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Effect of the extraction technique on the recovery of bioactive compounds from eucalyptus (*Eucalyptus globulus*) wood industrial wastes

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ABSTRACT

Eucalyptus (*Eucalyptus globulus*) wood veneer trimmings, a waste product from the wood board industry, were studied as source of bioactive compounds. Two extraction techniques, maceration in an orbital bath and microwave-assisted extraction (MAE), were compared. In the conventional one, the effect of solvent (water, MeOH, EtOH, 50% MeOH and 50% EtOH), temperature (50 and 75 °C) and particle size (ground or unground material) on extraction yield and extract properties (total phenols content (TPC) and FRAP, DPPH and ABTS antioxidant activities) were analyzed. Extraction yield increased with increasing temperature and with reducing particle size. However, extract properties decreased when temperature was increased. Extracts obtained with EtOH at 50 °C showed the highest antioxidant properties. MAE experiments were planned according to an incomplete 3^3 factorial design to study the influence of temperature (50–70 °C), liquid–solid ratio (5:1–10:1 mL/g) and time (5–15 min) on extraction yield and extract properties. The optimal condition selected were 65 °C, liquid–solid ratio of 8.8:1 (mL/g) and 10 min. Comparing both techniques, maceration led to the extract with the best antioxidant properties, but MAE allowed to reduce significantly the extraction time. The aqueous extracts obtained under conventional extraction were able to inhibit the growth both bacteria and fungi. Gallic acid esters of glucose, gallic acid, ellagic acid and small proportions of quercetin-3-O-rhamnoside were found in the aqueous and ethanolic extracts.

1. Introduction

Eucalyptus is one of the main forest species in Galicia (NW of Spain) covering an area of 380,000 ha. Together with Portugal, it is the main global area of *Eucalyptus globulus* plantations. In Galicia, the main uses of eucalyptus wood are the production of cellulose pulp and wood boards. During wood industrial processing several wastes are produced such as the bark or wood rejects. These by-products could be re-used to reduce their environmental impact and also to obtain an economic benefit. The extraction of bioactive compounds, such as phenolic compounds, from these residues could be a possible valorization way. The interest in phenolic compounds is related with their antioxidant and antimicrobial capacities (Rauha et al., 2000; Moure et al., 2001) and several eucalyptus by-products have been studied as natural sources of these kind of compounds. At these respect, Vázquez et al. (2008, 2009, 2012a) have reported the antioxidant activity of the eucalyptus bark; antioxidants from wood were reported by González et al. (2004); eucalyptus leaves were studied by El-Ghorab et al. (2003) and the antimicrobial activity of essential oil from eucalyptus has been demonstrated by Tyagi and Malik (2011).

Solvent extraction is the most widespread procedure to extract bioactive compounds. Important factors in the process are the type of solvent, extraction time and temperature (Moure et al., 2001; Dai and Mumper, 2010). The efficiency of the process is also strongly dependent on the extraction technique used. Traditional techniques, such as maceration or Soxhlet extraction, usually require long extraction times and large amounts of solvent (Wang and Weller, 2006; Dai and Mumper, 2010; Aspé and Fernández, 2011). Alternative techniques, such as microwave-assisted extraction (MAE) or ultrasounds assisted extraction (UAE) are being developed to improve the efficiency and reduce the environmental impact of the extraction process. MAE presents some additional advantages such as improved yields of extractable compounds and faster processes (Liazid et al., 2010). MAE technique is based on the absorption of microwave energy by the water contained on the plant matrix. The internal heating promotes the cell disruption facilitating the liberation of compounds into the solvent (Wang and

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Weller, 2006). The effect of the MAE process depends of many factors such as the dielectric susceptibility of solvent and matrix, the solvent concentration, the particle size, the liquid–solid ratio, the temperature, the extract time and extract power (Wang and Weller, 2006; Li et al., 2011). Ethanol, methanol or water are susceptible to be used as solvents under microwave conditions.

The aim of this work was the valorization of eucalyptus wood trimmings obtained from veneers used for the finishing of wood panels as source of bioactive compounds. Nowadays, these veneer trimmings are used for the manufacturing of particleboards; however, the obtaining of high added value compounds, such as phenolic compounds, could be an alternative or complementary exploitation way. The effect of the extraction technique, conventional (maceration) and not conventional (MAE), together with the extraction conditions on the antioxidant properties of the eucalyptus wood extracts obtained was analyzed. The aqueous extracts were also tested against bacteria and fungi to analyze their antimicrobial capacity. Finally, the polyphenolic profile of the extracts was determined by RP-HPLC-ESI-TOF.

2. Materials and methods

2.1. Reagents and standards

Folin–Ciocalteu reactive, sodium carbonate, gallic acid, sodium acetate, $FeCl_3 \cdot 6H_2O$, HCl, L-ascorbic acid, methanol, ethanol and potassium persulfate were purchased from Panreac (Barcelona, Spain). 2,4,6-Tripyridyl-s-triazine (TPTZ), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were from Fluka (Steinheim, Germany). ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid), sodium chloride and agar-agar were obtained from Sigma (Steinheim, Germany). Glucose was purchased from Merck (Darmstadt, Germany). Yeast extract, peptone and tryptone were obtained from Himedia (Mumbai, India). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Raw material

Eucalyptus (*E. globulus*) veneer trimmings were supplied by the wood board industry Aserpal S.A. (Grupo Losán S.A., Galicia, NW Spain), specialized in the elaboration of fine wood surfaces. In the factory, the veneers were obtained from slicing a block of eucalyptus wood lengthways, which was previously pretreated in water at 75 °C for 16 h. Afterwards, in the laboratory, the veneer trimmings were air-dried till equilibrium humidity and prepared in pieces of 0.60 mm × 10 mm × 20 mm. Part of the material was ground in a mill, sieved and the fraction of particle size between 0.1 and 1 mm was selected.

2.3. Extraction and concentration

2.3.1. Conventional extraction

Eucalyptus wood was extracted in an orbital shaker with temperature control (UNITRONIC-OR, Selecta, Spain). The solid/liquid ratio was fixed at 10/1 v(mL)/w(g) and the shaking speed was 90 rpm. In a first stage the influence of the solvent used was studied. Thus, the unground material was extracted using water, MeOH, EtOH and 50% aqueous solutions of MeOH and EtOH at 50 °C for 90 min. In a second stage, extractions with water, 50% MeOH and 50% EtOH were performed at 75 °C, keeping constant the rest of conditions, to analyze the effect of temperature. A preliminary experiment at 25 °C (unpublished) led to a very low extraction yield, accordingly, the lowest temperature assayed was 50 °C. Finally, the grounded material was extracted under the best conditions selected in the previous stages (water, EtOH and 50% EtOH) at 50 °C to evaluate the influence of particle size. The extracted material was recovered by vacuum filtration. The solvent was evaporated in a Büchi R-210 rotavapor (Flawil, Switzerland) for the alcoholic extractions and in a Büchi mini spray dryer B-191 (Flawil, Switzerland) for the aqueous ones to obtain a dry power. Extraction yield was calculated as the weight loss percentage of the starting material.

2.3.2. Microwave assisted extraction (MAE)

Extracts of eucalyptus veneers were obtained in a Discover SP-CEM microwave system (CEM Co., USA) using EtOH as solvent. The extraction temperature, liquid/solid ratio and time were fixed for each experiment according to an incomplete 3^3 factorial experimental design (Table 3). The microwave conditions were: power of 150 W, extraction pressure of 250 psi, high stirring level and cooling was left on. The extracted material was recovered by vacuum filtration and the extraction yield was calculated as the weight loss percentage of the starting material.

2.3.3. Experimental design

In the microwave assisted extraction, the influence of the operational conditions, temperature (x_1, 50–60–70 °C), liquid/solid ratio (x_2, 5:1–7.5:1–10:1 mL/g) and time (x_3, 5–10–15 min) on the extraction yield and extract properties was analyzed using an incomplete factorial design 3^3. The experimental design selected consisted of 12 experiments and three replicates in the central point (Table 3).

The dependent variables (Y_j) selected were the extraction yield (Y_1, g extract/100 g eucalyptus veneer), total phenols content (Y_2, g gallic acid equivalents (GAE)/100 g extract) and FRAP antioxidant activity (Y_3, nmol ascorbic acid equivalents (AAE)/mg extract). Experimental results were analyzed by linear regression using the backwards elimination method (PASW Statistics 18 software) and fitted to polynomials of the form:

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

where x_1^*, x_2^*, and x_3^* are the independent variables coded at three levels −1 (lower limit), 0 (central point) and +1 (upper limit).

2.4. Total phenols content

Total phenols content was determined by the Folin–Ciocalteu method (Singleton and Rossi, 1965) carried out as described in a previous work (Vázquez et al., 2012b). The results were expressed as g gallic acid equivalent (GAE)/100 g of extract (on dried basis).

2.5. Antioxidant activity

2.5.1. Ferric reducing antioxidant power (FRAP)

The FRAP assay was done according to Szöllösi and Szöllösi-Varga (2002). The results were expressed as nmol ascorbic acid equivalent (AAE) per mg of extract (on dried basis).

2.5.2. DPPH scavenging activity

The radical scavenging ability of the extracts was monitored using the stable free radical DPPH following a modification of the method proposed by Barreira et al. (2008) described in Vázquez et al. (2012b). The results were expressed as mmol Trolox equivalent (TRE) per gram extract on dried basis and as the EC_{50} value, or extract concentration necessary to achieve a 50% radical DPPH inhibition.