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Extraction of polyphenols from *Eucalyptus nitens* and *Eucalyptus globulus*: Experimental kinetics, modeling and evaluation of their antioxidant and antifungical activities

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ABSTRACT

The species *Eucalyptus globulus* and *Eucalyptus nitens* have become of great importance in Chile and the world, due to their rapid growth and because compounds with greater added-value can be obtained from their waste. This study aimed at obtaining extracts rich in phenolic compounds from the bark of *E. globulus* and *E. nitens* and at evaluating the extraction kinetics and the resistance provided by these extracts to the degradation of pine wood attacked by decay fungi. For this purpose, bark samples were taken from *E. nitens* and *E. globulus* at a commercial site and then dried and ground. The extraction of the hydrophilic components was optimized by using a Box-Behnken design that considered the influence of temperature, solid/liquid ratio and methanol concentration on the maximization of the following responses: extraction yield, total phenols (Folin-Ciocalteu) and antioxidant capacity (DPPH). Under optimum conditions, the reaction kinetics of phenolic compounds at three temperatures were modeled and determined. Two antifungal assays were performed with the extracts: determination of the minimum inhibitory concentration (MIC) and a mass loss test for three decay fungi. The optimum was obtained at a solid/liquid ratio (1:60) at a 51% concentration of methanol and water for both species and at a temperature of 319 K and 326 K for *E. globulus* and *E. nitens*, respectively. The Fick diffusion model of two extractive phases successfully represented the extraction kinetics of the phenolic compounds from the eucalyptus bark at different temperatures. Bark extractions from both species were validated as possible antifungals against the fungal species.

In future studies the technical-economic feasibility of replacing commercial antifungals with solutions of the extracts obtained in this study will be analyzed, taking the extraction process to a pilot scale.

1. Introduction

Species of eucalyptus are playing an increasingly important role in the production of wood and pulp in the world and are the greatest source of fiber in Europe, South Africa, Japan and South America. According to FAO (2017) (Food and Agriculture Organization of the United Nations), in the year 2000, species of eucalyptus covered 17.9 million ha worldwide. Specifically in Chile, the Forest Institute (INFOR, 2016) reported that approximately 32% of the trees planted were eucalyptus, with 774,000 ha; then the species *Pinus radiata* had the highest plantation rate in Chile with 1,400,259 ha (58.4%). The industrial activities developed in the Biobío Region utilize most of the trees harvested in the country's forest plantations, namely 74% (8,933,000 m^3 ssc) for eucalyptus species and 52% (15,906,000) for pinus species. These industries generate large amounts of waste from biomass, particularly bark, which in most cases is burned to produce energy. However, this process has a low efficiency due to the high content of resins in the bark, causing the formation and deposition of pitch in industrial equipment (Back and Allen, 2000). This is why the exploitation of by-products derived from the forest industry is one of the most successful examples of biorefinery (Huang et al., 2008; Kamm et al., 2008) and can also be applied to the generation of valuable products from bark. The exploitation of some of these by-products is already being implemented in some foreign pulp industries (Fernandes and Cabral, 2007).

Miranda et al. (2013) found that *E. globulus* bark consisted of 83.2% holocellulose, 34.1% lignin and 6.5% extractives. Wegener (1984) showed that the bark possesses a large quantity of extractives including lipophilic compounds and polyphenolic compounds. Some of these lipophilic compounds are beneficial to health and objects of demand from food, cosmetic and pharmacological industries for their antimicrobial, hypoglycemic, anti-inflammatory and antitumor properties

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(Braga et al., 2007; Horiuchi et al., 2007; Sato et al., 2007). Phenolic compounds have potential application such as a substitute for phenols in the formulation of adhesives for wood derivatives, a substitute for chromium in leather tanning, and as a source of antioxidant compounds (Vázquez et al., 2009).

In this study, the kinetics of the hydrophilic extraction process of phenolic components from the bark of *E. globulus* and *E. nitens* will be determined and their phenolic content and associated antioxidant capacity will be characterized. In addition, the application of the hydrophilic extract as a possible antifungal for the inhibition of fungal growth in pine wood will also be validated.

2. Materials and methods

2.1. Plant material and bark characterization

The bark of six *E. globulus* and *E. nitens* trees with ages between 10 and 12 years was obtained from Comaco Forest Company (Concepción, Biobío Region, Chile), being harvested on December 17, 2015 from Los Castaños farm, Concepción, Biobío Region (36°46′16.53″S, 73°6′16.31″W). Prior to extraction, the bark was dried at room temperature (292–300 K) and subsequently was ground in a mill to a size smaller than 2 mm, passing through six sieves from 0.08 to 2 mm in diameter for the determination of the particle size distribution. To study the influence of the particle diameter on the rates of extraction and equilibrium concentrations, four ranges of particle diameter were used: 0.08–0.35 mm, 0.35–0.59 mm, 0.59–0.85 mm and 0.85–1.00 mm (Figs. 1 and 2, section S1 supplementary data). In the figures, it can be observed that at any particle size between 0.08 and 0.59 mm, there was no variation in the concentration of phenols at the equilibrium for a 95% confidence level, confirming that solute retention phenomena inside the solid matrix (within it, generating a concentration gradient inside the solid material; or by material trapped inside the solid walls) and also the length of diffusive pathways were minimized (Hostettmann et al., 2014). The particle size used ranged between 0.08 and 0.59 mm and their average size was determined by the weighted arithmetic mean between these sieves. The densities of the ground barks of each species were determined by the mechanical compression method, introducing a sample of the ground bark into a press. The tablet formed was weighed and measured (height and diameter for volume calculation (Saldarriaga et al., 2014)). The bark pore size and pore distribution area were measured by N_2 adsorption at 81 K (Sample Degas System, Micromeritics, Flowprep 060) for both eucalyptus species. The powder was previously multi-extracted and vacuum cleaned. The semi-quantitative analysis based on the *t*-graphic was used for the study of micropore distribution. The bark pore size was calculated using the method of Barrett et al. (1951) (BJH method).

2.2. Reagents and standards

Folin & Ciocalteu's phenol, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Supplemented Agar, malt reagents and Ketoconazole were purchased from Sigma Aldrich (St. Louis, MO, USA). Sodium carbonate was purchased from Winkler and Zawadzky (Chile). Analytical grade methanol was purchased from Merck (Darmstadt, Germany). The bidistilled water used in all solutions was purified to HPLC grade using a Millipore Milli-Q (MQ) as a fast water system (Bedford, MA, USA). Vermiculite was purchased from Sodimac (Chile).

2.3. Hydrophilic batch extraction

Each of the bark samples was suspended in a methanol/water mixture (%v/v), using 80 mL Erlenmeyer flasks. These flasks were put into a Bench Top Incubator NBL-205 (200 rpm) at the solid/liquid ratio, methanol concentration and temperature given by the experimental design. Flasks were sealed with Parafilm paper and lined in aluminum paper to avoid the degradation of compounds caused by light. After the extraction, the suspension was vacuum filters with filter paper (Sartorius, 3 hw, Germany) to obtain the free supernatant of the worn-out bark. Methanol from the water soluble extracts was evaporated in a rotary evaporator at 303 K and 6.5 rpm. The remaining extract was frozen (12 h refrigeration at 269 K) and subsequently lyophilized to powder in a lyophilizer (Heto Drywinner, Denmark). Dried samples were stored in the dark in sealed jars at a temperature between 275 K and 281 K. Extraction yields obtained in this study were normalized and expressed on the basis of dry bark for all experiments.

The bioactive compounds of the bark were identified by HPLC technique (see section S3.1 of Supplementary data).

2.4. Experimental design

A Box-Behnken experimental design with three central points and one replica was performed. The studied factors were: liquid/solid ratio (x_1), methanol concentration (x_2) and temperature (x_3). In response, the extraction yield, total phenolic content and antioxidant capacity (DPPH) were evaluated. The levels of each factor are specified in Table 1. Results were adjusted to a second order polynomial, as observed in Eq. (1). Thus, Y_i represents the independent variable and x_i and x_j are the dependent variables codified between −1 and 1, a_0 is the constant of the model, a_i, a_{ij} and a_{ii} are the coefficients of the model for both linear and quadratic interactions of the independent variables.

$$Y_i = a_0 + \sum_{i=1}^{3} a_i x_i + \sum_{i=1}^{2} \sum_{j=2,j>1}^{3} a_{ij} x_i x_j + \sum_{i=1}^{3} a_{ii} x_i^2 \quad (1)$$

For the optimization of multiple responses, a desirability function was defined for each dependent variable. The desirability function d(y) is defined in a scale of 0–1 and expressed in order to maximize the responses (Eq. (2)).

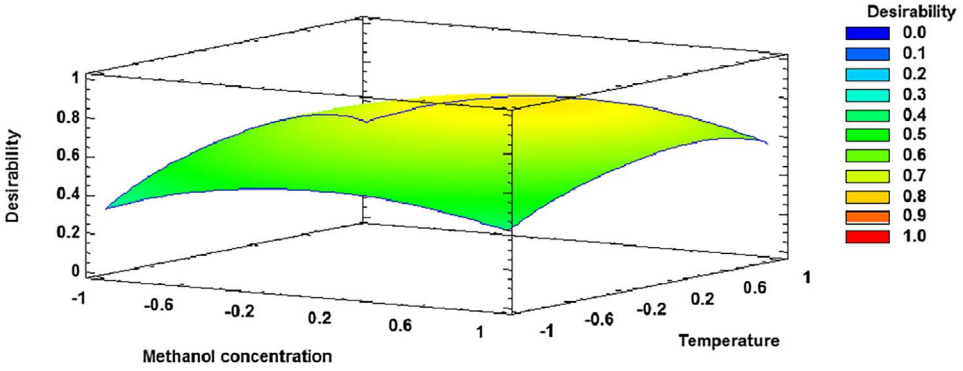

Fig. 1. Response surface of the desirability with the solid/liquid ratio at its optimum for *E. nitens*.