

# Aqueous two-phase systems for the extraction of phenolic compounds from eucalyptus (*Eucalyptus globulus*) wood industrial wastes

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## Abstract

**BACKGROUND:** Aqueous two-phase extraction is recognized as an effective, versatile and important emerging green-technique for the downstream processing of biomolecules. Extraction of phenolic compounds from trimmings of *Eucalyptus globulus* wood veneers was studied using aqueous two-phase systems (ATPS) (water + polymer + salt) based on PEG 2000 and ammonium sulphate. The aim was the recovery of phenolic compounds to be used as natural antioxidants. Experiments were planned to optimize the extraction process. The influence of several operational conditions (time, temperature, ATPS composition, solid–liquid ratio and settlement time) on phenolics recovery was studied.

**RESULTS:** It was found that phenolic compounds have preference for the top PEG-rich phase. Settlement time had no significant impact on phase composition. Total phenols yield increased with temperature, extraction time and amount of solvent used. However, ATPS composition did not significantly influence total phenol yield. Extract analysis by RP-HPLC-ESI-TOF confirmed the presence of phenolic compounds with potential antioxidant activity, namely, mono and digalloyl glucose, (–)-gallic acid, ellagic acid and quercetin 3-O-rhamnoside.

**CONCLUSION:** Results demonstrated that extraction with ATPS (PEG 2000 and ammonium sulphate) is an efficient way for recovering phenolics from eucalyptus wastes without requiring previous purification (total phenols yield of up to  $1.88 \pm 0.04$  mg GAE/100 o.d. wood).

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**Keywords:** aqueous two-phase systems; green engineering/products; waste treatment and waste minimisation; *Eucalyptus globulus*; phenolic compounds

## INTRODUCTION

In recent years there has been growing public concern about sustainability practices, green chemistry and inherent safe design. Consequently, an urgent need is emerging for the efficient use of natural resources.

Approximately one half of the total area of Galicia (NW of Spain) (48%) is forested with two dominant species: *Pinus* sp. and *Eucalyptus* sp. Pine and eucalypt woods are mainly used to produce lumber wood and cellulose pulp and, to a lesser extent, panels and boards.<sup>1,2</sup> A large amount of residues, mostly used for energy production, is generated during the first stage of processing. The valorization of this waste to obtain high added value products is an increasingly important challenge for industries that aim for economically sustainable and environmentally friendly processes.<sup>2</sup>

Antioxidants are an example of these high added value products. These are a group of chemical derivatives that act by extending the shelf life of different products, such as foods, cosmetics, stabilizing lubricants or by preventing the oxidative degradation of rubbers, plastics and adhesives.<sup>3,4</sup> In general, they protect these products against the deterioration caused by oxidation.

There are many ongoing research efforts to obtain natural antioxidants from both agricultural and industrial waste to replace the less safe synthetic antioxidants.<sup>3,5–12</sup> Antioxidants are commonly and efficiently extracted by aqueous mixtures of

organic solvents such as ethanol, methanol or acetone,<sup>5,10</sup> but the use of green extraction technologies is rare. Besides conventional solvent extraction, new methods based on more advanced extraction techniques, such as pressurized solvent, high hydrostatic pressure, sub- and supercritical fluids, microwave and ultrasound assisted extraction or membrane processes, have been reported.<sup>13</sup> Limitations of these novel techniques include the requirement for special equipment, severe operating conditions, high energy consumption and higher industrial production costs.<sup>13</sup>

Aqueous two-phase extraction is emerging as an effective and versatile green-technique for the downstream processing of biomolecules.<sup>13</sup> Aqueous two-phase systems (ATPS) are low volatility systems with high versatility. That is, a large variety may be obtained using substances that follow the Green Chemistry principle on ecotoxicity, biodegradability, bioaccumulation and

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persistence, minimizing waste and maximizing yields. In addition, they comply with the principle of substitution of intrinsically safe design to allow work under atmospheric pressure. Since the 1970s many in-depth studies have reported the purification of proteins and other biologic materials using ATPS,<sup>14</sup> and many researchers have studied various applications of ATPS for the extraction and purification of biological products.<sup>15–18</sup> However, the use of such systems for the recovery of phenolic compounds from plant materials is very limited.<sup>8,13–19</sup> Moreover, there is extensive literature about the thermodynamic properties of ATPS but, to the best of our knowledge, their application to raw unpurified samples has been quite limited.

There are two main types of ATPS: polymer–polymer and polymer–salt systems. The choice of polymer–polymer ATPS is usually determined by economic considerations. The high cost of some forming phase polymers (e.g. dextran) limits the application of these systems, only justified when the cost of the product of interest is considerable. Therefore, the selection of the more economical polymer–salt systems is highly recommended.<sup>14,16,19</sup>

This study proposes an alternative way of processing residues from eucalyptus wood veneers used for the finishing of wood panels. The objective was to study the extraction of phenolic compounds from eucalyptus wood using an ATPS (water + polymer + salt) based on PEG 2000 and ammonium sulphate. In order to optimize the extraction process, the influence of several operational conditions on phenolics recovery was evaluated. In particular, we studied extraction time and temperature, ATPS composition, solid–liquid ratio and settlement time for phase separation. Moreover, RP-HPLC-ESI-TOF mass spectrometry was used to identify the phenolic compounds in the selected final extract.

## EXPERIMENTAL

### Raw material

Eucalyptus (*Eucalyptus globulus*) wood veneer trimmings were supplied by the company Aserpal S.A. (Grupo Losán S.A., Galicia, NW Spain) specialized in the elaboration of fine wood surfaces. In the factory, veneers are obtained by slicing a block of eucalyptus wood lengthways, which was previously pretreated in water at 75 °C for 16 h. Then, in the laboratory, veneer trimmings were air-dried till humidity reached equilibrium and prepared in pieces of 0.60 mm × 10 mm × 20 mm.

### Chemicals

Sodium carbonate, ammonium sulphate, gallic acid-1-hydrate, and Folin-Ciocalteu's phenol reagent were purchased from Panreac (Barcelona, Spain). Acetic acid, acetonitrile and polyethylene glycol (PEG) 2000 from Merck (Darmstadt, Germany). HPLC standards: (+)-catechin hydrate, (–)-epicatechin, procyanidin B2, quercetin-3-β-D-glucoside, quercetin-3-O-rhamnoside, ellagic acid, isorhamnetin, kaempferol and tannic acid were purchased from Fluka (Steinheim, Germany); galocatechin was from Sigma (Steinheim, Germany) and (–)-gallic acid was from Riedel-de Haën (Seelze, Germany).

### Extraction and separation procedure

PEG 2000 and ammonium sulphate were selected as components of the ATPS because both are suitable for the separation of bioactive materials, compatible with products for human use, environmentally friendly and not classified as dangerous. The aqueous two-phase systems (water + polymer + salt) based on

**Table 1.** Composition of the ATPS based on PEG 2000 and ammonium sulphate

System	Composition				PEG2000/ (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ratio (w/w)
	TLL (% w/w)	PEG 2000 (%)	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (%)	H <sub>2</sub> O (%)	
1	39.73	16.52	12.90	70.58	1.28
2	31.36	12.87	12.28	74.85	1.05
3	45.37	16.37	15.21	68.42	1.08

TLL, tie line length; system total weight, 50 g.

PEG 2000 and ammonium sulphate were prepared using the phase diagram of the ternary system found in the literature.<sup>20</sup> A predetermined quantity of ammonium sulphate was dissolved in water and the corresponding quantity of PEG according to the composition selected (see Table 1) was added into the ammonium sulphate aqueous solution to form the ATPS. Finally, the eucalyptus wood pieces were added to the ATPS solution in accordance with the selected solid–liquid ratio. The extractions were performed in a water bath with orbital shaking (UNITRONIC-OR, Selecta, Spain) at a shaking rate of 90 rpm. The variables examined were temperature (25, 45 and 65 °C), solid–liquid ratio (0.2:10, 0.5:10, 0.7:10, 1:10, 1.2:10 and 1.5:10 (w/w)), extraction time (30, 90, 270 and 390 min) and composition of ATPS (see Table 1). The amount of liquid used in the extraction process was 50 g. After the extraction, the wood pieces were removed and the phases were separated in a separatory funnel under gravity. Afterwards, the total phenols content was determined in both the top and bottom phases. In order to establish the settlement time, the separation was performed for different times (1, 4, 8 and 15 h). All experiments were carried out in triplicate.

### Total phenols content

Total phenols content was determined by the Folin–Ciocalteu method:<sup>21</sup> to 0.5 mL of an aqueous solution of the extract, 2.5 mL of Folin–Ciocalteu reagent previously diluted with water (1:10, v/v) and 2 mL of a 75 g L<sup>-1</sup> sodium carbonate aqueous solution were added. The mixture was kept for 5 min at 50 °C and, after cooling, the absorbance was measured at 760 nm in a Jasco V-530 UV-visible spectrophotometer. The phenols content was calculated as gallic acid equivalent (GAE) from the calibration curve of gallic acid standard solutions (2–40 mg mL<sup>-1</sup>). The results were expressed as total phenols concentration (mg GAE L<sup>-1</sup>) and as total phenols yield (mg GAE per 100 mg of oven-dried (o.d.) wood), which takes into account both the amount (g) and the phenols concentration of each phase. All analyses were carried out in triplicate.

### RP-HPLC-ESI-TOF mass spectrometry analysis

The top PEG-rich phase of selected samples, once separated from the bottom phase as previously indicated, was lyophilized in a ScanVac Coolsafe 100-9PRO lyophilizer (LaboGene, Denmark). For this, the sample was frozen at –30 °C and then lyophilized under the following conditions: temperature, from –80 °C to –85 °C and pressure –0.088 hPa. The lyophilized sample was analyzed for its phenolic composition by RP-HPLC-ESI-TOF using an HPLC Agilent Technologies 1100 (Agilent Technologies, Germany) and the Bruker Microtof ESI-TOF analyzer (Bruker Daltonics, Germany). Phenolic compounds were separated using a Zorbax Eclipse

XDB-C18 4.6 × 150.0 mm, 5 μm column (Agilent Technologies, Germany). A binary gradient of 2% aqueous acetic acid for mobile phase A and 0.5% acetic acid in water–acetonitrile (1:1, v/v) for mobile phase B at a flow rate of 1 mL min<sup>-1</sup> was applied. The linear gradient was from 10 to 55% B for the time range from 0 to 50 min, from 55 to 100% B from 50 to 60 min and from 100 to 10% B from 60 to 65 min. The mass spectrometry analysis was performed in negative ion mode under the following conditions: analyzer TOF (time-of-flight), ionization source ESI (electrospray), capillary voltage at +4.5 kV, nebulizer gas pressure at 32 psi, dry gas flow at 12 L min<sup>-1</sup>, injection volume 10 μL. The sample and the standards were dissolved in water to a concentration in the range 100–200 mg L<sup>-1</sup>.

### Statistical analysis

Data were reported as mean ± SD (standard deviation) of triplicate determinations. The existence of significant differences among the results for total phenols concentration and total phenols yield depending on the extraction conditions was analysed. For this, one-way analysis of variance (ANOVA) was used followed by the Tukey's HSD or Dunnett T3 test, depending on whether equal variances could be assumed or not. All statistical tests were performed at a 5% significance level using PASW Statistics 18 software.

## RESULTS AND DISCUSSION

The recovery of phenolic compounds from eucalyptus wood veneer waste using aqueous two-phase systems based on PEG 2000 and ammonium sulphate was studied. Equilibrium data was available in the literature.<sup>20</sup> This allowed optimizing time and resources; compositions were selected to cover different tie line lengths (TLL), polymer and salt concentrations and polymer/salt ratios (Table 1). The extraction pH depends on the salt selection. In the present work, ammonium sulphate was chosen and this led to pH values in the range from 5.3 to 5.6 depending on its concentration in aqueous solution. In addition, the influence of the settlement time, extraction temperature, solid–liquid ratio, and extraction time on the total phenols content of both phases was analyzed. The selection of the variables and their intervals was done based on previous experiments with conventional extraction systems (Fernández-Agulló A, unpublished). Total phenols yields obtained from eucalyptus wood using PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ATPS were in the range of those obtained using conventional extraction with water and aqueous solutions of MeOH and EtOH (Fernández-Agulló A, unpublished).

After each extraction process two immiscible liquid phases were formed in all the experiments: a polymer-enriched top phase and a salt-enriched bottom phase. The partition coefficients (K) of phenolic compounds were calculated as the ratio of the total phenol concentration in the top phase to that in the bottom phase. As shown in Tables 2–6, the highest phenols content values were attained in the top phase, showing that phenols tend to concentrate in the PEG-rich phase, as also indicated by the high K values (Tables 2–5). Previous results<sup>22</sup> showed that aromatic molecules prefer the PEG-rich phase of a PEG–salt system. The affinity of phenolic compounds for the top phase can be explained considering hydrophobic and hydrogen bond interactions between the phenolic compounds and the PEG phase-forming components.

The present work deals with a raw material with a complex composition. As stated below, phenolic compounds with varying

**Table 2.** Influence of settlement time on the recovery of phenolics from *Eucalyptus globulus* wood veneers (extraction temperature, 45 °C; S/L, 1:10; extraction time, 90 min; PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.28 (w/w))

Settlement time (h)	Top phase	Bottom phase	F	K
	Total phenols concentration (mg GAE L <sup>-1</sup> )	Total phenols concentration (mg GAE L <sup>-1</sup> )		
			g top phase g <sup>-1</sup> bottom phase	
1	2462 ± 51 <sup>a</sup>	34.7 ± 0.4 <sup>a</sup>	0.55 <sup>a</sup>	71.0
4	2459 ± 50 <sup>a</sup>	30.0 ± 1.3 <sup>b</sup>	0.57 <sup>ab</sup>	82.0
8	2377 ± 153 <sup>a</sup>	26.6 ± 2.0 <sup>bc</sup>	0.61 <sup>b</sup>	89.4
15	2360 ± 113 <sup>a</sup>	24.0 ± 1.9 <sup>c</sup>	0.60 <sup>ab</sup>	98.3

Values are presented as mean ± SD (n = 3).

<sup>a,b,c</sup>In each column, values with different letters are significantly different (P < 0.05).

molecular weights were identified in the extract (Table 7). This makes it difficult to explain more clearly the changes observed in the partition coefficient and in the ratio between the masses of the two phases (F = mass top phase (g)/mass bottom phase (g)).

The distribution of components between the two phases depends on a group of factors that are themselves not independent from each other, and that might present synergic or antagonistic effects depending on the case. In PEG–salt systems the partitioning of biomolecules depends on the volume exclusion effect of the polymer in the polymer-rich (top) phase and on salting out in the salt-rich (bottom) phase. An increase in the concentration and/or the molecular weight of the polymer increases the effect of volume exclusion. In this case, the space for biomolecules in the top phase is reduced and the biomolecules tend to partition to the bottom phase. The solubility of biomolecules in the salt-rich (bottom) phase decreases with increase in salt concentration, which results in increased partitioning of biomolecules to the top phase. The reduced free volume affects high molecular weight molecules more significantly and, therefore, the partition coefficient of these compounds might undergo a greater variation. Conversely, the influence of the available free volume on low molecular weight molecules can be negligible.<sup>23</sup> Partition behavior and K values can also be explained in terms of polarity. Polarity can affect K if compounds to be extracted contain oxygen that can form hydrogen bonds with PEG.<sup>24</sup> Temperature, in addition to affecting TLL as explained below, influences diffusion and mass transfer phenomena.

### Effect of the settlement time

It is well known that phase separation of ATPS under gravity is not as rapid as in water–organic solvent systems. It may vary from a few minutes to a few hours due to the rather low difference in the densities of the two phases (about 0.05–0.15 g cm<sup>-3</sup>).<sup>25</sup>

In the present study the extractions were performed at 45 °C for 90 min at a 1/10 solid/liquid ratio and a 1.28 (w/w) PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ratio. The two phases formed were separated under gravity with varying settlement times. Table 2 shows the total phenols concentration of the separated phases. The total phenols concentration of the top phase varied between 2462 ± 51 mg GAE L<sup>-1</sup> for a settlement time of 1 h and 2360 ± 113 mg GAE L<sup>-1</sup> for 15 h, without significant differences between them, whereas the partitioning coefficient (K) increased continuously. In addition, mass ratio of the two phases (F) did not

**Table 3.** Influence of temperature on the extraction of phenolic compounds from *Eucalyptus globulus* wood veneers (settlement time, 1 h; S/L 1:10; extraction time, 90 min; PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.28 (w/w))

Temperature (°C)	Top phase		Bottom phase		F (g top phase g <sup>-1</sup> bottom phase)	K
	Total phenols concentration (mg GAE L <sup>-1</sup> )	Total phenols yield (mg GAE per 100 mg o.d. wood)	Total phenols concentration (mg GAE L <sup>-1</sup> )	Total phenols yield (mg GAE per 100 mg o.d. wood)		
25	1795 ± 150 <sup>a</sup>	0.69 ± 0.06 <sup>a</sup>	17.5 ± 0.1 <sup>a</sup>	0.009 ± 0.000 <sup>a</sup>	0.72 <sup>a</sup>	102.6
45	2462 ± 51 <sup>b</sup>	0.79 ± 0.01 <sup>a</sup>	34.7 ± 0.4 <sup>b</sup>	0.020 ± 0.001 <sup>b</sup>	0.55 <sup>b</sup>	71.0
65	4778 ± 147 <sup>c</sup>	1.21 ± 0.03 <sup>b</sup>	59.9 ± 1.6 <sup>c</sup>	0.036 ± 0.001 <sup>c</sup>	0.39 <sup>c</sup>	79.8

Values are presented as mean ± SD (n = 3).

<sup>a,b,c</sup>In each column, values with different letters are significantly different ( $P < 0.05$ ).

**Table 4.** Total phenols concentration of the separated phases and total phenols yield depending on the ATPS composition (settlement time, 1 h; S/L, 1:10; extraction time, 90 min; extraction temperature, 65 °C)

PEG2000/(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ratio	Top phase		Bottom phase		F (g top phase g <sup>-1</sup> bottom phase)	K
	Total phenols concentration (mg GAE L <sup>-1</sup> )	Total phenols yield (mg GAE per 100 mg o.d. wood)	Total phenols concentration (mg GAE L <sup>-1</sup> )	Total phenols yield (mg GAE per 100 mg o.d. wood)		
1.28	4778 ± 147 <sup>a</sup>	1.21 ± 0.03 <sup>a</sup>	59.9 ± 1.6 <sup>a</sup>	0.0368 ± 0.0010 <sup>a</sup>	0.39 <sup>a</sup>	80
1.05	5450 ± 222 <sup>b</sup>	1.27 ± 0.15 <sup>a</sup>	120.0 ± 3.5 <sup>b</sup>	0.0787 ± 0.0028 <sup>b</sup>	0.34 <sup>b</sup>	45
1.08	5569 ± 196 <sup>b</sup>	1.15 ± 0.09 <sup>a</sup>	47.8 ± 1.0 <sup>c</sup>	0.0300 ± 0.0020 <sup>c</sup>	0.30 <sup>b</sup>	117

Values are presented as mean ± SD (n = 3).

<sup>a,b,c</sup>In each column, values with different letters are significantly different ( $P < 0.05$ ).

change significantly as settlement time varied between 1 and 4 h, but increased as settlement time increased from 4 to 8 h (Table 2).

Although a thermodynamic equilibrium was not reached in the current conditions, since K values increased with increasing settlement time, it remains to be evaluated if on an industrial scale the increased yield can compensate for the increased process duration. In consequence, a settlement time of 1 h was selected for the rest of the experiments.

### Effect of temperature

To analyze the influence of temperature on the extraction of phenolics from eucalyptus wood, experiments were performed at temperatures of 25, 45 or 65 °C for 90 min at a 1/10 solid/liquid ratio; a 1.28 (w/w) PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ratio and a 1 h settlement time. The composition of the resulting separated phases are shown in Table 3.

As previously indicated, phenolics concentrated preferentially in the top PEG-rich phase and the extraction of phenolics increased with increasing temperature in both phases. This can be attributed to the extracted substances higher diffusion rates and solubilities as the temperature is increased.<sup>26</sup> The highest total phenols concentration in the top phase, 4778 ± 147 mg GAE L<sup>-1</sup>, was obtained at the highest temperature, 65 °C, whereas at 25 °C a significantly different concentration was obtained, 1795 ± 150 mg GAE L<sup>-1</sup>.

An increase in temperature causes in turn an increase in the tie line length and, moreover, the slope of the equilibrium tie lines also increases. This leads to a significant change in the composition of the phases at equilibrium.<sup>20,22</sup> Thus, raising the temperature resulted in an increase of the polymer concentration in the top phase, while the bottom phase at equilibrium was more diluted.

As shown in Table 3, temperature also influenced the mass ratio of the two phases and F decreased with increasing temperature.

A similar tendency was observed for the partition coefficient, although little effect was found when temperature was increased from 45 to 65 °C. As explained previously, higher temperatures increased TLL resulting in higher PEG concentrations in the PEG-rich phase. This also resulted in higher salt concentrations in the salt-rich phase, but not high enough to counteract the effect of the excluded volume in the top phase, and, thus, K decreased.<sup>27</sup> However, total phenols yield followed the same trend as total phenols concentration. At 65 °C, the total phenols yield in the top phase was 1.21 ± 0.03 mg GAE per 100 mg of oven-dried (o.d.) wood and decreased to 0.69 ± 0.06 mg GAE per 100 mg o.d. wood at 25 °C. Then, to analyze the rest of the variables, experiments were carried out at 65 °C.

### Effect of equilibrium phase composition

Extraction experiments were carried out at 65 °C for 90 min at a 1/10 solid/liquid ratio, 1 h settlement time, and variable equilibrium phase compositions of the ATPS. The experiments were performed at PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ratios of 1.28 (system 1), 1.05 (system 2) or 1.08 (system 3) (w/w) (see Table 1). This choice was made so that systems 2 and 3 had approximately the same PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ratio, system 3 higher concentrations of polymer and salt, and system 1 a higher PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ratio than the others.

As shown in Table 4, for the top phase, there were no significant differences among the total phenols yields of the different systems used. The highest value was obtained for the 1.05 PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ratio. On the contrary, for the total phenols concentration, systems 2 and 3, with the lower PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ratios, led to higher values while not showing significant differences between them. The highest value was that of system 3, with the highest PEG/salt ratio in the tie line upper

**Table 5.** Influence of extraction time on the extraction of phenolic compounds from *Eucalyptus globulus* wood veneers (settlement time, 1 h; S/L, 1:10; extraction temperature, 65 °C; PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.08 (w/w))

Extraction time (min)	Top phase		Bottom phase		F (g top phase g <sup>-1</sup> bottom phase)	K
	Total phenols concentration (mg GAE L <sup>-1</sup> )	Total phenols yield (mg GAE per 100 mg o.d. wood)	Total phenols concentration (mg GAE L <sup>-1</sup> )	Total phenols yield (mg GAE per 100 mg o.d. wood)		
30	3860 ± 406 <sup>a</sup>	0.74 ± 0.16 <sup>a</sup>	35.7 ± 1.1 <sup>a</sup>	0.0223 ± 0.0010 <sup>a</sup>	0.30 <sup>a</sup>	108.1
90	5569 ± 197 <sup>b</sup>	1.15 ± 0.09 <sup>b</sup>	47.8 ± 1.0 <sup>b</sup>	0.0300 ± 0.0020 <sup>b</sup>	0.30 <sup>a</sup>	116.5
270	7367 ± 515 <sup>c</sup>	1.25 ± 0.17 <sup>b</sup>	77.8 ± 5.9 <sup>c</sup>	0.0486 ± 0.0032 <sup>c</sup>	0.27 <sup>ab</sup>	94.7
390	8803 ± 279 <sup>d</sup>	1.32 ± 0.14 <sup>b</sup>	83.3 ± 2.2 <sup>c</sup>	0.0506 ± 0.0029 <sup>c</sup>	0.26 <sup>b</sup>	105.7

Values are presented as mean ± SD (n = 3).  
<sup>a,b,c</sup>In each column, values with different letters are significantly different (P < 0.05).

**Table 6.** Total phenols yield depending on the solid–liquid ratio (settlement time, 1 h; extraction temperature, 65 °C; extraction time, 270 min; PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.08 (w/w))

Solid–liquid ratio (w/w)	Top phase	Bottom phase
	Total phenols yield (mg GAE per 100 mg o.d. wood)	Total phenols yield (mg GAE per 100 mg o.d. wood)
0.2:10	1.88 ± 0.04 <sup>a</sup>	0.046 ± 0.013 <sup>a</sup>
0.5:10	1.64 ± 0.09 <sup>a</sup>	0.045 ± 0.004 <sup>a</sup>
0.7:10	1.59 ± 0.08 <sup>a</sup>	0.043 ± 0.002 <sup>a</sup>
1:10	1.25 ± 0.17 <sup>abc</sup>	0.049 ± 0.003 <sup>a</sup>
1.2:10	0.84 ± 0.01 <sup>b</sup>	0.042 ± 0.001 <sup>a</sup>
1.5:10	0.55 ± 0.05 <sup>c</sup>	0.037 ± 0.002 <sup>a</sup>

Values are presented as mean ± SD (n = 3).  
<sup>a,b,c</sup>In each column, values with different letters are significantly different (P < 0.05).

**Table 7.** Phenolic compounds identified in the *Eucalyptus globulus* wood extract obtained under the selected conditions: extraction temperature, 65 °C; extraction time, 90 min; PEG 2000/ (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.05 (w/w); settlement time, 1 h; S/L, 1:10

Compound	[M-H] <sup>-</sup> (m/z)	Retention time (min)
Monogalloyl glucose ( <b>3</b> )	331 <sup>a</sup>	2.1 <sup>a</sup>
Digalloyl glucose ( <b>3</b> )	481 <sup>a</sup>	2.1 <sup>a</sup>
(-)-Gallic Acid ( <b>4</b> )	169 <sup>a</sup>	3 <sup>a</sup>
Ellagic Acid ( <b>13</b> )	301 <sup>a</sup>	26.4 <sup>a</sup>
Quercetin 3-O-rhamnoside ( <b>14</b> )	447 <sup>a</sup>	32.6 <sup>a</sup>
Myricetin 3-O-rhamnoside ( <b>11</b> )	463 <sup>b</sup>	18.9 <sup>b</sup>
Quercetin 3-glucoside ( <b>11</b> )	463 <sup>b</sup>	18.9 <sup>b</sup>
Quercetin 3-glucuronide ( <b>12</b> )	477 <sup>b</sup>	26.0 <sup>b</sup>
Isorhamnetin ( <b>16</b> )	315 <sup>b</sup>	34.8 <sup>b</sup>

<sup>a</sup> According to standards.  
<sup>b</sup> based on molecular weight.

phase composition according to the equilibrium data, which also showed the highest partition coefficient. As TLL increased when changing the PEG/salt ratio, both PEG concentration in the top phase and salt concentration in the bottom phase increased. Thus, the high salt concentration could counteract the effect of the excluded volume in the PEG-rich phase, which would explain the

higher K values for systems 3 and 1.<sup>27</sup> Looking back on the original equilibrium data, the highest total phenols concentration was attained for one of the lower PEG/salt ratio overall compositions and for the highest PEG/salt ratio in the tie line upper phase composition. This kind of information is very useful to select mixtures swiftly.<sup>20</sup> As a consequence, system 3 was selected to continue experimentation.

#### Effect of extraction time

The recovery of phenols depends on the extraction time and thus the highest values are generally obtained with extended extraction times.<sup>26</sup> The influence of extraction times in the range of 30 to 390 min was analyzed in combination with the previously selected values for extraction temperature, 65 °C, PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ratio, 1.08 (w/w), and settlement time, 1 h, at a 1:10 S/L ratio.

As shown in Table 5, the total phenols concentration in the top phase increased significantly with increasing extraction time, from 3860 ± 406 mg GAE L<sup>-1</sup> for 30 min to 8803 ± 279 mg GAE L<sup>-1</sup> for 390 min. F did not change when extraction time varied between 30 and 90 min but decreased when extraction time was increased to 270 min (Table 5). Consequently, the total phenols yield increased significantly up to 90 min, but further increase to 390 min did not improve the results. The partition behaviour also did not change markedly when the extraction time was increased to 390 min.

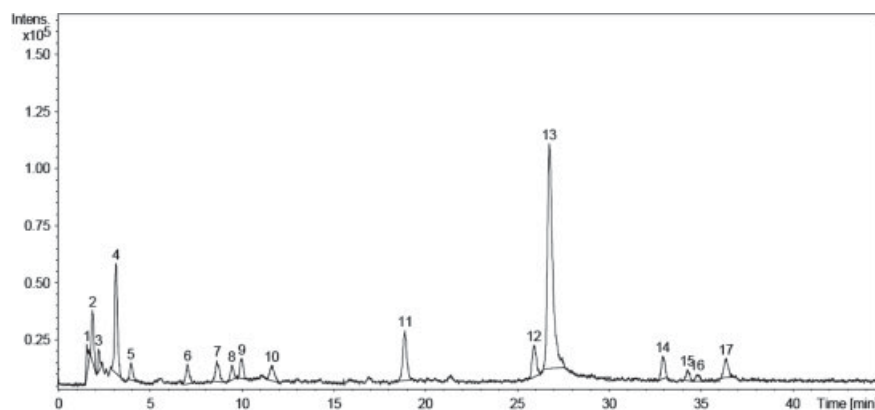
#### Effect of the solid–liquid ratio

The extraction of phenolic compounds is generally influenced by the solid–liquid ratio.<sup>7,28</sup> Extractions were performed at 65 °C for 270 min with a 1.08 (w/w) PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ratio and a 1 h settlement time while varying the solid/liquid ratio from 0.2:10 to 1.5:10 (w/w). The results are shown in Table 6.

The total phenols yield did not change significantly when the solid–liquid ratio was varied from 0.2:10 to 1:10 but its value decreased when the ratio was increased to 1.5:10. Indeed, a lower solid to solvent ratio resulted in a larger concentration gradient during the diffusion from the interior of the material into the solution, that is, the extraction efficiency increased.<sup>29</sup>

#### RP-HPLC-ESI-TOF mass spectrometry results

The chromatograms of all the top-phase extracts analyzed showed the same phenolic compounds. As an example, Fig. 1 shows the HPLC chromatogram for the top-phase extract obtained using a 1.05 (w/w) PEG 2000/(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> ratio at 65 °C for 90 min, a 1:10 solid/liquid ratio and 1 h settlement time.



**Figure 1.** HPLC chromatogram of the top-phase of the eucalyptus (*Eucalyptus globulus*) wood extract (extraction temperature, 65 °C; extraction time, 90 min; PEG 2000/ (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.05 (w/w); settlement time, 1 h; S/L, 1:10) with identified peaks numbered: (3) monogalloyl glucose and digalloyl glucose, (4) (–)-gallic acid, (11) myricetin 3-O-rhamnoside and quercetin 3-glucoside, (12) quercetin 3-glucuronide, (13) ellagic acid, (14) quercetin 3-O-rhamnoside and (16) isorhamnetin.

The following phenolic compounds were identified based on their molecular weight and/or the retention time of the standard compounds used: monogalloyl glucose (peak 3), digalloyl glucose (peak 3), (–)-gallic acid (peak 4), ellagic acid (peak 13) and quercetin 3-O-rhamnoside (peak 14) (Table 7, Fig. 1). All these compounds typically occur in bark, leaves or needles of eucalyptus sp.<sup>30,31</sup> Conde *et al.*<sup>30</sup> detected high concentrations of low molecular weight phenolics (gallic, vanillic and ellagic acids and syringilic, sinapic and vanillic aldehydes) and a great variety of ellagitannins in the *Eucalyptus globulus* wood extracts.

Based on molecular weight, the following compounds might also be present: myricetin 3-O-rhamnoside (peak 11), quercetin 3-glucoside (peak 11), quercetin 3-glucuronide (peak 12) and isorhamnetin (peak 16) (Table 7).

It is worth noting that all the phenolic compounds found have demonstrated antioxidant capacity.<sup>5,32–35</sup> Therefore, due to the presence of these phenolic compounds, *Eucalyptus globulus* wood is an important source of potential natural antioxidants.

Also noteworthy is that, as evidenced from Fig. 1, ellagic acid appears to be one of the predominant polyphenolic compounds in the eucalyptus wood extract. It has been demonstrated that ellagic acid is a bioactive molecule with beneficial characteristics in human and animal physiology and health. Its ability to prevent the formation of various tumors and as an antiviral and antimicrobial agent are some of its benefits.<sup>36</sup>

## CONCLUSIONS

This paper proposes an efficient way of valorizing a waste product from the wood industry, i.e. trimmings of eucalyptus wood veneers used for wood panels finishing. With this purpose, an alternative to conventional solvent extraction for recovering phenolic compounds from vegetable materials using a green technology was studied. Thus, the capacity of ATPS (water + polymer + salt) based on PEG 2000 and ammonium sulphate for phenolics extraction was demonstrated and phenolic compounds with potential antioxidant activity were identified in the recovered extracts. It is worth highlighting the innovative improvement of applying an ATPS directly over a raw material without purification, an economic value for commercial exploitation.

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