Energy and environmental analysis of flavonoids extraction from bark using alternative solvents

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ARTICLE INFO

ABSTRACT

Tree barks are rich in extractive compounds, among which the flavonoids are considered as products of interest. Due to the increase in the demand for these natural products, the development of efficient and sustainable extraction processes is needed. This work aimed to study the selective extraction of flavonoids from *Larix decidua* bark using an environmentally friendly process. For this purpose, different extraction techniques as well as different solvents were used in order to achieve the highest flavonoid content. The characterisation results revealed improvements in extraction yield not only with the use of intensification processes, but also with the use of ionic liquids as solvents with a proven selectivity for flavonoids. [C$_4$C$_1$im]Br and [C$_4$C$_1$im][BF$_4$] considerably improved the total flavonoid content in comparison with the other extraction methods. The antioxidant capacities of all the extracts obtained were very high, confirming their potential for different applications. The [C$_4$C$_1$im]Br (25 wt%) was selected as the best solvent not only because of its good flavonoid extraction ability, but also because of the good antioxidant properties of the extract, and simultaneous microwave-ultrasound assisted extraction was the most energy saving process.

1. Introduction

Tree bark is a widely generated waste due to the debarking process that the wood-based industry has as a pre-treatment. Therefore, bark is considered as a cheap feedstock (Rezaei and Sokhansanj, 2018). Bark, as well as wood, is mainly constituted by structural compounds (cellulose, hemicellulose and lignin). However, unlike wood, bark is rich in extractive content. Among the different families that constitute this fraction, in recent years the flavonoids have been gaining greater importance. This is mainly due to the great benefits that these compounds have, such as antioxidant, anti-allergenic, anti-inflammatory and vasoprotective properties among others (Kesarkar et al., 2009). Flavonoids are phenolic compounds constituted of two aromatic rings joined by a three atom carbon unit, C6–C3–C6 (Tanase et al., 2019a), which can be divided into 6 sub-groups (Fig. 1): flavonols, flavones, isoflavones, flavanones, chalcones and anthocyanins (Panche et al., 2016).

Nowadays, many works are focused on the valorisation of the different fractions of lignocellulosic biomass as possible alternatives to fossil resources (Morales et al., 2020). It is considered an interesting raw material because it is abundant, cheap, renewable, available worldwide, and it does not compete with food supply. The complex structure of the lignocellulosic materials, as well as their lack of homogeneity, presents a significant challenge for biorefineries. Thus generating the need for further research to improve the cost-effectiveness of the process. To improve these limitations, the greatest possible benefit should be gained from each lignocellulosic material. Therefore, it is important not only to take advantage of the structural compounds, but also to exploit the smaller fractions, such as extractives. Tree barks are good candidates for this, since they generally have a high extractive content (Sillero et al., 2019).

Conventional or traditional methods are the most exploited technologies for the extraction of polyphenolic compounds from plants, but they require large amounts of solvent and energy (Bouras et al., 2016; Panja, 2018). Therefore, with the aim of improving the extraction, in recent years both the industrial and the academic communities have been studying the intensification of the processes through the use of new techniques such as microwave, ultrasound and pulsed electrical fields, among others (Perino and Chemat, 2019). However, although these new techniques permit the reduction of extraction time, solvent and energy

consumption, as well as the improvement of extraction yield (Bursać Kovačević et al., 2018), it is true that there is still a lot to do in order to fulfil the principles of green chemistry. Many of the used solvents are volatile organic compounds (VOCs), which generate a large impact on the environment and also pose a health at risk (Thuy Pham et al., 2010). Due to their impact, more environmentally friendly solvents are required to replace them. For that purpose, the use of ionic liquids for the extraction of polyphenolic compounds is being studied. They are generally considered as 'green solvents' mainly due to their non-volatile and non-flammable nature (Singh and Savoy, 2020), in addition they are known as 'designer solvents', which facilitates a more selective extraction of the compounds (Passos et al., 2014). Hence, these new solvents are being studied to carry out more selective extractions of flavonoid compounds from plants (Cui et al., 2018; Dróżdż and Pyrzynska, 2018; Meng et al., 2018).

The aim of this work is to study the selective extraction of flavonoids from *Larix decidua* bark in the most environmentally friendly way. For that, different extraction techniques as well as different solvents are studied. The obtained extracts were characterised by the measurement of total flavonoid content, as well as different antioxidant activities (DPPH, ABTS and FRAP).

2. Material and methods

2.1. Raw material

Errekondo Egur-Zerra Company (Basque County, Spain) provided *Larix decidua* (henceforth "pine") tree bark. It was dried at room temperature, cleaned and ground, to obtain a homogeneous batch of particle size smaller than 0.5 × 0.5 mm. According to our previous work with this raw material, it is known that the chemical composition of the used pine bark was 3.5 wt% ash, 20.0 wt% extractives, 2.0 wt% suberin, 36.8 wt% total lignin, 7.6 wt% hemicelluloses and 25.7 wt% cellulose (Sillero et al., 2020).

2.2. Selected extraction methods to obtain flavonoid compounds

Four different extraction techniques were chosen. The first was the conventional extraction (CE) technique, used as a reference method for the extraction yield. The other three were ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), and simultaneous microwave-ultrasound assisted extraction (SMUAE). The conditions used in the extraction with the CE, UAE and MAE methods were those previously optimised by Sillero et al. (2018). For the extraction using SMUAE, the conditions used were those optimised by Sillero et al. (2020). Table 1 summarises the conditions employed for the different extraction techniques.

Furthermore, in order to study the influence of the used solvents for the extraction of flavonoid compounds, the use of modern and more selective solvents was also studied. For that, CE were carried out by replacing the EtOH/H_2O solvent with different mixtures of IL/H_2O. The extractions were carried out with several aqueous mixtures of ILs at different concentrations (0, 25, 50 and 75 wt%). The used operation conditions were the same as those used for CE (Table 1). In all cases, after the extraction time was over, the solid was separated from the liquid phase by vacuum filtration. Then, the solid phase was washed with distilled water and dried. The extraction yield was gravimetrically calculated using the Equation (1).

$$\text{Extraction yield } (\%) = 100 - \left[\left(\frac{W_{dried\ solid\ without\ extracts}\ (g)}{w_{dry\ bark}\ (g)} \right) x\ 100 \right]$$
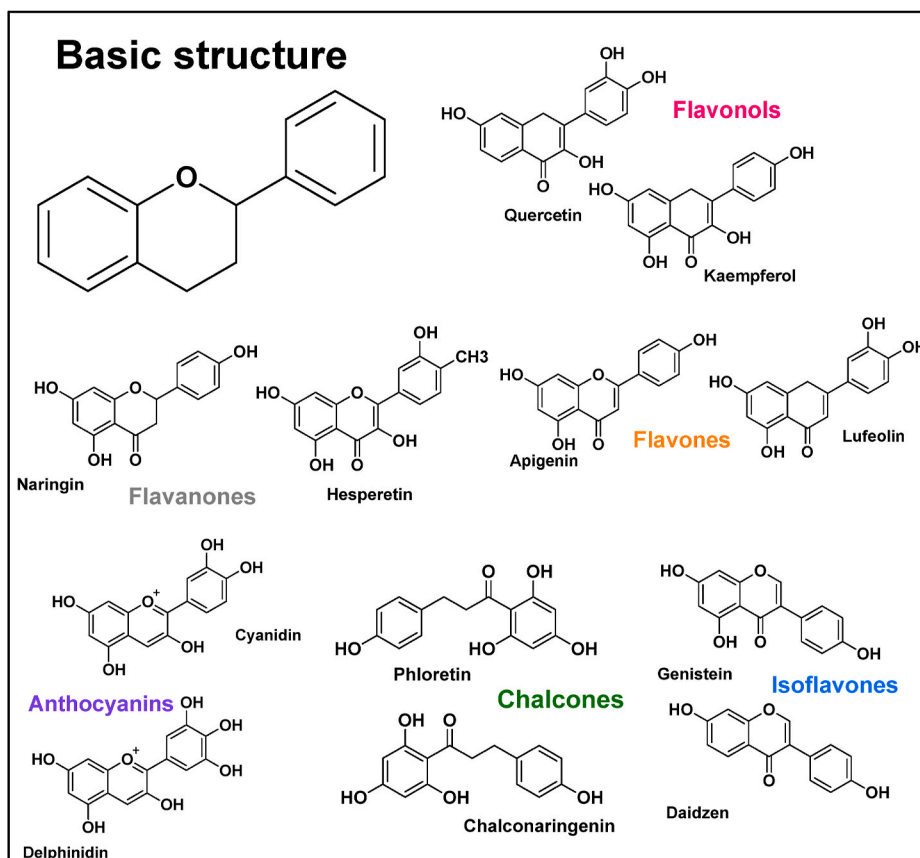
Equation (1)

Fig. 1. Flavonoid sub-groups and its basic structure.

Table 1
Selected extraction techniques and their extraction conditions. In all methods the solid/liquid ratio was 1/10 (w/v).

Extraction method	Solvent	Extraction conditions	Equipment
CE	EtOH/H$_2$O (50/50 (v/v))	Temperature: 58 °C	Heidolph Unimax 1010 + Heidolph Incubator 1000 (Orbital shaker)
		Extraction time: 94 min	
		Shaking speed: 120 rpm	
MAE	EtOH/H$_2$O (50/50 (v/v))	Mw power: 100 W	CEM microwave Discover system model (Open vessel)
		Extraction time: 63 min	
UAE	EtOH/H$_2$O (50/50 (v/v))	Temperature: 65 °C	Elmasonic 570 H, Elma (ultrasonic bath)
		Extraction time: 95 min	
SMUAE	EtOH/H$_2$O (50/50 (v/v))	Mw power: 300W	MILESTONE flexiWAVE + HIELSCHER UIP500hdT (open vessel microwave with an added ultrasonic unit)
		Ultrasound amplitude: 100%	
		Extraction time: 120 seg	
CE	IL/H$_2$O	Temperature: 58 °C	Heidolph Unimax 1010 + Heidolph Incubator 1000 (Orbital shaker)
		Extraction time: 94 min	
		Shaking speed: 120 rpm	

2.3. Characterisation of IL/H$_2$O mixtures

The pH of all the prepared IL/H$_2$O mixtures as well as distilled water were measured using a pH meter (pH-2005 SELECTA). The polarity study was conducted using the most commonly used solvatochromic polarity scale, the multiparameter polarity scale developed by Kamlet and Taft based on three solute-solvent interactions. The studied parameters are: polarisability, π* (Kamlet et al., 1977); hydrogen bond accepting ability (basicity), β (Kamlet and Taft, 1976); and hydrogen bond donating ability (acidity), α (Taft and Kamlet, 1976).

2.4. Synthesis of the ionic liquids (IL)

The three chosen selective ionic liquids (ILs) were 1-butyl-3-methyl-imidazolium bromide ([C$_4$C$_1$im]Br (IL 1)), 1-butyl-3-methylimidazo-lium acetate ([C$_4$C$_1$im][OAc] (IL 2)) and 1-butyl-3-methylimidazolium tetrafluoroborate ([C$_4$C$_1$im][BF$_4$] (IL 3)), and all of which were synthesised in the laboratory before their use for the extraction.

The synthesis of [C$_4$C$_1$im]Br followed the method described by Brandt et al. (2010) with minor modification. Briefly, 90 g of 1-methyl-imidazole freshly distilled was transferred into a 1L two-neck round--bottomed flask. The reagent then was stirred and 70 g of acetonitrile was added. Then, freshly distilled 1-bromobutane in excess (217 g) was added dropwise. Once the addition was finished, the mixture was heated to 75 °C and was left at these conditions for 24 h. The IL crystallised upon cooling down to −20 °C overnight. Finally, the IL was recrystallised with acetonitrile under nitrogen atmosphere, and it was dried and stored until it was used (96.15% yield).

The synthesis of the [C$_4$C$_1$im][OAc] was carried out in 2 steps. The first step was performed in a 600 ml stainless steel 4545 Parr reactor with a 4848 Parr controller. 121 g of previously distilled N-butylimi-dazole was introduced into the reactor with 194 ml of MeOH. Then

dimethyl carbonate in excess (263 g) was added and the mixture was heated to 140 °C, under mechanical stirring for 24 h. Once it was verified that the reaction was complete, the next stage of the synthesis was carried out. The entire mixture was transferred into a 2 L two-neck round-bottomed flask, and it was placed in an ice bath. Later, 59 g of acetic acid was added dropwise, and then left stirring overnight. Finally, the solvent was removed with a rotary evaporator, and the ionic liquid was dried under vacuum at 30 °C overnight resulting in a pale-yellow liquid with a yield of 94.59%.

[C$_4$C$_1$im][BF$_4$] was synthesised by a metathesis following the general method reported by Ab Rani et al. (2011) with the difference of the used starting material. Sodium tetrafluoroborate (115 g) was added to a flask which contained [C$_4$C$_1$im]Br (222 g) in 250 mL of dichloromethane under N$_2$. The mixture was stirred under N$_2$, at room temperature for 24 h. Once the synthesis was completed, it was left to settle, whereupon a white precipitate (NaBr) appeared, which was separated from the IL by cannula filtration. In order to carry out the complete removal of the NaBr, the IL was washed twice more with dichloromethane (2 × 50 ml). Once cleaned, the IL was filtered through acidic and basic alumina, and finally it was dried in vacuum at 45 °C overnight, resulting in a colourless liquid with a yield of 71.63%.

Before being used, the ILs were characterised to verify their correct synthesis using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Nuclear Magnetic Resonance (NMR). All solvents were characterised by ^{1}H-NMR and ^{13}C-NMR. The IL 3 was also subjected to ^{19}F-NMR to verify its structure. The IL characterisation results are shown in Supplementary data.

2.5. Chemical characterisation of bark after extractions

The cleaned and dried solids were subjected to a quantitative acid hydrolysis (QAH) (NREL/TP-510-42618) to determine their lignin, hemicelluloses and glucan content following the methodology described by Sillero et al. (2019).

2.6. Characterisation of bark extracts

The total flavonoids content (TFC) was measured using spectro-photometry, following the procedure described by Sillero et al. (2019) using catechin as standard, and reporting the results as catechin equivalents (CE)/g of dried bark extract (DBE).

Three different methods were used to study the antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH) 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) equivalent antioxi-dant capacity assay (ABTS), and ferric reducing antioxidant power assay (FRAP). They were measured following the methodology described by Sillero et al. (2018), using a Jasco V-630 UV-VIS spectrophotometer. The results obtained in these measurements were expressed as mg of Trolox equivalent (TE)/g of dried bark extract (DBE).

2.7. Energy efficiency and environmental analysis of the extraction methods

The energy consumption and the solvent toxicity of all the different extraction methods used in this work were studied. For the measurement of energy consumption, a Zaeel power meter (an energy consumption meter) was employed to determine the total energy consumption (kW/h) of each process, taking into account both the reaction and the heating process.

The toxicity analysis was done analysing the components of the mixtures separately. First, the toxicity of the ethanol and water was studied by consulting the solvent selection guide produced by Prat et al. (2016). Then, an exhaustive study of each of the ILs used in this work was carried out separately. In this work, not only the final properties of the ILs have been taken into account, but also the processes of the synthesis of these solvents.

Table 2
Kamlet–Taft parameters, using the dye set Reichardt's Dye, N,N-diethyl-4-nitroaniline and 4-nitroaniline.

Solvent		α	β	π*	Reference
H₂O		1.23	0.47	1.14	Jessop et al. (2012)
[C₄C₁im] Br	IL 1	0.36	0.87	–	Lungwitz et al. (2008)
[C₄C₁im] [OAc]	IL 2	0.48	1.20	0.96	Ab Rani et al. (2011)
[C₄C₁im] [BF₄]	IL 3	0.63	0.37	1.05	Ab Rani et al. (2011)

2.8. Statistical analysis

All the measurements were replicated three times, and the results are expressed as mean ± standard deviation. A statistical analysis was performed for the extraction yield by one-way analysis of variance (ANOVA) with IBM SPSS Statistic 24 software. The study of the significance was done using Tukey's range test. Values of $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Evaluation of the employed solvents

The use of water as the solvent for the extraction of different compounds from lignocellulosic biomass is generally the best option. However, due to the properties of water itself, as well as those of the target compounds, its use is not always suitable. Unfortunately, many bioactive compounds, including flavonoids, have limited water solubility (Ali et al., 2019). The aim of this work was the selective extraction of flavonoid compounds, which has been demonstrated to be facilitated by the use of H₂O/organic solvent mixtures (Cho et al., 2016; Tanase et al., 2019b), especially with EtOH. Therefore, in this work, apart from using EtOH/H₂O as a solvent, the use of different ILs to replace the EtOH was also studied in order to improve the extraction of flavonoid compounds. The use of water in mixtures with ILs decreases the viscosity of the solvent, which facilitates the extraction of the target compounds (Dai et al., 2013). This mixture also modifies their polarity, which has a direct effect on the extraction.

The polarity of the solvent is defined as the sum of all possible intermolecular interactions that occur between the solute and the molecules of the solvent. Therefore, it should be expected that the polarities of the ILs studied are not equal due to the different degrees of intermolecular interactions experienced resulting from structural differences of their ions. Proof of this can be found in the solvatochromic parameters measured for each of the solvents listed in Table 2.

The values of π* are affected by both cation and anion. For these types of solvents, the values of π* trend to be higher than for most organic solvents due to the contribution of Coulomb forces. Taking water as a reference, the lowest π* value reported was for IL 2. This may be due to the fact that in the case of the ILs, when more atoms are introduced into the anion there is a decrease in the strength of the Coulombic interactions between the solute and the ion due to the increased dispersion of the delocalised charge (Harris, 2008). The parameter α is mainly influenced by the cationic component of the IL, so it is expected to remain unchanged in the studied cases. However, as can be seen in Table 2 it is not the case. The reason for this is that α values are controlled by the ability of the compounds to act as a hydrogen bond

donor, which in turn is moderated by what they have around them (anions). The β parameter, which describes the ability of the solvent to donate electron density to form a hydrogen bond with the protons of a solute, is more dependent on the anion. In this case, all anions are different, so the values are different in all cases.

The solvents used in this work were a mixture of ILs with water, so the polarities were not the same as those reported in Table 2. Therefore, a theoretical estimation of these polarities is discussed in following sections.

Regarding the toxicity of the solvent mixtures, it is necessary to examine each element separately. Water represents the most appropriate solvent, based on all the consulted solvent guidelines. EtOH is usually considered as a recommended solvent, but its classification is not so clear. This is mainly due to the risk of flammability that compromises the safety of users. In conclusion, it could be said that the EtOH/H₂O mixture is an environmentally friendly solvent.

ILs are often considered as 'green solvents' due to their specific properties, especially their low volatility, which means that they have almost no risk of air pollution and/or flammability (Hospido and Rodríguez, 2019). Nevertheless, it is important to consider other factors to be able to affirm that they are environmentally friendly compounds.

The biodegradability of IL is poor; hence, they could accumulate in soil or water. Płotka-Wasylka et al. (2017) confirmed the toxicity of imidazolium-based IL in aquatic environments, with higher toxicity for IL 3 than for IL 1 (Frade and Afonso, 2010). The adverse effect on the populations of some soil bacteria for IL 3 was also proven in that work. As far as live cycle assessment (LCA) is concerned, the synthesis, the use and the degradation of ILs in ecosystems must be taken into account to understand their real impact. The lack of data makes it difficult to conduct an LCA for IL. Looking at the synthesis process used in this paper, it is confirmed that this step is a serious limiting factor for considering ILs as 'green solvents'. In general, they are processes that require a lot of energy and a considerable amount of reagents, including different VOCs. IL 2 and IL 3 are high-energy consuming products, so their impact is considered to be high. With regard to the effect of ILs on ecosystems, this is closely related to the toxicity and biodegradability explained earlier. According to Thuy Pham et al. (2010), the most desired properties of ILs in industry, non-volatility and thermal stability, are potential problems of degradation or persistence in the environment. From this work, it is also concluded that the use of oxygen containing functional groups, such as acetate, facilitates degradation, whereas halides are more stable and therefore less biodegradable ([BF4]⁻ is worse than Br⁻). Therefore, ILs could have a higher environmental impact during their life cycle than conventional solvents (Zhang et al., 2008).

3.2. Extraction yield

Table 3 shows the results obtained for the extraction yield of the different extraction techniques used in this work. The UAE was the technique with the lowest extraction yield, while the CE and MAE obtained similar values. SMUA was the best extraction technique with the highest extraction yield, with a significant difference compared to the other extraction methods.

Regarding the IL/H₂O extractions, ten different solutions, based on three ILs, were prepared and tested for the evaluation of the selective extraction of flavonoids from pine bark. Table 4 presents the average

Table 3
Comparison of extraction yield obtained at the optimum point for the different extraction methods and extracts characterisation. The values are presented as average ± SD (n = 3). Superscript letters in the yield column depict significant differences (Tukey's test, $p < 0.05$).

Extraction Method	Yield (%)	TFC (mg CE/g DBE)	DPPH (mg TE/g DBE)	ABTS (mg TE/g DBE)	FRAP (mg TE/g DBE)
CE	8.2 ± 0.5[a]	417 ± 16	749 ± 26	807 ± 7	330 ± 30
UAE	6.1 ± 0.4[b]	412 ± 14	750 ± 37	677 ± 35	351 ± 29
MAE	8.3 ± 0.2[a]	430 ± 10	748 ± 38	906 ± 31	390 ± 9
SMUAE	15.7 ± 0.1[c]	433 ± 17	834 ± 8	1173 ± 24	457 ± 18

Table 4

Extraction yield obtained by the different IL/H$_2$O mixtures and characterisation of the obtained extracts. The values are presented as average $\pm$ SD (n = 3). Superscript letters in the yield column depict significant differences (Tukey's test, p < 0.05)).

Solvent	[IL] (wt.%)	pH	Yield (%)	TFC (mg CE/g DBE)	DPPH (mg TE/g DBE)	ABTS (mg TE/g DBE)	FRAP (mg TE/g DBE)
H$_2$O	0	5.80	9.31 $\pm$ 0.17[a]	34 $\pm$ 2	22.3 $\pm$ 0.3	106 $\pm$ 2	31 $\pm$ 4
IL 1	25	3.95	15.15 $\pm$ 0.63[b,c]	779 $\pm$ 32	1075 $\pm$ 21	1933 $\pm$ 89	608 $\pm$ 6
	50	4.51	15.33 $\pm$ 0.44[b,c]	540 $\pm$ 12	728 $\pm$ 20	1095 $\pm$ 27	403 $\pm$ 3
	75	4.91	15.55 $\pm$ 0.23[b,c]	431 $\pm$ 34	650 $\pm$ 28	903 $\pm$ 61	384 $\pm$ 12
IL 2	25	6.53	14.74 $\pm$ 0.70[b,c]	96 $\pm$ 4	102.5 $\pm$ 0.6	599 $\pm$ 58	57 $\pm$ 18
	50	7.57	14.80 $\pm$ 0.06[b,c]	371 $\pm$ 18	368 $\pm$ 16	711 $\pm$ 15	202 $\pm$ 5
	75	9.55	19.28 $\pm$ 0.38[d]	369 $\pm$ 11	306 $\pm$ 9	610 $\pm$ 11	155 $\pm$ 2
IL 3	25	1.93	14.55 $\pm$ 0.48[b,c]	435 $\pm$ 20	279 $\pm$ 19	1053 $\pm$ 43	432 $\pm$ 8
	50	0.90	14.27 $\pm$ 1.83[b]	532 $\pm$ 93	316 $\pm$ 56	1136 $\pm$ 5	493 $\pm$ 57
	75	1.19	15.99 $\pm$ 0.24[b,c]	431 $\pm$ 9	251 $\pm$ 8	1034 $\pm$ 23	368 $\pm$ 12

extraction yield obtained for each of the experiments.

The extraction yield obtained varies from 9% to almost 20% of the dry weight of the bark. The lowest value measured was recorded for water as the solvent, while the highest yield was obtained for IL 3 (75 wt %). All the extractions carried out are significantly different from the one with water, as shown in Table 4. It can also be seen that among all the extractions carried out with the different mixtures of ILs the results obtained do not show significant differences, except in the case of IL 2 (75 wt%).

Regarding the pH of the different solvents (Table 4), no general trend was observed in the influence on the extraction yield. Mixtures of ILs with water showed pH values from very acidic to basic; however, the extraction yield does not seem to be directly affected by this factor under the selected working conditions.

In the case of IL 2, the extraction yield remains stable at concentrations of 25 and 50 wt%, but at 75 wt% of IL, the yield rises. This may be due to a greater variation in the α and β polarity parameters, which are decreased and increased, respectively. This suggests that a higher hydrogen bond basicity enhances the extraction. A similar trend is observed for different concentrations of IL 3, with the difference that the yield with the highest concentration of IL is lower than that measured for IL 2 (75 wt%). However, in this case the polarity parameters studied vary, mainly because of the β parameter, which decreased, perhaps confirming the link between this parameter and the extraction yield.

Comparing the values obtained for the IL/H$_2$O mixtures with those obtained for the conventional solvent mixture (EtOH/H$_2$O), a considerable increase in the extraction yield is noted compared to the extracts obtained by CE, UAE and MAE (Table 3). None of the extraction yields obtained by these techniques are greater than 10%, while all the extractions carried out with the different mixtures of IL do exceed this value. The extraction yield obtained for SMUAE extracts was 15.7%, which is similar and even higher than some of the yields obtained by IL/H$_2$O mixtures.

Studies conducted by Yang et al. (2012) and Sun et al. (2013) for the extraction of Larix bark have shown that [C$_4$C$_1$im]Br is the best IL for extraction compared to other IL or conventional solvents. Furthermore, in both cases the best extraction yield was obtained with an aqueous solution of 1.25 M (27 wt%). Table 4 illustrates that among the ILs investigated here, IL 1 is the one that provides the best extraction yield for concentrations of 25 and 50 wt%, while for the concentration of 75 wt%, the best value is obtained with IL 2.

In order to confirm that only extractive compounds have been extracted, the characterisation of the solids was carried out once the extraction was completed. In this characterisation, the acid-insoluble lignin (AIL), cellulose (represented as glucan content) and hemicellulose content were measured directly by QAH (Table 5).

In general, it can be said that there is no solubilisation of glucan in any of the extractions carried out. In the case of the total hemicellulose content, it is observed that the variation is not very high either, although some trend can be seen. For example, in the case of mixtures with IL 1, a greater solubilisation of this fraction is observed when the concentration

of IL increases. In the cases of IL 2 and IL 3, a decrease in solubilisation of hemicelluloses was shown upon increasing the concentration of IL. AIL is the predominant component and the one with the highest variation observed after extractions. The solid that was extracted with water shows a 50.2% content in AIL. However, this value may be somewhat overestimated due to the presence of extracts and suberin in the sample. In general, for the extractions carried out with mixtures of IL 2, it can be seen that the solubilisation of the AIL increases with increasing IL concentration. However, in the case of the IL 1 mixture, the solubilisation decreased with increasing IL. Since the measurement of AIL can be affected by the presence of extracts (Sluiter et al., 2012), it cannot be confirmed that in the extractions carried out under these conditions there is a real solubilisation of lignin.

3.3. Total flavonoid content

To verify the selectivity of the extraction, the TFC of the extracts was measured (see Tables 3 and 4). It can be noticed that all the studied solvents extracted higher amounts of flavonoids than water. This confirms the low affinity of this type of compound with water (Meng et al., 2018).

The TFC determined for the IL/H$_2$O extractions varies from 96 to 779 mg CE/g DBE. In general, the best values were obtained with the IL 1 mixtures, while the lowest value was determined for IL 2 (25 wt%), not even reaching 100 mg CE/g DBE. IL 2 was the worst IL. IL 1 and IL 3 mixtures extracted the most flavonoid compounds, coinciding with the lowest pH values. In the case of IL 1, it is observed that the increase of pH above 4 led to a decrease of TFC. In the case of IL 3 the opposite is observed, since the highest TFC was determined with the lowest pH. Therefore, it can be confirmed that the pH of the mixture affects the extraction of flavonoids, although other factors such as polarity certainly have an effect. The influence of the anion on the extraction is evident. This suggests that the bigger the β, the more favoured is the flavonoids extraction. However, the highest β value is found in IL 2, but its TFC are lower. It is explained by the dispersion of the charge on the anions with longer chain which reduces the force of solute-IL interaction (Harris, 2008).

Table 5

Chemical composition of the pine bark after the extractions (all results are expressed as wt%).

Solvent	[IL]	Acid-insoluble lignin	Glucan	Hemicelluloses
H$_2$O	0	50.2 $\pm$ 3.2	27.7 $\pm$ 0.6	11.1 $\pm$ 0.1
IL 1	25	43.3 $\pm$ 0.7	28.5 $\pm$ 0.6	10.8 $\pm$ 0.2
	50	44.3 $\pm$ 1.8	29.0 $\pm$ 0.5	10.7 $\pm$ 0.4
	75	44.7 $\pm$ 0.5	28.3 $\pm$ 0.4	10.0 $\pm$ 0.5
IL 2	25	45.9 $\pm$ 1.1	27.2 $\pm$ 0.7	10.0 $\pm$ 0.1
	50	42.9 $\pm$ 0.1	27.6 $\pm$ 0.2	9.4 $\pm$ 0.9
	75	42.6 $\pm$ 1.6	27.7 $\pm$ 0.7	10.0 $\pm$ 0.8
IL 3	25	42.6 $\pm$ 0.6	27.7 $\pm$ 0.6	10.0 $\pm$ 0.1
	50	44.4 $\pm$ 2.0	27.4 $\pm$ 0.1	11.5 $\pm$ 0.3
	75	43.7 $\pm$ 3.4	27.1 $\pm$ 0.1	12.1 $\pm$ 0.1

Comparing the results obtained using the different ILs with those obtained using EtOH/H$_2$O in combination with other techniques (UAE, MAE or SMUAE), it is observed that at least three of the extractions with ILs obtained TFC values higher than those obtained with EtOH/H$_2$O.

Comparing the values obtained in this work with the values reported by other authors, in general it can be said that good results have been obtained. The value obtained by IL 1 (25 wt%) is higher than the value provided by Soto-García and Rosales-Castro (2016) for hydroalcoholic extracts from the bark of *Pinus durangensis* (615 mg CE/g extract). Furthermore, the values measured with IL 2 (50 and 75 wt%) are in the range of the ones reported for acetone/H$_2$O extracts from *Pinus durangensis* (379 mg CE/g extract) (Rosales-Castro et al., 2017), and the ones reported for hydroalcoholic extracts from *Qercus sideroxyla* (386 mg CE/g extract) (Soto-García and Rosales-Castro, 2016). Finally, all the TFCs of the different mixtures of IL 1 and IL 3 reported in Table 4 were better than the TFC determined by Chupin et al. (2015) for the ethanolic extracts from maritime pine (403 mg CE/g extract).

Considering all the aforementioned, it can be seen that the extraction yield is not linked to the flavonoid compounds' concentrations in the final extract regarding the extraction with ILs. This could be because the used solvents are not very selective. All the studied solvent mixtures contain water, which as shown in Table 4, also extracts compounds from pine bark by itself. Nevertheless, these compounds are generally not flavonoids, since the TFC value reported for aqueous extracts is only 34 mg CE/g DBE. Therefore, although ILs enhance the extraction of flavonoids, as well as other phenolic compounds, the presence of water in the mixture will allow the solubilisation of other compounds.

It is observed that the use of ILs in general provides better flavonoid extractions, especially IL 1. This result is in agreement with that obtained by Ma and Row (2017). In that work, they studied the extraction of three flavonoid compounds from *Herba Artemisiae Scopariae* using different ILs and DESs, including IL 1 and IL 3, with IL 1 extracting the greatest amount of flavonoids, 10275.92 μg/g rutin, 899.73 μg/g quercetin, and 554.32 μg/g scoparon.

According to Table 4, the best extraction yield for the IL mixtures used here was obtained for IL 2 (75 wt%). However, this yield is not consistent with a higher TFC. This may result from a lower selectivity of these solvents, since they have also been studied for the delignification of different lignocellulosic materials (Espinoza-Acosta et al., 2014; Kalhor and Ghandi, 2019; Prado et al., 2018). This indicates that the use of the mixture IL 2, together with the extraction of flavonoids, can also solubilise part of the lignin.

3.4. Antioxidant capacities

From the data in Table 4, the low antioxidant capacity reported for the pine bark extracts obtained with water should be highlighted. This is consistent with the low TFC, which suggests that the use of ILs as additives for the extraction has a strong effect on the extraction of bioactive molecules. DPPH has a wide range of values for the different tested extracts, from 102 to 1075 mg TE/g DBE. In general, there is a tendency: the higher the TFC, the higher the DPPH value. This suggests that the solvent properties affecting this parameter are the same that for TFC.

Comparing these values with those reported for the extracts obtained by EtOH/H$_2$O from pine bark (Table 3), it could be seen that in most cases, the reported capacities are lower. The values determined for the extractions carried out using CE, UAE MAE and SMUAE are in the range that 740–840 mg TE/g DBE. Only the DPPH of the IL 1 (25 wt%) extract was higher, and the IL 1 (50 wt%) extract was similar. Since the antioxidant capacities occur not only due to flavonoid compounds, the amounts of other compounds that also provide antioxidant capacity may have decreased (e.g. other phenolic compounds). Hence, the reported values are lower.

The ABTS antioxidant capacity in general were higher than those reported for scavenging capacity against the radical DPPH. The best values were determined for the extracts obtained by the mixtures of IL 1 and IL 3. The best value was determined for IL 1 (25 wt%) extracts, 1933 mg TE/g DBE. This result was better than those reported for CE, UAE, MAE and SMUAE extracts (Table 3).

Regarding FRAP assay, the extracts obtained by IL 2 mixtures have the lowest antioxidant capacities. These values are far below the ones reported for the EtOH/H$_2$O extracts obtained by different extraction techniques, which are in the range of 330–460 mg TE/g DBE. The worst results, as with the other antioxidant capacities, were obtained with the lowest IL 2 concentrations. The values in this case are especially low, since they did not even reach 100 mg TE/g DBE. The extracts of IL 1 (25 wt%) were the ones with the best measured antioxidant capacity (608 mg TE/g DBE).

In the case of pine bark, no other work has been carried out apart from those described in this study. Therefore, a comparison with the values reported for other raw materials has been made cautiously (Table 6).

In the work conducted by Bibi Sadeer et al. (2019) to obtain methanolic extracts from three different tree stem barks, the highest ABTS value was determined for the methanolic extracts of *Sterculia tragacantha* (943 mg TE/g extract), which was exceeded by the pine bark extracts obtained with the mixtures of IL 1 and IL 3. In the case of DPPH, *Macaranga hurifolia* and *Sterculia tragacantha* reported values close to 495 mg TE/g extract, far below those calculated for the extracts obtained with IL 1 mixtures (650–1075 mg TE/g DBE). Analysing the values measured for FRAP, the value reported for the methanolic extract of *Macaranga hurifolia* (622 mg TE/g extract) was higher than the highest

Table 6

Comparison of the antioxidant capacities of the best results measured in this work with those obtained by other authors.

Raw material	Solvent	DPPH[a]	ABTS[a]	FRAP[a]	Reference
Larix decidua	IL 1 (25%)	1075 ± 21	1933 ± 89	608 ± 6	Present work
Macaranga hurifolia	Methanol	494 ± 1	784 ± 14	622 ± 20	Bibi Sadeer et al. (2019)
Sterculia tragapantha		491 ± 2	943 ± 14	583 ± 8	
Zanthoxylum gilletii		83 ± 1	178 ± 4	163 ± 7	
Fagus sylvatica L.	Water	676 ± 20	472 ± 67	625 ± 10	Tanase et al. (2019b)
	Ethanol/water (50:50)	741 ± 59	620 ± 21	783 ± 31	
	Ethanol/water (80:20)	505 ± 42	464 ± 37	593 ± 44	
Eucalyptus globulus	Ethanol	n.f.	n.f.	489 ± 10	Neiva et al. (2020)
	Water	n.f.	n.f.	402 ± 4	
Picea abies	Ethanol	n.f.	n.f.	530 ± 3	
	Water	n.f.	n.f.	346 ± 3	
Acacia melanoxylon	Ethanol	n.f.	n.f.	323 ± 9	
	Water	n.f.	n.f.	292 ± 6	
Acacia dealbata	Ethanol	n.f.	n.f.	1295 ± 32	
	Water	n.f.	n.f.	1132 ± 10	

n.f.: not found.

[a] The measurement units are: mg Trolox equivalent/g dry extract.

value obtained in this work.

Tanase et al. (2019b) characterised the extracts obtained with different solvents using MAE from the bark of *Fagus sylvatica*. The reported values of FRAP were in the range of 592–784 mg TE/g extract, being higher than those reported in this work, except for the IL 1 (25 wt %) extracts. The DPPH values (505–620 mg TE/g extract) were higher than those reported in Table 4, except for all the extracts obtained by IL 1 mixtures, which were higher. Regarding ABTS, the antioxidant capacity reported for 80% EtOH extracts of *Fagus sylvatica* bark, was 472 mg TE/g extract. These values were exceeded in all the experiments of this work.

Neiva et al. (2020) also characterised the potential of different barks using FRAP test. From their results, it is concluded that the range of values for ethanolic and aqueous extracts from different barks is large, from 323 to 1295 mg TE/g extract. Comparing these results with those measured in this work, only the extracts obtained with the IL 1 and IL 3 mixtures are in the same range, although all the values calculated in this work are lower than the best antioxidant capacities given by Neiva et al. (2020).

In conclusion, it could be said that the best flavonoids extractions from pine bark were those carried out with different concentrations of IL 1. Not only because they had higher TFC but also because they showed very high values for antioxidant capacities. All IL 1 mixtures had similar extraction yields (15.15–15.55%), but IL 1 (25 wt%) extracted more flavonoid compounds and reported the best antioxidant capacity. Therefore, it is selected as the best choice. This is in line with the optimisation carried out by Zhang et al. (2014) for the extraction of isoflavones compounds from *Radix puerariae*, where the solvent with the best yield was also IL 1, with a concentration of 1.2 mol/L (26 wt%). Yang et al. (2012) also established IL 1 as the best solvent for proanthocyanidins extraction from *Larix gmelini bark*. The optimal concentration in this case was a little bit higher, 1.25 mol/L (27 wt%).

3.5. Energy consumption analysis of the processes

The energy consumption of each treatment has been studied, since energy consumption is considered one of the most important factors to take into account for a 'green' process. It is well known that CE is a technique that consumes a lot of energy, as has been confirmed in this work (0.65 kW/h). From the CE performed with different solvents, it can be deduced that there is no difference in the energy consumptions. Therefore, it can be said that in this case the solvent does not affect the energy consumption. However, it is true that for the same energy consumption the extraction yield obtained was higher in some cases over others (see Tables 3 and 4).

UAE was the technique with the greatest impact after CE, with a consumption of 0.54 kW/h. In the two cases studied so far, there was a problem of inefficient systems. This is due to the need to heat more water/space than what was really needed. In the case of UAE, which is carried out in an open bath, there was an added difficulty in maintaining constant temperature. MAE had lower energy consumption (0.33 kW/h), and a higher extraction yield. However, SMUAE was the technique that showed the lowest energy consumption, at less than 0.03 kW/h. SMUAE was considered the most environmentally friendly alternative due to its high extraction yield coupled with its energy efficiency. In this way, SMUAE could take over from MAE, which is currently used in industry.

4. Conclusions

The extraction of flavonoid compounds from *Larix decidua* bark was successfully completed using different extraction techniques and solvents. In addition, three different ILs have been successfully synthesised in this work.

It has been proved that aqueous mixtures of ILs can be used as alternative solvents for selective extraction of flavonoid compounds

from pine bark. All the studied cases presented an improvement in the extraction yield compared to the aqueous extraction. Furthermore, these solvents obtained higher extraction yields than those obtained with EtOH/H_2O using intensification techniques such as UAE and MAE. Thus, the potential of these alternative solvents is demonstrated. However, as far as TFC is concerned, only mixtures of IL 1 and IL 3 showed an improvement compared to the results reached with the conventional solvent. Throughout this work, the influence of polarity and pH on the extraction of flavonoid compounds was also confirmed.

IL 1 (25 wt%) was chosen as the optimal solvent not only because of its good flavonoid extraction ability, but also because of the good antioxidant properties of the obtained extracts. The characterisation of these extracts showed that the extract had a high flavonoid content, considerably higher than that measured for extracts obtained using the different intensification methods (MAE, UAE and SMUAE). The increased biological activity of the extracts has also been confirmed, which is very good for different applications in fields as varied as cosmetics, food industry or bio-based materials, among others.

Although the use of ILs for the extraction of flavonoid compounds is promising, especially IL1, there is still room for improvement from an energy and environmental point of view. CE is the most energy-intensive method, so the behaviour of ILs in the different studied intensification processes should be investigated to assess the possible benefits on extractions. In addition, it was noted that IL1 is not very environmentally friendly, so it is necessary to carry out studies for the total recovery of IL and its possible reuse.

CRediT authorship contribution statement

Leyre Sillero: Investigation, Validation, Writing – original draft, Preparation. **Raquel Prado:** Conceptualization, Supervision, Writing – original draft. **Tom Welton:** Methodology, Supervision, Writing – review & editing. **Jalel Labidi:** Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2021.127286.

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