Bark Characterisation of the Brazilian Hardwood *Goupia glabra* in Terms of Its Valorisation

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The bark of *Goupia glabra* trees grown in a native forest area in the Amazon region of Brazil was anatomically and chemically characterised for potential use as a chemical source for bio-refineries. The bark is silvery-grey to reddish-grey, with a scaly rhytidome composed of 2 to 3 periderms with a small phellem content. The phloem has abundant sieve tube members and a conspicuous presence of sclerified nodules of fiber-sclereids or sclereids; no fibers were observed. The bark had the following average composition (dry mass): 5.2% ash, 24.6% total extractives, 1.1% suberin, and 43.8% total lignin. The polysaccharide composition showed a high ratio of xylan hemicelluloses to cellulose. The ethanol-water bark extract showed high antioxidant capacity. The chemical characterisation of different granulometric fractions showed that extractives were present preferentially in the finest fractions, particularly with enrichment in ethanol solution.

Keywords: Goupia glabra; Bark; Anatomy; Chemical composition; Fractionation; Extractives

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INTRODUCTION

Goupia glabra Aublet (family Celastraceae) is a tropical tree species that produces high-value solid wood for the international timber market (Berni *et al.* 1979). *G. glabra* is native to tropical South America and is widespread in Brazil, Guyana, Colombia, Venezuela, Peru, and Suriname (Loureiro *et al.* 1979; Schwengber and Smiderle 2005). In Brazil, the species occurs in the Amazonian regions of Acre, Amapá, Amazonas, Mato Grosso, Pará, and Rodônia (Oliveira *et al.* 2005; Gurgel *et al.* 2015) and is commonly known as "cupiúba," "cachaceiro," "peniqueiro," or "peroba-rosa" (Souza *et al.* 2002).

G. glabra is a fast-growing and large evergreen or semi-deciduous tree that can reach 50 m in height and more than 2 m in diameter. The wood is heavy and hard, with suitable mechanical strength and good workability, and it may be used for high-quality sawn wood (Oliveira *et al.* 2005; Sales *et al.* 2011; Kubitzki 2014; Gurgel *et al.* 2015). This species is also harvested for local use in medicinal purposes using different parts of the plant *e.g.* flowers and leaves (Roth and Lindorf 2002). The bark is traditionally applied for toothache soothing, to treat chickenpox and eczema, and for the treatment of malaria (DeFilipps *et al.* 2004).

Bark has gained increasing attention as a potential substrate for the production of fuel, chemicals, and bio-materials, particularly within a bio-refinery platform (*e.g.*, Le Normand *et al.* 2014). Barks are rich in chemicals with pharmaceutical and bioactive

compounds, green polymers, and bio-based materials (Conde *et al.* 1996; Pietarinen *et al.* 2006; Sen *et al.* 2010; Valentín *et al.* 2010). However, bark valorisation requires knowledge of structural and chemical characteristics that are complex and different from those of wood (Fengel and Wegener 1984; Pereira *et al.* 2003). Only a small number of species have been studied from this perspective, mostly those of temperate regions (Miranda *et al.* 2012, 2013; Ferreira *et al.* 2015) or of tropical regions with high commercial exploitation (Baptista *et al.* 2013; Miranda *et al*. 2016).

Little information is available on the bark of *G. glabra*. The bark anatomy in the Celastraceae family, which is very heterogeneous because it includes lianas, shrubs, and trees (Schweingruber *et al.* 2011), has been reported for various genera (Archer and van Wyk 1993; Qi and Gao 1994); Roth (1981) made anatomical observations of the bark of some species, including *G. glabra*. No information has been found for the chemical composition of *G. glabra* bark.

This study investigates the valorisation of *G. glabra* bark, thereby contributing to the sustainable exploitation of tropical forests, particularly in the Amazon region. It aims to provide chemical and structural knowledge that can support its potential use as a chemical source within a biorefinery route. One goal is also to analyse the extraction of potentially bioactive and functional compounds, *e.g.*, phenolics with high antioxidant activity.

EXPERIMENTAL

Sampling

Bark samples were collected from *G. glabra* trees in the native forest area of the Amazon region, in the Itaúba municipality of the north of Mato Grosso State, Brazil. Three trees were randomly selected from those legally harvested, *i.e.*, under the Brazilian legislation for Amazonian low-impact forest exploitation. The sampled trees had the following over-bark diameters at breast height and age, respectively: 52.5 cm and 158 years, 71.9 cm and 161 years, and 95.2 cm and 141 years. A 10-cm-thick stem disc was taken at the base, and the bark was manually separated.

Anatomical Characterisation

Bark samples were impregnated with DP1500 polyethylene glycol (AGROS New Jersey, USA), and transverse and longitudinal sections of approximately 17-μm thickness were cut with a Leica SM 2400 (Leica Biosystems, Nussloch, Germany) microtome using TesaFilm 106/4106 (Beiersdorf AG, Hamburg, Germany) (Quilhó *et al.* 1999). The sections were stained with chrysodine/astra blue and mounted on Kaiser glycerin. After 24 h, the sections were submerged in xylol, dehydrated in 96% and 100% ethanol, and mounted in Eukitt.

Individual specimens were taken from the cambium towards the periphery and macerated in a 1:1 solution of 30% H_2O_2 and CH_3COOH at 60 °C for 48 h and stained with astra blue. A light microscope (Leica DM LA) (Leica Microsystems, Wetzlar Germany) was used; photomicrographs were taken with a digital camera (Leica DFC 320) (Leica Microsystems Imaging Solutions, Cambridge, UK), and image acquisition was performed with Leica software Qwin V3.5.0 (Leica Microsystems Imaging Solutions, Cambridge, UK). The terminology followed that of Junikka (1994) and Richter *et al.* (1996).

Bark Density

Bark basic density (ρ_p) was estimated using the water immersion method by determining the green saturated volume and the oven-dry weight (TAPPI 258 om-02, 2002).

Fractionation of Bark

The fractionation procedure was made using a composite sample including the barks of the three trees. The barks were fractionated using a cutting mill SM 2000 (Retsch, Haan, Germany) with a 10 mm x 10 mm output sieve, and particle size distribution was determined according to ASAE S319.3. The granulometric fractioning was made using a vibratory sieving apparatus AS 200 basic (Retsch, Haan, Germany) with U.S. standard wire sieves numbers 10, 15, 20, 40, 60, and 80 (opening sizes: 2.00, 1.00, 0.850, 0.425, 0.250, and 0.180 mm, respectively) with a 10-min shaking time. The mass retained on each sieve was weighed, and the corresponding mass fraction yields were determined. Granulometric analysis was repeated three times.

Chemical Characterisation

Chemical summative analysis was made on the 40 to 60-mesh (0.250 to 0.450 mm) particle size fraction, and included the determination of ash, extractives, suberin, Klason and acid soluble lignin, and the monomeric composition of polysaccharides. The composition was reported in terms of percentage of oven-dry mass. The granulometric fractions with particles of size less than 0.180 mm and more than 2 mm, corresponding to fine (F) and coarse (C) particles were also analysed. The coarse fraction was ground prior to chemical analysis to obtain particles that passed through the 40-mesh sieve.

The ash content was determined according to TAPPI 211 om-93 (1993). The extractives content was determined gravimetrically after successive Soxhlet extraction by dichloromethane (6 h), ethanol (16 h), and hot water (16 h). Suberin content was determined in the extractive-free material by use of methanolysis for depolymerisation according to the method described by Pereira (1988). The lignin content was determined on the extracted and desuberinised material as Klason lignin (TAPPI 222 om-02 (2002)) and acid-soluble lignin by UV-absorbance at 205 nm (TAPPI UM 250 (1991)).

The polysaccharides were calculated based on the amount of neutral sugar monomers released in the hydrolysate obtained for lignin determination. The neutral monosaccharides were quantified by high-performance anion exchange chromatography (HPAEC, Dionex ICS-3000 Sunnyvale, CA, USA, equipped with an electrochemical detector) using Aminotrap plus CarboPac SA10 anion-exchange columns.

Ash Composition

The ash content determined by combustion in a muffle furnace at 500 °C was analysed for macro- and micro-element concentrations. The ash was dissolved in HCl, and the concentrations of Ca, Mg, Fe, Cu, Mn, Zn, Na, and K were determined by atomic absorption spectrophotometry in a Pye Unicam SP-9 apparatus (Cambridge, UK) equipped with a GF95 graphite furnace.

Phenolic Content of Bark Extract

The extraction of bioactive compounds was carried out with ethanol/water (50/50, v/v) with a solid-liquid ratio of 1:10 (m/v) for 60 min at 50 °C using an ultrasonic bath.

The total phenolics content of the ethanol/water extract was estimated according to the Folin–Ciocalteu method using gallic acid as a standard (Singleton and Rossi 1965; Miranda *et al.* 2016). Total flavonoids were quantified by an aluminium chloride colorimetric assay, and the results were expressed as mg of (+)-catechin equivalents on a dry extract base (Jia *et al.* 1999; Miranda *et al.* 2016). Tannin content was determined by the vanillin-H_2SO_4 method, and the results were expressed as mg of (+)-catechin equivalents on a dry extract base (Abdalla *et al.* 2014; Miranda *et al.* 2016).

Antioxidant Activity of Bark Extract

The antioxidant activity of the bark ethanol/water extracts was measured in relation to hydrogen-donating or radical scavenging ability using 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) (Sharma and Bhat 2009; Miranda *et al.* 2016) and were expressed in terms of: a) the amount of extract required to reduce 50% of the DPPH concentration (IC_{50}); and b) Trolox and catechin equivalents on a dry extract base.

RESULTS AND DISCUSSION

The bark of *Goupia glabra* is silvery-grey to reddish-grey (Fig.1A), with a hard and orange inner bark and a rough and cracked outer bark, in agreement with what has been reported (Gurgel *et al.* 2015). The average bark thickness was 8.4 ± 1.7 mm and included rhytidome, periderm, and phloem; the phloem constituted the main part of the entire bark width, with 7.4 ± 1.5 mm thickness, and comprised the non-collapsed phloem, which was distinguished from the collapsed phloem by its different colour (Fig. 1B). The bark surface of *G. glabra* is longitudinally fissured.

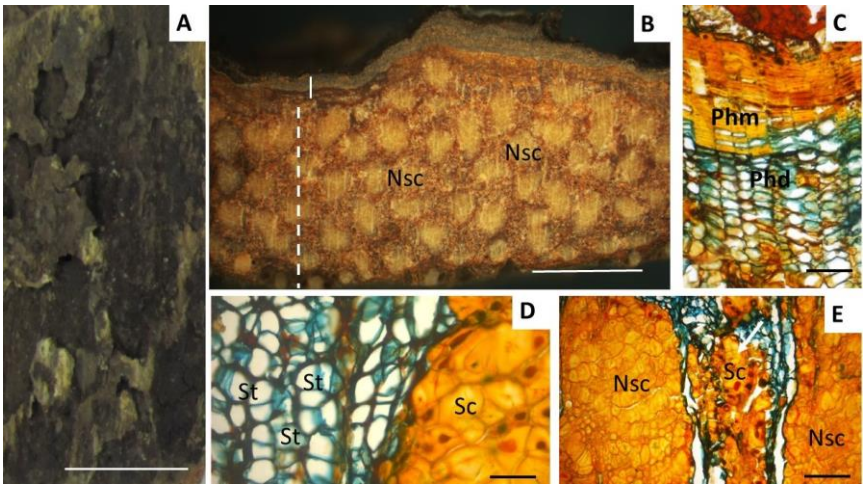

Fig. 1. Bark of *Goupia glabra*: external appearance (A) and transverse section observed under a binocular stereoscope (B) and a microscope (C–E). B) phloem (dashed vertical line) with nodules of sclereids (Nsc) and rhytidome (solid vertical line); C) periderm with sclerified cells in the phellem (Phm) and phelloderm (Phd); D) sieve tubes (ST) and sclereids (Sc); E) sclerified cells (arrow) between nodules of sclereids. Scale bar: (A) 2 mm; (B) 4 mm; (C– E) 100 µm; (C) 40 µm

Anatomical Characterisation

The rhytidome is scaly and composed of two to three periderms, with a ramified, net-like disposition. Lenticels were not observed. The periderm showed a layer of

thickened phellem cells often alternating with a uniseriate layer of thin-walled cells, and the phelloderm was thin with scattered sclereids (Fig. 1C).

The phloem was very regular, and the dilatation tissue was weakly developed. Prominent nodules of sclereids were observed under low magnification or even the naked eye that gave a characteristic pattern to this bark (Fig. 1B). Annual growth increments were not well-detected. The transition from non-collapsed to collapsed phloem was marked by a layer of stretched, obliterated, unlignified cells.

The phloem is composed of abundant sieve tube members with companion cells, some axial and ray parenchyma cells, as well as the sclerenchyma tissue (Fig. 1C-E) in the form of fiber-sclereids or sclereids; no fibers were observed. The absence of fibers was reported in various genera within Celastraceae (Archer and van Wyk 1993). In transverse view, the sieve tubes were large and conspicuous with a polygonal to round form, arranged in groups scattered between the axial parenchyma cells (Fig. 1D). They can be distinguished from the axial parenchyma by the inclined sieve plates with numerous sieve areas. The axial parenchyma cells have thin unlignified walls and appear rectangular and polygonal in the transverse section, while in the outer part of the phloem between the sclereid nodules, and they have thick cell walls with strong lignification (Fig. 1E). The rays were non-storied, 1-2 seriate heterocellular with procumbent to upright cells. They followed a more or less straight course in the inner phloem but became distorted near or across the sclereid nodules. The rays did not dilate toward the bark outside, contrary to other genera of the same family, *e.g.*, *Zinowiewia* sp. and *Maytenus* sp. (Roth 1981).

The large nodules of sclereids (Fig. 1E) that are more or less arranged in tangential rows in the inner phloem enlarged and became numerous outwards, perhaps supporting the radius growth change. Conspicuous nodules of sclereids were also observed in the phloem of other genus, *i.e.*, *Quercus* spp. (Sen *et al.* 2011; Quilhó *et al.* 2013) and thick-walled sclereids arranged in tangential bands were reported for other celastraceous members (Archer and van Wyk 1993; Schweingruber *et al.* 2011).

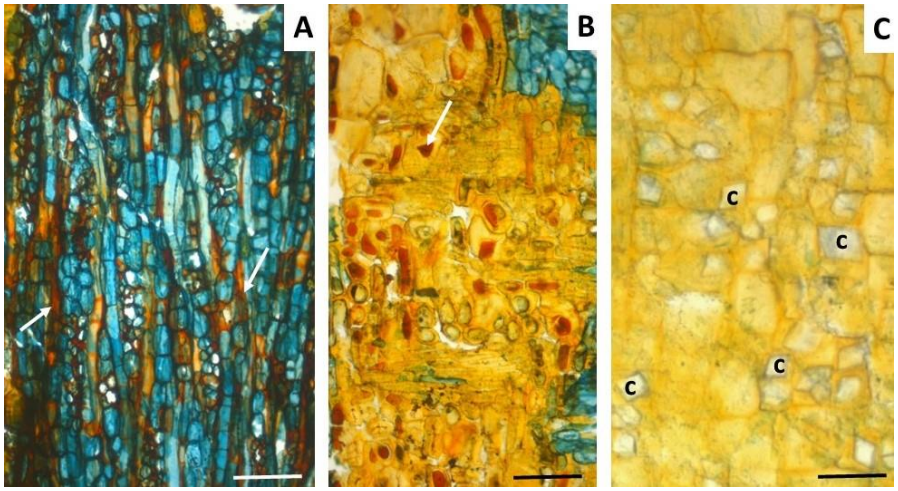

Fig. 2. Longitudinal sections of the phloem of *Goupia glabra*. (A) Phenolic compounds in parenchyma cells (arrows, tangential section); (B) phenolic compounds in sclereids (arrows, radial section); and (C) crystals (c) within the sclereids. Scale bar: (A–B) 40 µm; (C) 20 µm.

Phenolic compounds were observed by colour staining in the phellem, parenchyma cells, and sclereids (Figs. 1C, 2A,B). Solitary rhomboid crystals occurred within the sclereids (Fig. 2C), but septate crystal strands were not observed.

Bark Density

The basic density of the bark was on average 690.1 kg/m^3. The density of *G. glabra* bark was within the range of values found for other tropical barks, *e.g.*, 618 kg/m^3 for *Tectona grandis* (Baptista *et al.* 2013) and 781.4 kg/m^3 for *Copaifera langsdorffii* (Carmo *et al.* 2016), but higher than the values for barks from temperate regions such as 517 kg/m^3 to 559 kg/m^3 for *Betula pubescens* and *Betula pendula* (Bhat 1982), and 374 to 454 kg/m^3 for *Eucalyptus globulus* (Quilhó and Pereira 2001).

The high density of *G. glabra* bark is the result of its anatomical features, namely the large amount of sclereids scattered in the phloem (Fig. 1(a)).

Chemical Composition

The chemical composition of the bark from *G. glabra* is reported here for the first time (Table 1). The non-structural component corresponding to the extractives represented 24.6% of the bark.

Table 1. Chemical Composition, Monosaccharide Composition, and Elemental Constituents of Ash of the Bark of *Goupia glabra*

Chemical Composition	**% of Total Dry Mass**	
Ash	5.2	
Extractives total	**24.6**	
Dichloromethane	3.4	
Ethanol	15.0	
Water	6.2	
Suberin	**1.1**	
Lignin total	**43.8**	
Klason lignin	42.5	
Soluble lignin	1.3	
Holocellulose	**25.9**	
Monosaccharide Composition	**% of Total Dry Mass**	**% of Total Neutral Sugars**
Glucose	12.1	46.6
Xylose	12.4	47.9
Galactose	0.6	2.4
Arabinose	0.6	2.3
Mannose	0.2	0.8
Ash Composition	**% of Total Dry Mass**	**% of Total Ash**
Calcium	1.9534	90.48
Potassium	0.1165	5.71
Sodium	0.0138	0.48
Magnesium	0.0793	3.81
Iron	0.0153	0.71
Copper	0.0001	0.00
Zinc	0.0014	0.07
Manganese	0.0136	0.65

The high extractives content is similar to that of *C. langsdorffii* bark from the same Amazon zone (21.3%, Carmo *et al.* 2016), but higher than most hardwood barks of other species, such as *B. pendula* (6.5%) and *E. globulus* (17.6%) (Miranda *et al.* 2013), or of 12 *Eucalyptus* spp. (6.1 to 18.9%, Neiva *et al.* 2015), *Quercus laurina* (19.2%) and *Quercus crassifolia* outer bark (12.7%) (Ruiz-Aquino *et al.* 2015) or *T. grandis* bark (10.7%, Baptista *et al*. 2013).

Regarding the proportion of extractives solubilised by the different solvents, the main contribution came from polar compounds solubilised by ethanol and water, representing on average 86% of the total extractives (21.2% of the bark). The non-polar compounds extracted by dichloromethane corresponded to only 14% of the total extractives (3.4% of the bark). The polar extractives include mainly phenolic compounds, flavonoids, and tannins. This high content of polar compounds is in accordance with the phenolic deposits found in microscopic observations (Fig. 2A,B).

The *G. glabra* bark had very little suberin (1.1% of the bark) in direct relation with its anatomical structure, *i.e.* the small amount of phellem tissue in the periderms (Fig. 1B,C). Suberin is the chemical fingerprint of phellem (cork) cells, and when the proportion of phellem is small, the suberin content is correspondingly small. Therefore, bark containing small amounts of cork tissues, as *G. glabra* bark, have low suberin content, *e.g.*, 11.36 mg/g in *Arbutus andrachne* and 15.95 mg/g in *Platanus orientalis* bark (Dönmez et al. 2016), 1.9% in *T. grandis* (Baptista *et al.* 2013), 1.0% in *E. globulus* (Miranda *et al.* 2013), and 0.8% in *C. langsdorffii* (Carmo *et al.* 2016). On the contrary, bark with a substantial proportion of cork have high suberin content, *e.g.*, 22.0% in the outerbark of *Pseudotsuga menziesii* (Ferreira *et al.* 2015).

The total lignin content was very high (43.8%, Table 1), in accordance with the large proportion of highly lignified sclereids (Fig. 1B). This extent of lignification is significantly higher than values reported for the barks of other hardwood species, *e.g.*, *T. grandis* (20%, Baptista *et al.* 2013), *B. pendula* (27.9%, Miranda *et al.* 2013), *Salix* spp. (20 to 26%, Serapiglia *et al.* 2009), *Fagus crenata*, and *Quercus mongolica* (respectively, 34.6% and 24.9% (Kofujita *et al.* 1999)), *E. globulus* (19.2%, Vázquez *et al.* 2008, and 18.6%, Sakai 2001), and in 12 *Eucalyptus* species (21.6 to 30.8%, Neiva *et al.* 2015).

The total content of polysaccharides (*i.e.* holocellulose) accounted for only 26% of the bark. The monomeric composition of polysaccharides showed mainly glucose and xylose, with 46.6% and 47.9%, respectively, of total neutral monosaccharides, with only minor amounts of arabinose and galactose (2.3% and 2.4% of total neutral monosaccharides) and of mannose (0.8%). The hemicelluloses are therefore mainly of the glucuronoxylan-type. Thus, the glucan content represented 12.1% of the total dry bark, and hemicelluloses content, comprising xylan arabinan, galactan, and mannan chains, represented 13.8% of the bark. The xylose proportion in *G. glabra* bark is significantly higher when compared with other types of bark. The ratio of glucose to xylose was approximately 1, while it is generally between 1.5 and 3 in most hardwoods barks, *e.g.*, 3.0 in *T. grandis* (Baptista *et al.* 2013), 2.9 in *E. globulus*, 1.4 in *B. pendula* (Miranda *et al.* 2013), 2.8 in *C. langsdorffii* (Carmo *et al.* 2016), 1.6 to 2.0 in *Salix* (Serapiglia *et al.* 2009); in wood, this ratio varied between 2.4 and 3.1 in different *Eucalyptus* species (Neiva *et al.* 2015).

The proportion of lignin, hemicelluloses, and cellulose is an important criterion for selecting the best conversion pathway and targeted products. In the case of *G. glabra* bark, the high content of polar extractives motivates their removal and valorisation as a first step in the conversion process, while the high content of lignin could be of interest for, *e.g.*,

biofuel production, and the xylans can be a source of xylo-oligosaccharides and other sugars (Moniz *et al.* 2013, 2014, 2015).

The ash content of *G. glabra* bark was 5.2%. Most inorganic elements were Ca (91% of total ash), and K and Mg (5.7% and 3.8%, respectively). Overall, the high content of mineral nutrients in this bark (especially Ca and K) makes it a potential bio-element source for soil or substrate enrichment.

Phenolic Content

The yield of ethanol-water extraction and the extract characterisation are given in Table 2. The 17.5% yield was only slightly lower than the content of polar extractives determined by sequential solvent extraction (21.2%, Table 1).

Table 2. Chemical Composition of *Goupia glabra* Bark

Extraction yield (%)	17.5
Total phenolic content (mg GAE/g of extract)	158.2
Tannins (mg catechin/g of extract)	24.2
Flavonoids (mg catechin/g of extract)	74.8
Antioxidant capacity TEAC (mg Trolox/g of extract)	563.4
Antioxidant capacity TEAC (mg Trolox/g of bark)	98.6
IC_{50} values (μg extract/mL)	5.51
IC_{50} Trolox in ethanol-water (μg Trolox/mL)	2.71
IC_{50} Trolox in ethanol-water (μg catechin/mL)	5.39

The phenolic content corresponding to 158.2 mg GAE/g extract (27.8 mg GAE/g of bark) was lower or in the range of previously published values for barks of other hardwood species. Carmo *et al.* (2016) referred 589.2 mg GAE/g extract for the ethanol water extract of bark of *C. langsdorffii* from the Amazon. Santos *et al.* (2012) reported 386, 347, and 204 mg GAE/g extract in the ethanol-water of *E. grandis*, *E. urograndis*, *and E. maidenii* barks, respectively. Sultana *et al.* (2007) found 93, 165, 120, and 120 mg GAE/g extracts in the ethanol-water of *Eugenia jambolana*, *Acacia nilotica*, *Azadirachta indica*, and *Terminalia arjuna*, respectively. Luis *et al.* (2014) reported 383 mg GAE/g extract in 70% ethanol for *E. globulus* stemp bark. Puttaswamy *et al.* (2014) reported for *E. tereticornis* bark 198 mg GAE/g aqueous methanolic extract.

The flavonoid content in the extract of *G. glabra* bark was 74.8 mg CE/g extract (13.1 mg CE/g of bark). A large range of values has been reported for other hardwood barks, such as *C. langsdorffii* (442 mg CE/g extract, Carmo *et al.* 2016), *Delonix elata* (75 mg quercetin equivalent/g extract in ethanol, Krishnappa *et al.* 2014), *Eugenia jambolana*, *A. nilotica*, *A. indica*, and *T. arjuna* (respectively, 21, 49, 31, and 35 mg CE/g extract in ethanol-water, Sultana *et al.* 2007), *E. globulus* stump (12 mg quercetin equivalents/g extract in ethanol-water, Luis *et al.* 2014), or *Eucalyptus tereticornis* (160 mg rutin equivalents/g of bark, Puttaswamy *et al.* 2014).

The tannin content of *G. glabra* bark (24.2 mg CE/g extract, 3.6 mg CE/g of bark) was low when compared with barks of other species: The values for the ethanol-water extract of *Alnus incana* and *Alnus glutinosa* barks were in the range of 434 and 343 mg/g of extract, respectively (Janceva *et al.* 2011) and 55 mg CE/g extract for *C. langsdorffii* bark (Carmo *et al.* 2016). The acetone-water extract of bark of the *E. globulus* stump contained 29 mg GAE/g extract (Luis *et al.* 2014) and *E. tereticornis* bark 103 mg tannic acid equivalents/g of bark (Puttaswamy *et al.* 2014).

The free radical-scavenging activity of *G. glabra* bark ethanol to water extract was expressed in terms of the amount of extract required to reduce the DPPH concentration by 50% (IC_{50}) and also in terms of Trolox equivalents (TEAC) on a dry extract base (mg Trolox/mg extract). The radical scavenging activity corresponded to an IC_{50} value of 5.5 μg/mL (Table 2). This value compares very favorably with the IC_{50} values of well-known antioxidant standards such as catechin (5.4 μg/mL) and Trolox (2.7 μg/mL), the latter of which is considered to have excellent antioxidant activity. The antioxidant activity of the *G. glabra* bark extract expressed using Trolox as a reference corresponds to 563.4 mg Trolox/g extract, or 98.6 mg Trolox/g of bark).

The bark extract of *G. glabra* therefore shows potential as an antioxidant additive in food, drugs, or other products.

Effect of Particle Size on Chemical Composition of Bark

The milled *G. glabra* bark samples were chemically characterised and Table 3 gives the results for three fractions: < 0.180 mm (fine), 0.250 to 0.450 mm (medium), and > 2 mm (coarse), which represented respectively 3.9%, 14.3% and 56.8% of the total bark fractions.

Table 3. Summative Chemical Composition (% of Total Dry Mass) and Monosaccharide Composition (% of Total Neutral Monosaccharides) of the Bark of *Goupia glabra* Fractionated in Three Granulometric fractions after Milling: fine (F. < 0.180 mm), medium (M. 0.250 to 0.450 mm), and coarse (C. > 2 mm)

	% of Total Dry Mass		
	F	**M**	**C**
Ash	5.3	5.5	4.7
Extractives total	45.3	21.1	14.6
dichloromethane	4.9	2.7	3.8
Ethanol	34.6	15.0	6.0
Water	5.8	3.1	4.8
Suberin	0.7	1.6	0.6
Lignin total	36.0	38.9	55.4
Klason lignin	34.2	37.4	54.6
Soluble lignin	1.8	1.5	0.8
Holocellulose	13.0	38.4	29.4
Monosaccharide	**% of Total Neutral Sugars**		
Composition	**F**	**M**	**C**
Glucose	46.6	46.5	46.6
Xylose	47.9	47.9	47.9
Galactose	2.5	2.4	2.5
Arabinose	2.3	2.4	2.3
Mannose	0.8	0.8	0.8

Extractives were present preferentially in the fines that contained three times more extractives than the coarse fraction (45.3 *vs.* 14.6%). There was also an enrichment in polar extractives (ethanol and water solubles) in the fine fraction, while non-polar (diclorometane solubles) were similar in the three fractions. This means that in the case that this bark is processed for the recovery of extractives, the fines should not be discarded.

For the structural components, a difference between the fractions was found in relation to the lignin content, which was lower in the fines: 36.0% and 55.4% in the fine

and coarse fractions, respectively. However this difference is a result of the difference in extractives: if expressed in extractive-free bark, the lignin content is similar in both fractions (respectively, 73.9% and 69.2% in fine and coarse fractions).

Similar compositional changes with changes in particle size have been reported: Baptista *et al.* (2013) found in fractionated *T. grandis* bark that extractives increased with decreasing particle size, while lignin content did not show a clear trend. Miranda *et al.* (2013) and Carmo *et al.* (2016) also reported a large increase in extractives content in the fine fraction for fractionated *E. globulus* and *C. langsdorffii* barks, respectively.

The chemical differences of the bark fractions are related to the bark's anatomical features, since the grinding behaviour depends on the structural characteristics and the fractions may therefore differ in composition (Vázquez *et al*. 2001; Miranda *et al.* 2012, 2013; Baptista *et al.* 2013). In the case of *G. glabra*, bark has a rather homogeneous structure, with a very small proportion of rhytidome (Fig. 1) which explains the compositional similarity of the different fractions regarding the structural components.

CONCLUSIONS

1. The bark of *G. glabra* was chemically and anatomically characterized for the first time.
2. The bark has a high extractives content that included mainly polar compounds with a high antioxidant capacity. The lignin content was found to be high, as was the ratio of xylan hemicelluloses to cellulose.
3. Bark grinding and fractionation by particle size may be used to selectively enrich the fine fractions in soluble materials.

ACKNOWLEDGMENTS

Jair Figueiredo do Carmo was supported by a PhD Sandwich Scholarship (CAPES 11692/2013-08), Programa de Pós-Graduação em Ciências Ambientais e Florestais da Universidade Federal Rural do Rio de Janeiro (UFRRJ). Centro de Estudos Florestais is a research unit funded by Fundação para a Ciência e a Tecnologia (Portugal) under UID/AGR/00239/2013. Thanks are due to Sofia Cardoso and Vicelina Sousa for help in anatomical characterisation and to Lídia Silva and Joaquina Silva for chemical analysis.

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Article submitted: February 2, 2016; Peer review completed: March 18, 2016; Revised version received: March 23, 2016; Accepted: April 3, 2016; Published: April 14, 2016.
DOI: 10.15376/biores.11.2.4794-4807