Industrial Crops and Products 82 (2016) 81–87

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

The bark of *Eucalyptus sideroxylon* as a source of phenolic extracts with anti-oxidant properties

Isabel Miranda*, Leandro Lima, Teresa Quilhó, Sofia Knapic, Helena Pereira

Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 8 September 2015
Received in revised form
18 November 2015
Accepted 5 December 2015
Available online 22 December 2015

Keywords:
Bark
Eucalyptus sideroxylon
Anatomy
Chemical composition
Phenolic extracts
Anti-oxidants
Tannins

ABSTRACT

Barks are today viewed as a potential resource for biorefineries given their chemical richness and diversity. This paper describes for the first time the chemical composition of *Eucalyptus sideroxylon* bark and the antioxidant properties of its polar extractives. The bark is thick, deeply furrowed and dark colored. Large pockets of kino were observed formed by the breaking down of tissues of the outer phloem. The mean chemical composition of *E. sideroxylon* bark was: ash 1.3%; total extractives 55.7%, mainly corresponding to polar compounds that were soluble in ethanol and water, lignin 13.1% and suberin 1.9%. The polysaccharides composition showed predominance of glucose and xylose (80.0% and 11.0% of total neutral monosaccharides respectively). The ethanol–water bark extract had a high content of phenolics: total phenolics 440.7 mg gallic acid equivalent/g extract, flavonoids 204.4 mg catechin equivalent/g extract and tannins 395.0 mg catechin equivalent/g extract. The antioxidant activity corresponded to 648.8 mg Trolox/g of extract, and FRAP values to 5247 mM Fe^{2+}/g of extract. *E. sideroxylon* bark can therefore be extracted with green solvents to yield polar extractives with a potential valorization based on their chemical functionalities and bioactivity.

1. Introduction

Tree barks are complex biomass components with a large structural and chemical diversity among species. Barks have been used traditionally e.g., as a source of drugs, materials and energy, and are today viewed as a potential resource for biorefineries (e.g., Le Normand et al., 2014). Knowledge on bark characteristics is however limited to a small number of species, mostly those with high wood commercial exploitation.

Eucalyptus sideroxylon A. Cunn. ex Woolls (Myrtaceae family) (also known as Red Ironbark, Mugga Ironbark or Mugga) is a species native to Australia where it exists in open forests in New South Wales, extending to Queensland and Victoria. It is a small to medium sized, occasionally tall, tree that can be easily recognized by its hard, deeply furrowed bark of dark grey to black color, and impregnated with kino, and by its white, pink or red flowers (Bean, 2010). The species has been introduced in various regions where it grows well withstanding dry climates, poor soils and frost, sometimes used in arid zones (Jayawickrama et al., 1993)

E. sideroxylon has a hard and dense wood (*sideroxylon* means iron wood) with high durability that is used in construction and outdoor uses e.g., sleepers, posts, piers, boatbuilding. A few phytochemical and toxicological studies were carried out on extracts of *E. sideroxylon* heartwood and leaves in relation to chemical composition and biological activities (Hillis and Hasegawa, 1962; Hillis and Isoi, 1965; Hart and Hillis, 1974; Hillis et al., 1974), as well as on its essential oil (Ahmadouch et al., 1985; Dellacassa et al., 1990; Satoh et al., 1992; Ashour, 2008; Vuong et al., 2015).

Little information was found on the bark of this species. The mature bark of *E. sideroxylon* is persistent to the small branches, hard and deeply furrowed, dark brown to black (Boland et al., 1992). This species belongs to the "Ironbark" group of eucalypts with others e.g., *Eucalyptus paniculata*, *Eucalyptus crebra*, or *Eucalyptus siderophloia* as described by Chattaway (1955a) as a furrowed bark, with cracks extending through the rhytidome to the outermost phloem. Information on the bark structure of *E. sideroxylon* is however scarce in spite of the numerous anatomical studies of barks in the genus *Eucalyptus* (Chattaway, 1955b,c,d; Alfonso, 1987; Quilhó et al., 1999, 2000).

The presence of kino in *E. sideroxylon* bark is well recognized (Boland et al., 1992). Kino is a wood exudate found in many Myrtaceae species including the eucalypts that is characterized by a deep rich coloring, with high polyphenols and tannin content, and

* Corresponding author.
E-mail address: imiranda@isa.ulisboa.pt (I. Miranda).

http://dx.doi.org/10.1016/j.indcrop.2015.12.003
0926-6690/

astringency. Tippett (1986) studied the pathological anatomy of 28 *Eucalyptus* species that exhibited kino veins in the phloem, including *E. sideroxylon*. The mechanism of kino formation was recently reviewed (Locher and Currie, 2010) and still a matter of discussion. A number of kinos have been used for centuries as astringents for control of diarrhea and the Australian aborigines used eucalypt kinos to tan skins into leather (Hillis 1986, 1989; Von Martius et al., 2012; Locher et al., 2013). The tannin content of *E. sideroxylon* bark has also been considered namely for production of adhesives (Fechtal and Riedl, 1993).

In recent years, the composition of bark extractives has been studied for several *Eucalyptus* species, focusing on the extraction of phenolic compounds as natural antioxidants in *Eucalyptus globulus* (Vázquez et al., 2012; Santos et al., 2012 and Mota et al., 2012; Conde et al., 1995), *Eucalyptus camaldulensis* and *Eucalyptus rudis* (Cadahía et al., 1997; Conde et al., 1996), *Eucalyptus exserta* (Li and Xu, 2012), *Eucalyptus astringens*, *Eucalyptus cladocalyx*, *Eucalyptus occidentalis* and *E.* sideroxylon (Fechtal and Reidl, 1991) as well as on the lipophilic composition of bark extracts in *E. globulus* (Domingues et al., 2010; Freire et al., 2002) *E. grandis*, *Eucalyptus* urograndis and *Eucalyptus* maidenii (Domingues et al., 2011). Studies on bark as a feedstock for fermentable sugars of *Eucalyptus urophylla* × *Eucalyptus grandis* and *E. grandis* clones were also made (Bargatto, 2010; Lima et al., 2013). Bark as potential fiber supply to the pulp industry was studied for *E. globulus* (Miranda et al., 2013).

In this paper we describe the anatomy and chemical composition of *E. sideroxylon* bark with the objective to analyze its potential within a biorefinery route of bark use, namely related with the presence of extractives, and their high phenolic content and antioxidant properties.

2. Material and methods

The bark of *E. sideroxylon* was taken from three trees harvested with 6 years of age, with an average breast height diameter of 16.2 cm, from an eucalypt arboretum located in the fields of the School of Agriculture, University of Lisbon (ULisboa), at Tapada da Ajuda, Lisboa, Portugal (38°42′N; 09°10′W). The bark was separated manually from a disc taken at breast height.

2.1. Microscopic observations

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. The terminology follows Richter et al. (1996).

2.2. Chemical characterization

The bark samples were fractionated using a cutting mill (Retsch SM, 2000) with an output sieve of 1 × 1 mm^2. The ground material was sieved in a vibratory apparatus, and the 40–60 mesh fraction was used for chemical analysis.

Mineral content was calculated gravimetrically according to TAPPI Standard Method (T 211 om-93). 2 g of the bark material was incinerated in a muffle furnace at 525 °C overnight and the combustion residue was weighed and reported as ash content of the original sample.

Extractives were determined by successive Soxhlet extraction of approximately 2 g of the sample with dichloromethane, ethanol and water during 6 h, 16 h and 16 h respectively. The extractives solubilized by each solvent were determined using the mass difference from the mass of the solid residue after drying at 105 °C, and reported as percent of the original sample (TAPPI T204 om-88).

Suberin content was determined on 1.5 g of the extractive-free sample by refluxing with 100 mL of a 3% $NaOCH_3$ solution in CH_3OH during 3 h (Pereira, 1988). The sample was filtrated, washed with methanol, again refluxed with 100 mL CH_3OH for 15 min and filtrated. The combined filtrates were acidified to pH 6 with 2 M H_2SO_4 and evaporated to dryness. The residue was suspended in 50 mL water and the alcoholysis products recovered with dichloromethane in three successive extractions, each with 50 mL dichloromethane. The combined extracts were dried over anhydrous Na_2SO_4 and 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.

The determination of lignin content was made by acid hydrolysis as Klason lignin (TAPPI T222 om 98) and acid soluble lignin (TAPPI UM 250). Klason lignin was determined on 0.35 g of the material after suberin removal that were treated with a 72% sulphuric acid solution in a 30 °C water-bath for 1 h, after which the acid concentration was reduced to 3% with water and the hydrolysis was completed in an autoclave at 120 °C (1.2 bar) for 3 h. The reaction mixture was filtered in a G3-porosity glass filter, the residue, dried in an oven at 105 °C and weighed as the Klason lignin. Aliquot from the aqueous acidic filtrate was used for soluble-lignin determination by spectroscopy at 250 nm wavelength (absorptivity coefficient of 110 g L^{-1} cm^{-1}). The remainder of the acidic solution was kept for sugar analysis. Klason lignin and acid-soluble lignin were reported as percent of the original sample and combined to give the total lignin content.

The polysaccharides were estimated by determining the neutral monosaccharides monomers released by the total acid hydrolysis used for lignin determination in the extractive-free and suberin-free samples. The neutral sugar monomers were determined by high performance anion exchange chromatography (HPAEC) using a Dionex ICS-3000 system equipped with an electrochemical detector. The separation was performed with Aminotrap plus CarboPac SA10 anion-exchange columns. The mobile phase was an aqueous 2 nMNaOH solution at a flow rate of 1.0 mL/min at 25 °C. The polysaccharide content was determined by the sum of the individual sugar masses.

2.3. Phenolic content of the bark extract

Approximately 1 g of the ground bark was extracted with ethanol/water (50/50, v/v) with a solid–liquid ratio 1:10 (m/v) for 60 min at 50 °C using an ultrasonic bath. The insoluble materials were removed by filtration and the supernatant extract was stored at 4 °C. The solid residue was dried and the extraction yield was calculated as the percent mass loss of the starting material.

Total phenolic content was determined by the Folin–Ciocalteu method using gallic acid as standard (Singleton and Rossi, 1965). An aliquot (100 μL) of the extract was mixed with 4 mL of the Folin–Ciocalteu reagent and after 6 min, 4 mL of a 7% Na_2CO_3 solution was added. After 15 min of incubation in a bath at 45 °C, absorbance at 760 nm was read versus a prepared blank. A calibration curve was built using gallic acid as a standard (0–150 μg/mL). The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE)/g of the dry bark extract.

Total flavonoids content was determined by an aluminium chloride colorimetric assay (Zhishen et al., 1999). An aliquot (1.0 mL) of the extract was mixed with 4.0 mL of deionized water followed by 0.3 mL of a 5% $NaNO_2$ solution. After 5 min, 0.3 mL of a 10% $AlCl_3 \cdot 6H_2O$ solution was added to the mixture. After 5 min, 2.0 mL of 1 M NaOH solution was added, and the total volume was adjusted