesii*, α,ω-diacids represent 39.9 %, ω-hydroxyacids 12.5 %, and alkanoic acids 6.2 % [28], and in *Kielmeyeria coriacea* 49.8 % ω-hydroxyacids, 17.9 % α,ω-diacids and 1.3 % alkanoic acids [24]. A substantial compositional difference of suberin was also reported for the cork of the monocot *Beaucarnea recurvata* with 20.7 % α,ω-diacids, 15.2 % ω-hydroxyacids and 22.5 % alkanoic acids [35].

This comparative analysis of suberin composition clearly shows that the proportion of the three chemical families of long chain monomers i.e. diacids, hydroxyacids, and acids, varies between species and therefore may translate onto different 3-D assembly of the suberin polymer.

4.4. Monomeric composition of lignin

The lignin in the *P. curatellifolia* cork samples (Table 6) has a preponderance of G-units (71.7 % of the three precursors), but with a relatively large proportion of S-units (S/G 0.32), and can therefore be considered a GS-lignin. The FTIR spectrum of the extractive-free cork (Fig. 7) also corroborates the importance of S units and the GS character given the relative intensities of the absorbances at 1607 cm^{-1}, 1510 cm^{-1}, and 1466 cm^{-1} [36]. In fact, in GS lignins the peak at 1466 cm^{-1} corresponding to S ring breathing increases in relation to the 1510 cm^{-1} corresponding to G ring breathing. The difference in relation to the G lignin of *Q. suber* cork is clear when both spectra are compared (Fig. 7). Most studies on cork lignin composition have been made for *Q. suber* and research on isolated lignin (milled cork lignin) reported an almost total composition of G-units with 95.4 % of the three lignin precursors (S/G 0.03 [37]) or 90.9 % (S/G 0.10 [38]), while reports on the analytical pyrolysis directly on the extractive-free cork refer a proportion of G-units of 65.6 % of the three precursors (S/G 0.12 [39]). Lignin composition for extractive-free corks of other species was reported for *Betula pendula* and *Quercus cerris,* showing that the monomeric proportion varies with species: G-unit content of 95.2 % (S/G 0.03) for *Q. cerris* cork, and 85.8 % (S/G 0.14) for *B. pendula* [40].

The pyrolysis of cork presents some specificities in relation to that of wood, namely in the temperature-dependence of the results, because of the presence of suberin [41]. To avoid the inclusion in the pyrograms of the suberin aliphatic-derived compunds, the present Py-analysis was made on desuberinized cork. A previous Py-TMAH study on cork and on desuberinised cork reported a G-unit proportion of 93.3 % and 88.8 %, respectively (S/G of 0.04 and 0.09, respectively) and suggested that overestimation of S-units may occur in desuberinised cork due to inclusion of gallic/ellagic acids [42]. However this was not the case here, as the composition of the pyrolytic products show (Table ASup).

Therefore the present results on *P. curatellifolia* cork reinforce the overall species' variation of the cork lignin composition.

4.5. Potential environmental of cork features

The cellular characteristics of cork are key factors for providing insulation and impermeability properties, which are important for commercial applications, as it is the case in *Q. suber* cork products, as well as for protection against biotic and abiotic stress, namely in environmental harsh conditions [4,5]. The most important features are the regular cellular arrangement, the closed cell structure, without intercellular voids or cell wall openings, and the small dimensions of the cells that give the material a very low heat transfer due to the small solid fraction and low thermal conductivity of the air enclosed in the cells, while convection is eliminated. These features were found here in the cork of *Parinati curatellifolia* (Figs. 4–6, Tables 1 and 2) and therefore contribute to establish this bark as a barrier with enhanced insulation properties in the hot season of the miombo.

The chemical features of the cork cell wall also confer appreciable heat resistance given the thermal stability of suberin and the small content in polysaccharides that are more heat sensitive [31,43]. Even at elevated temperatures, the cellular structure is maintained which facilitates the insulating performance. This is particularly important in case of fire to keep an insulation layer that preserves the cellular features of the cork layers in the bark. Wild fires are recurring in the miombo and for the preservation of the woodland characteristics it is important to have fire resistant trees [11].

The permeation of vapors and liquids through cork is also an important characteristic since it is related to water vapor loss by transpiration and water evaporation, aspects that are of high relevance in drought prone regions or seasons, as it is the case of the miombo. The permeability to gases, vapors and liquids of cork from *Q. suber* has been studied and reported to be very low, and shown to be related to its anatomical features (namely the cell wall plasmodesmata) in conjunction with the cell's dimensions [44] while the permeation of vapors and liquids is associated with the cell wall chemical composition and topochemistry [45,46]. In the dry miombo conditions, the cork layers of *P. curatellifolia* will enhance the protective functions of the bark against water loss by transpiration and evaporation.

5. Conclusions

The bark structure of *Parinari curatellifolia* includes a rhytidome with substantial cork layers in successive periderms that provides an enhanced protection to heat and drought. The cellular and chemical characteristics of cork are key factors for providing insulation and impermeability, which are important for protection in environmental harsh conditions, as it is the case in the miombo ecosystem.

The cork of *Parinari curatellifolia* was studied here for the first time regarding cellular and chemical features. Overall it resembles

corks from other species, although species variation occur both regarding cell dimensions and topology, as well as in chemical summative composition and in specific monomeric composition of suberin and lignin.

The results presented here increase the existing knowledge on corks, thereby enlarging the insight into this bark-component protective barrier.

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Author contribution statement

Helena Pereira: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Abílio Santos Malengue; Isabel Miranda; Rita Simões; Ana Lourenço; Jorge Gominho: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. Material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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