Characterisation of bark of six species from mixed Atlantic forest

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Abstract

Bark is one of the most available by-product derived from the wood-base industry because of the total volume of the tree that comprised. This study aimed at evaluating the chemical composition of barks of six typical species of the mixed Atlantic forest of the Basque Country and the potential of their extractives. The used species were Northern red oak (*Quercus rubra*), Common oak (*Quercus robur*), Common ash (*Fraxinus excelsior*), Iberian White birch (*Betula celtiberica*), Sweet chestnut (*Castanea sativa*) and Black locust (*Robinia pseudoacacia).* Differences between chemical compositions of all the barks were noted. Extractive content was very high for all the barks remarking Sweet chestnut and Common ash with the highest content with 31.89 and 29.44% respectively. The suberin content was higher than 3% with a maximum value for Black locust of 16.37%. Variation of EtOH/H_2O was high depending on studied species with a range of extraction yield of 3.08-15.77%. Total phenolic content of the bark extracts ranged from 178.11 to 635.08 mg GAE/g of dry bark extract and total flavonoid content from 439.19 to 1021.78 mg CE/g of dry bark extract. The antioxidant capacity of the bark extracts was measured by DPPH, ABTS and FRAP and the obtained values were ranged from 167.23 and 1912.38 mg TE/g dried bark extract, 561.92 to 1556.57 mg TE/g dried bark extract 146.11 to 640.30 mg TE/g dried bark extract, respectively. The structural differences were confirmed by GPC and FT-IR, where it was observed an average molecular weight differences and different spectra. The obtained results confirm the high interest in barks source as biomolecules for specific uses such as cosmetics or pharmaceuticals among others.

Keywords

Bark, Chemical composition, Antioxidant capacity, Phenolic compounds, Structural analysis

1. Introduction

The use of renewable sources for the production of energy, chemicals and materials is a growing tendency due to the society concern about the environmental problems, such as, climate change, pollution, biodiversity loss and energy (Álvarez-Álvarez et al., 2018; Carmo et al., 2016b; Gabaston et al., 2017; Komakech et al., 2017; Miranda et al., 2016; Neiva et al., 2018). The general motivation is to reduce the use of fossil fuels as principal source of commodities. The scientific community is searching for more sustainable processes following the green chemistry principles. Thus, the biorefinery technology is evolving exponentially to cover all its potential possibilities including the study of alternative biomass resources from different origins.

Biomass is defined as the organic material that comes from vegetables or animals, including agricultural crops and wastes, forest residues, animal wastes, municipal and industrial wastes among others (Prado et al., 2018). Biomass stems from plants are an attractive source of different products due to their chemical composition, which can be classified as: primary metabolites (nucleic acids, sugars and amino acids), secondary metabolites (phenolic compounds, fatty acids, terpenes, lignans, flavonoids, tannins, waxes, etc. (Dou et al., 2016)) and high-molecular polymeric materials (cellulose, hemicellulose and lignin). Lignocellulosic biomass is composed mostly by cellulose, hemicellulose and lignin in addition to a small amount of ash and extractives (Feng et al., 2013), but the composition mainly depends on the species as well as environmental conditions (age, growth site, etc.) (McKendry, 2002).

Trees are a very used resource around the world, usually at wood-based industries. There are 3,900 million of forest in the world, and more than 900 million of m^3 forest are assigned to that sector (FAO, 2016). In the Basque Country the 68% of the total area is considered as forest, and nearly 55% of the total area of the country is accounted as tree-

covered forest area (HAZI, 2017). 21% of the total area of the Basque Country is considered protected according to Nature 2000, and the 25.5% of the total forest mass (HAZI, 2017). It means that nearly 41% of the total area of the country can be considered as potential resource. The forests of the Basque Country consist of a mixture of different species of softwood and hardwood. Radiata pine is the most extended specie of tree with 123.921 ha of the area and the most developed one but there are many other species. The most important hardwood is beech, and the eucalyptus is becoming more important. During the last years, the extension of mixed Atlantic forest is being increased as a consequence of the abandonment of grasslands and cleared of pine forest (HAZI, 2017), consisting mixed Atlantic forests of a heterogeneous mixture of hardwoods.

Branches, leaves and trunk of the trees mainly compose forest biomass. The trunk and the branches are composed of wood and bark, and their compositions are different due to their different functions at the tree. Wood and bark have the main basic composition, cellulose, hemicellulose, lignin, extractives and ash, however, bark is richer in extractives and suberin (Dou et al., 2016; Rezaei and Sokhansanj, 2018) that help on the protective function that the bark has.

One of the most available byproduct or residue stemmed by the wood-based industries is the bark, because it is removed from the tree before processing them (de-barking). Taking into account that the bark comprised about 9-15% of the total volume of the tree (Feng et al., 2013; Leite and Pereira, 2017), it is noticed that the amount of waste generated is high. Consequently, bark could be considered a cheap feedstock (Rezaei and Sokhansanj, 2018). Up to now, bark is used mostly as a source of energy (Holubcík et al., 2018; Lima et al., 2018; Miranda et al., 2013), but it is also applied in horticulture (Miranda et al., 2012). Nevertheless, even if the caloric value of bark is higher than the one for the wood, due to its content on ashes, it is not the best option to use bark as an energy source because

it can damage the equipment (Holubcík et al., 2018). Taking that into account, and the significant potential of this raw material for the extraction of high-value chemicals, the extraction appears the most suitable way of valorisation.

Tree barks have a chemical complex structure rich on extractives, polyphenolics and inorganic materials (Baptista et al., 2013) that make it an attractive potential feedstock. Historically barks have been used for multiple applications such as medicine, chemistry, materials among others (Leite and Pereira, 2017), and today they are considered as a potential feedstock for biorefinery. For their valorisation, the knowledge of the composition is needed, but currently there are few species that have been completely characterised, the ones that have a higher commercial exploitation. Therefore, the study of a wider range of species is necessary. Thus, in recent years, the composition of different barks extractives have been studied to understand the potential that they have as a source for value-added application (Baptista et al., 2013; Ferreira et al., 2017; Jerez et al., 2007; Miranda et al., 2016, 2012; Rosdiana et al., 2017).

Bark composition can be classified into extractives and non-extractives. Inorganic compounds, lignin, cellulose and suberin are present in non-extractives, and tannins, waxes, terpenes, fatty acids, lignans, flavonoids and extractable carbohydrates are present in extractives (Dou et al., 2016). Some of these biomolecules are bioactive, which can be good not only for health related application but also for food preservation among others. Due to that, the applicability of the extracted molecules from bark is very variable, from pharmaceutical and chemicals to bio-based materials and green polymers (Miranda et al., 2012; Neiva et al., 2018; Sartori et al., 2016).

The aim of this paper is to provide a chemical characterization of the bark of six typical species of the mixed Atlantic forest of the Basque Country, Northern red oak (*Quercus rubra*), Common oak (*Quercus robur*), Common ash (*Fraxinus excelsior*), Iberian White

birch (*Betula celtiberica*), Sweet chestnut *(Castanea sativa)* and Black locust *(Robinia pseudoacacia).* The objective of this characterisation is to analyse their potential as first stage within a biorefinery route. For this purpose, the chemical characterisation has been carried out, as well as the different analysis of the extractive part, in order to analyse the potential of those extractives in a biorefinery route.

2. Material and methods

2.1. Chemicals

Scharlau supplied Gallic acid, Folin-Ciocalteu's phenol reagent, sodium carbonate and ethanol absolute (synthesis grade). Sodium methoxide solution in methanol and iron (III) chloride hexahydrate were obtained from Acros Organics. Panreac AppliChem supplied sodium hydroxide, sodium chloride, potassium di-hydrogen phosphate, potassium chloride, potassium peroxodisulphate, acetic acid glacial technical grade, sodium acetate hydrochloric Acid 37% and sodium phosphate dibasic. Aluminium chloride hexahydrate, 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH), Trolox, Catechin hydrate, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,4,6-Tri(2-pyridyl)-*s*-triazine (TPTZ) were obtained from Sigma-Aldrich. Fisher Scientific supplied dimethylformamide, dichloromethane, and methanol.

2.2. Raw material

The Confederation of Foresters of the Basque Country provided the six different raw materials. The Northern red oak (*Quercus rubra*) was 60 years old, Common oak (*Quercus robur*), Common ash (*Fraxinus excelsior*), and Iberian White birch (*Betula celtiberica*) were 17 years old, and the other two, Sweet chestnut *(Castanea sativa)* and Black locust *(Robinia pseudoacacia)* were 13 years old. All of them were collected in summer, in 2017 in Bizkaia (Spain). First, bark was separated of the wood, and both were dried at room temperature until constant moisture. Then, using a cutting mill, barks were ground and sieved to 0.5 x 0.5 mm in order to avoid differences at characterisation. Finally, the raw materials were stored in darkness at room temperature.

2.3. Chemical characterisation of bark

Moisture of the different samples was determined according to the Technical Report of National Renewable Energy Laboratory (NREL) TP-510-42621 using 1.0 g of material that was heated at 105 ± 3 ºC overnight and the residues were weighed. The ash content was calculated gravimetrically according to the NREL TP-510-42622 using 1.0 g of material that was incinerated at 575 ± 25 ºC for 24 hours and the combustion residue weighed and reported as ash content of the original dry sample.

The extractive content was measured with sequential soxhlet extraction of 5 g of sample with CH_2Cl_2, EtOH and distilled water for 6, 16 and 16 hours respectively following (Miranda et al., 2016). The total amount of solubilized extractives was determined by the mass difference from the mass solid residue after drying at 105 ºC and reported as a percentage of the original dry sample (NREL TP-510-42619).

Suberin content was measured by a modification of Pereira's method (Pereira, 1988). First of all, 1 g of extractive-free material was refluxed with 170 ml of $NaOCH_3$ 3% in MeOH during 3 h. Then the sample was filtrated, washed with MeOH and refluxed again with 70 ml MeOH for 15 min and filtrated. Both filtrates were combined and acidified until pH 6 with H_2SO_4 2 M and dried by evaporation. After that, the residue was suspended in 70 ml of H_2O and a successive liquid-liquid extraction was performed with 130 ml of $CHCl_3$. These extracts were dried over Na_2SO_4, filtrated and dried by evaporation. The total content of suberin was gravimetrically quantified, and the results were reported as a percentage of the original dry mass.

Klason lignin, acid soluble lignin and carbohydrates content were determined according to the Technical Report of National Renewable Energy Laboratory (NREL) TP-510-42618. Raw material, after suberin removal, was treated with 72% H_2SO_4 in a water-bath at 30 ºC for 1 h, after, the acid concentration was reduced to 4% with water and the hydrolysis was completed in the autoclave for 1 h at 121 ºC. The mixture was separated

by filtration and the obtained solid phase was oven-dried at 105 ºC for 24 h. The dried solid was considered as Klason lignin (AIL), and to determine the acid soluble lignin (ASL) an aliquot of obtained liquid phase was measured by UV-vis spectroscopy at 240 nm wavelength. Klason lignin and acid soluble lignin were reported as a percentage of the original dry mass.

The polysaccharides determination was carried out in the filtrated liquid phase by High Performance Liquid Chromatography (HPLC) with a Jasco LC Net II/ADC chromatograph equipped with a refractive index detector using a column Aminex HPX-87H with 300 x 7.8 mm (Bio-Rad Laboratories, USA). The mobile phase was H_2SO_4 0.005 M at a flow rate of 0.6 mL/min at 50 ºC. The polysaccharides were reported as a percentage of the original dry mass.

2.4. Characterisation of bark extracts

2.4.1. Extract acquisition

An EtOH/H_2O extraction has been done for the characterisation of the phenolic content and antioxidant activities of barks. 4 g of dry bark was extracted with EtOH/H_2O (50/50 (v/v)) mixture as solvent with a solid-liquid ratio of 1:10 (w/v) using an ultrasound bath with temperature control (Elmasonic 570 H, Elma) during 1 h at 50 ºC (Miranda et al., 2016). The extracts were filtrated under vacuum and supernatant was stored at 4 ºC. The yield of the extraction was calculated gravimetrically and referenced to a 100 g of dried bark. The extraction method was chosen not because it is a conventional method, but because it is the most used lately by different authors to carry out this type of characterisation. As it is cited before, Miranda and co-workers use this method to characterise Eucalyptus sideroxylon (Miranda et al., 2016). It was also used by Lima, Carmo and Ferreira in some of their researches to characterise bark extracts (Carmo et

al., 2016a, 2016b, 2016c; Ferreira et al., 2018; Lima et al., 2018). Besides being the most used method lately, it also has advantages with respect to conventional extractions. By the use of ultrasound assisted extraction, it is possible to reduce the extraction time (only 1 h) in addition to that fact, the extraction is favoured thanks to the disruption generated by ultrasound in the cells of the raw material.

2.4.2. Phenolic content of bark extract

Folin-Ciocalteu method (Singleton and Rossi Jr., 1965) was used for the determination of total phenolic content (TFC) using Gallic acid as standard. A diluted aliquot of the extract (300 µL) was mixed with 2.5 mL of the Folin-Ciocalteu reagent. Then 2 mL of Na_2CO_3 7.5% solution was added. After 5 min of incubation at 50 ºC in a bath, absorbance at 760 nm was measured. The results were expressed as mg of Gallic acid equivalents (GAE)/g of dried bark extract.

$AlCl_3$ colourimetric assay was used for the determination of total flavonoid content (TFC), using catechin as standard (Lima et al., 2018). 2 mL of a diluted aliquot of the extract was mixed with 0.3 mL of $NaNO_2$ 5% solution. After five minutes, 0.3 mL of $AlCl_3$ 10% solution was added, and after other 6 min, 2 mL of NaOH 1 N was added to neutralize the mixture. After 5 min, the absorbance at 510 nm was measured. The results were expressed as mg of catechin equivalents (CE)/g of dried bark extract.

2.4.3. Antioxidant activities of bark extract

In order to have a global vision of the real antioxidant capacity of each bark EtOH/H_2O extract three different types of antioxidant capacity assays (DPPH, ABTS and FRAP) were determined.

All methods are based on the reaction of specific radical with the extracts, and these reactions are measured by UV-VIS spectroscopy because of a colour change made during

177 the reaction. Taking that into account, DPPH, ABTS and FRAP were measured at this
178 work. FRAP is a method based in a reduction of the complex ferric ion-TPTZ, DPPH is
179 a method that measures the quality of hydrogen donors and ABTS is a method based on
180 the lost electron of the ABTS radical. Trolox was used as standard and the results were
181 expressed as mg of Trolox equivalent (TE)/g of dried bark extract.

182 The methodology described by Gullón and Sillero (Gullón et al., 2017; Sillero et al.,
183 2018) was followed to perform DPPH radical scavenging assay, ferric reduction
184 antioxidant power (FRAP) assay and ABTS assay.

185 *2.4.4. High Performance Size Exclusion Chromatography (HPSEC)*

186 Molecular weight (Mw), number-average (Mn) and the polydispersity index (Mw/Mn) of
187 the isolated extractives were analysed by high performance size exclusion
188 chromatography (HPSEC). The used chromatograph was a Jasco LC-NetII/ADC
189 equipped with a RI-2031Plus reflex index detector and provided with two PolarGel-M
190 columns in series (300 x 7.5 mm) and PolarGel-M guard (50 x 7.5 mm). The used
191 conditions were 0.7 mL per min flow, 20 µL of injection volume and temperature of 40
192 ºC using dimethylformamide with 0.1% of lithium bromide as eluent. Calibration was
193 carried out using polystyrene standards ranging from 266 to 70,000 g/mol (Sigma-
194 Aldrich).

195 *2.5. FTIR, Fourier transform infrared spectroscopy*

196 FTIR spectra of six different original material as well as obtained EtOH/H_2O extracts for
197 each raw material were determined on a PerkingElmer Spectrum Two spectrometer fitted
198 with a Universal Attenuated Total Reflectance accessory. The defined work range was
199 from 700 to 4000 cm^{-1} with 4 cm^{-1} resolution. 12 scans were recorded for each sample.

200

3. Results and discussion

3.1. Chemical composition

The six different hardwoods barks were chemically characterised and the calculated compositions are shown in Table 1. Even if all the analysed bark species are hardwoods, considerable differences have been found in their chemical composition.

In terms of the total ash content, Iberian white birch has the lowest ash content with 3.39%, which is higher than the *B. pendula* reported by Miranda (Miranda et al., 2013). Sweet chestnut, common oak and common ash have a similar ash composition with 5.14, 5.47 and 5.17% respectively, and northern red oak and black locust have the highest ash content (6.23 and 6.22% respectively). The comparison with other studies must be done cautiously due to the high heterogeneity of bark, whose composition differs among tree part and species, season and location (Dou et al., 2016). Carmo reported a value of 4.2% of ash content for *Copaifera langsdorffi* bark (Carmo et al., 2016b), 2.6% for *Quercus cerris* bark was reported by Şen (Şen et al., 2010) and between 1.3 and 5.4% of ash content was reported for different Eucalyptus species barks (Lima et al., 2018). Focusing on the oaks, between 2.68 and 3.83% of ash content was reported for *Q. laurina and Q. crassifolia* bark by Ruiz-Aquino (Ruiz-Aquino et al., 2015) and 14.56% was reported for *Q. faginea* by Ferreria (Ferreira et al., 2018).

The content of extractive differs a lot between the different bark species with the lowest concentration for northern red oak (12.11%), close to Black locust (12.72%), and the highest for Sweet chestnut (31.89%). This value is not in accordance with what was studied previously, which reported 14.55% of total extractives for Chestnut (Özgenç et al., 2017b) measured with alcohol-benzene. Focusing on the composition of different hardwood species, the existing differences are remarkable with the lowest content of

225 extractives just 5.5% for *E. resinifera* (Lima et al., 2018) and highest content 55.74% for
226 *E. sideroxylon* (Miranda et al., 2016). In the case of oaks, the reported concentrations
227 were between 12.7 and 31.7% (Ferreira et al., 2018; Ruiz-Aquino et al., 2015), which
228 fixed better with concentrations measured at this work not only for oaks but also for the
229 rest of species. 13.0% of total extractives was reported for Black locust bark by Putman
230 (Putman et al., 1989), similar to the obtained results in this study. In order to measure the
231 total extractive content three consecutive extraction were performed with CH_2Cl_2, EtOH
232 and water. The differences with the total extract content for each solvent have also a large
233 variation, but in five of the six studied barks, the highest total content of extractive has
234 been measured with H_2O as a solvent, even though the percentage between them differs.
235 Common ash is the only bark that the highest total extractive content is with EtOH as
236 solvent (18.49%). It is also the one that has the highest CH_2Cl_2 extractives (4.30%), which
237 means that is the bark with more non-polar extractives. Sweet chestnut is the richest in
238 water-soluble extractives content with 20.43%.

239 Suberin content in Black locust (16.37%) is remarkable high, close to 4 times higher than
240 for the other five barks but is lower than the reported by Putman (Putman et al., 1989).
241 Common ash is the one with the lowest suberin content (3.01%), for the others, the value
242 is very close to 4%. All these concentrations are greater than the ones reported by Miranda
243 and Lima for different Eucalyptus species, between 0.6 and 1.92% (Lima et al., 2018;
244 Miranda et al., 2016, 2013). Ferreira reported 2.94% (Ferreira et al., 2018) of total suberin
245 content for *Q. faginea* bark, close to the obtained concentration for common ash, and
246 Ruiz-Aquino (Ruiz-Aquino et al., 2015) has reported 3.57% of suberin content for *Q.*
247 *laurina* inner bark, which is similar to the obtained for the two oak species studied at this
248 work.

The total lignin content differs also between species from 18.64 to 36.42%, Common ash and Iberian white birch respectively. The obtained concentration are similar to the ones reported in the literature for other hardwoods species which are in the range of 13.1 (Miranda et al., 2016) – 39.7% (Ruiz-Aquino et al., 2015). The main difference at lignin content is for Klason lignin, where the concentrations vary between 13.13 and 30.82%, while acid soluble lignin content has not substantial differences, similar results as those reported by Lima (Lima et al., 2018).

The polysaccharides content, determined as the combination of sugars before and after acid hydrolysis, reveals small difference in composition between hardwood species, with a 41.90% for the highest content. 41.90% and 41.31% of total polysaccharide content have been obtained for Northern red oak and Common ash, respectively, which are similar to the results reported by Ferreira (Ferreira et al., 2018). However, other authors have reported higher concentration of polysaccharides, as for example, the concentration reported for *Eucalyptus globulus* bark, 61.14% by Neiva (Neiva et al., 2018), or the ones given for 11 different species of eucalyptus bark by Lima, which values are between 58.1 and 69.9% (Lima et al., 2018).

3.2. Phenolic content of bark extracts

The quantification of some phenolic compounds, such as total phenolic and total flavonoids, in bark extracts have been studied at this work. The used extraction method was carried out in an ultrasound bath with $EtOH/H_2O$ mixture as solvent, and the extraction yields are given in Table 2.

Common ash has the bark with the highest extraction yield, 15.77% and Black locust and Northern red oak are the two with the lowest extraction yield, 3.08 and 3.20% respectively. All the extraction yields obtained using this extraction method are lower

than the results obtained for the total polar extractives determined by successive Soxhlet extraction used for characterisation (Table 1).

The composition of extract varies among the different barks. Total phenolic content (TPC) differs from 178.11 to 635.08 mg GAE/g dried bark extract (Black locust and Sweet chestnut respectively). Common oak has also a high TPC, 610.63 mg GAE/g dried bark extract. The lowest TPC concentration coincides with the lowest extraction yield, but the greater TPC is not measured for the bark with the highest extraction yield. That is because the extraction method is not selective enough and there are not just phenolic compounds. The values for total flavonoid content (TFC) are ranged from 439.19 to 1021.78 mg CE/g dried bark extract (Common ash and Common oak respectively).

Even if TPC and TFC are not reported for the bark species that has been studied in this work, a wide range of results has been reported for other hardwood species for EtOH/H_2O extractions. In the case of different Eucalyptus bark species, the concentrations given for TPC are ranged from 282.5 to 916.7 mg GAE/g extract according to Lima (Lima et al., 2018). Miranda has also reported TPC for *Eucalyptus sideroxylon* within that range, 440.70 mg GAE/g extract (Miranda et al., 2016). The values reported by Sartori (Sartori et al., 2016) for different *Eucalyptus urophylla hybrids* species have a similar range than the ones reported by Lima, from 210.9 to 550.9 70 mg GAE/g extract. Neiva studied the phenolic content of *Eucalyptus globulus* industrial bark for the extracts removed with H_2O and EtOH and the reported range was from 144 to 403 mg GAE/g extract (Neiva et al., 2018). Other hardwood species barks were also studied, by Carmo: *Goupia glabra*, *Copaifera langsdorffii* and *Albizia niopoides* (Carmo et al., 2016a, 2016b, 2016c), 158.2 mg GAE/g extract, 589.23 mg GAE/g extract and 247.15 mg GAE/g extract respectively. Ferreira reported similar value (630.33 mg GAE/g extract) for *Quercus faginea* (Ferreira et al., 2018). Kähkönen and Santos studied TPC values for hardwood barks with other

298 extraction methods. MeOH/H_2O extraction was carried out for *E. grandis*, *E. urograndis*
299 and *E. maidenii* by Santos and co-workers (2012) and *Betua pendula* by Kähkönen
300 (Kähkönen et al., 1999). The reported concentrations were 385.63, 346.72, 203.86 and 2
301 mg GAE/g extract respectively.

302 Total flavonoids content in the EtOH/H_2O extract of studied barks were higher or in the
303 range of published results for barks of other hardwood species. For different Eucalyptus
304 barks the highest concentrations for TFC was reported by Sartori, with 550.9 mg CE/g
305 extract for *E. urophylla* × *E. camaldulensis hybrid* (Sartori et al., 2016). The lowest
306 concentration was reported for *E. ovata* by Lima (Lima et al., 2018), 121.0 mg CE/g
307 extract. When water is the only used solvent, lower TFC was reported for *Eucalyptus*
308 *globulus*, 73.5 mg CE/g extract (Neiva et al., 2018). In the case of Quercus family,
309 Ferreira reported a result of 204.7 mg CE/g extract for *Quercus faginea*, whose value is
310 below those obtained in this work for Northern red and Common oak. Other barks of
311 hardwood species have been studied, but only the TFC obtained for *Copaifera*
312 *langsdorffii* (Carmo et al., 2016b), 441.90 mg CE/g extract, was close to the results of
313 this work.

314 *3.3. Antioxidant capacity of bark extracts*

315 Concentrations obtained for scavenging capacity against the radical DPPH of EtOH/H_2O
316 extracts of each bark are ranged between 167.23 and 1912.38 mg TE/g dried bark extract
317 (Black locust and Iberian white birch respectively), which is a big range. Common oak
318 and Sweet chestnut have results above 1200 mg TE/g dried bark extract (1521.25 and
319 1217.18 mg TE/g dried bark extract respectively).

320 ABTS assay was carried out for EtOH/H_2O extracts of each bark and it is observed a
321 difference between the lowest and highest results. Common oak has the greater result,

1556.57 mg TE/g dried bark extract, and Northern red oak and Black locust the lowest, 561.92 and 584.85 mg TE/g dried bark extract respectively. Sweet chestnut and Iberian white birch have also values above 1000 mg TE/g dried bark extract.

The reducing ability of the EtOH/H_2O extracts of each bark was measured by FRAP and the obtained results differ from 146.11 to 640.30 mg TE/g dried bark extract. The lowest value corresponds with Black locust extracts and the highest with Common oak.

Few results are reported in the literature for the antioxidant properties of bark extracts, and usually, the only one that is used is DPPH. The most studied bark extracts are Eucalyptus, and the results given for Trolox equivalent antioxidant capacity (TEAC) are ranged between 277.3 (Sartori et al., 2016) to 1042.2 mg TEAC/g extract (Lima et al., 2018), *E. urophylla* × *E. grandis hybrid* and *E. rudis* respectively. Ferreira reported 1576.12 mg TEAC/g extract for *Quercus faginea* bark, very similar value to the result obtained in this work for Common oak. Other results have been published for other hardwood´s barks as 563.4 mg TEAC/g extract for *Goupia glabra* bark (Carmo et al., 2016a), 839.05 mg TEAC/g extract for *Albizia niopoides* bark (Carmo et al., 2016c) and 720.28 mg TEAC/g extract for *Copaifera langsdorffii* bark (Carmo et al., 2016b). Other extraction methods have been reported in the literature. Fernández-Agulló reported values from 6.98 to 9.67 mmol TE/g extract for different extraction with different solvents for *Eucalyptus globulus* wood (Fernández-Agulló et al., 2015). Francezon took out extracts of Black spruce bark with hot water, and the DPPH given are lower than those reported in this work, between 308 and 962 μmol TE/g dry extract (Francezon and Stevanovic, 2017).

The comparison of the results with other data from literature must be done carefully because of the differences in methods, calculations and standard. This problem is more noticeable for FRAP and ABTS. However, the extract of the six studied barks show a

lower reducing ability (FRAP) than the result reported by Ferrerira for *Quercus faginea*, 4.44 mM TEAC/g extract (Ferreira et al., 2018).

The interest for bioactives compound for different possible uses as pharmaceutical products, cosmetics and food is increasing at present. It allows considering studied barks as an interesting source for valorization taking into account that barks are rich in phenolic compounds and that they have high antioxidant capacity.

3.4. High Performance Size Exclusion Chromatography (HPSEC)

The molecular weight distribution of the six extracts has been analysed by GPC and the obtained results are summarized in Table 3. All extracts consisted of a heterogeneous mixture of compounds with differentiated fractions. The average molecular weight differs a lot between different bark extracts, and the average polydispersity (Mw/Mn) is very high. The highest average-molecular weight is obtained for Sweet chestnut, 57387 g/mol, with a polydispersity of 27.99. Analysing the different fractions, 86.69% of the total molecules have an average molecular weight of 66134 g/mol, which means that the extracted compounds have a high molecular weight. For this fraction, the polydispersity is also high. On the other hand, the other two fractions have an average molecular weight of 249 and 499g/mol. Iberian white birch bark extract has also a high average-molecular weight, followed by Common oak and Northern red oak (30972, 20288, 17211 g/mol respectively). Polydispersity for those extracts are also high as well as for Sweet chestnut. The extracts of the barks of Common oak and Iberian white birch has 4 differentiated fractions of molecular weight. Moreover, both of them have more than the 76% of the total molecular content in the highest fraction, with an average molecular weight of 26283 and 37470 g/mol respectively. The extracts of the barks of Black locust and Common ash have the lowest global average-molecular weight with low polydispersity 6334 and 3682 g/mol respectively). Common ash extract is the one that has more peaks, with 6, all of

them with a low polydispersity. 82.28% of the total molecular weight is lower than 500 g/mol in contrast to the high results of the others bark extracts.

Figure 1 shows the mean differences between the composition of the different bark extracts and also the big peak for the biggest molecular weights in the case of Sweet chestnut, Iberian white birch, Common oak and Northern red oak. In the case of Black locust and Common ash, the percentage of the difference obtained molecular weight fractions are more balanced and it can be seen represented at Figure 1.

Few articles have reported GPC characterisation of the extracts and the used extractions methods are not the same, because of that, the comparison with the literature must be made cautiously. Pan has reported the average molecular weight for lignin, tannin and cellulose fraction of two softwood, Douglas fir and Loblolly pine barks (Pan et al., 2013). According to this study, lignin and tannin fraction have a similar range between 5120 to 13100 g/mol, but for lignin, Douglas fir bark has the highest value and for tannin is the opposite. Focusing on the cellulosic fraction, the measured range was between $8.41 \cdot 10^5$ and $1.21 \cdot 10^6$ g/mol, with the highest value for Loblolly pine bark (Pan et al., 2013). Different authors have reported studies of molecular weight for different pines bark extracts. Bocalandro has studied the molecular weight of *Pinus radiata* bark extracts obtained with hot water, and he identified a peak around 300 g/mol assigned to some flavonoids, and other at 580 g/mol assigned to proanthocyanidin B-2 dimer (Bocalandro et al., 2012). Some commercial bark extract from *Pinus pinaster* and *Pinus massoniana* were analysed by Weber and co-workers (2007) and they concluded that *Pinus pinaster* bark extracts contain higher molecular weight proanthocyanidins, but in both samples, the majority of compounds have a molecular weight below 1180 g/mol (Weber et al., 2007). In the case of hardwood bark, the average molecular weight of *Eucalyptus globulus* acetylated bark extracts obtained with different solvents are from 314 to 1167 g/mol

(Vázquez et al., 2008). Taking into account all the different published results it can be concluded that the molecular weight of bark extracts depends on the species and the extraction conditions (Chen and Hatano, 1990).

3.5. FTIR, Fourier transform infrared spectroscopy

The spectra of the analysed raw material are presented in Figure 2 and the spectra of the EtOH/H_2O bark extracts are presented in Figure 3. The bands assignments are summarised in Table 4 for barks and Table 5 for bark extracts. The analysis of the spectra are based in reported results of other authors and they are summarised in each table.

According to the band assignment in Table 4, it is shown that the main detected band are common in all of the studied raw material. Nevertheless, some specific band only appear for some barks. For example, the band at 1631 cm^{-1}, identified by Traoré (Traoré et al., 2018) as absorbed O–H and conjugated C–O in polysaccharides, only appear for Black locust. However, the band range of 1603-1610 cm^{-1} is present at all barks except at Black locust. Other bands at the range of 1419 to 816 cm^{-1} are also different depending on the analysed bark. Back locust, Northern red oak and Common ash have a band at 1419 cm^{-1} that is identified as C–H asymmetric deformation in methoxyl and aromatic skeletal vibrations by Traoré. In the case of the bands fixed at 1264 and 1224 cm^{-1}, the bark that has the first one do not have the second one and vice versa. The last identified band is at 825 cm^{-1}, and Common ash and Common oak do not have it.

Analysing the results represented in Figure 2 it is noted that for Black locust the band defined for -CH stretch vibration in aromatic methoxy groups and in methyl and methylene groups of side chains (between 2850-2930 cm^{-1}) is relatively more intense than for the other barks. The band at 3300 cm^{-1} is narrower for Common ash, Sweet chestnut and Iberian white birch. Sweet chestnut and Iberian white birch have a similar spectrum

421 but it is evident that Sweet chestnut has relatively more intense band at 1610 and 1370
422 cm^{-1}. Comparing all the spectra it is noted that Common ash has a bigger band than the
423 rest barks at 1024 cm^{-1}.

424 Going over the results of FT-IR for bark extracts summarised at Table 5 it was noticed
425 that the main differences in band identification appear between bands 1500 and 900 cm^{-1}.
426 Common ash is the bark extract with more identified band at FT-IR and it has three
427 bands, which correspond to aromatics, that none else has at wavenumbers 1412, 1264 and
428 929 cm^{-1}. However, it does not have a band at 1275 a 1200 cm^{-1}. In other wavenumbers,
429 it has band identified but is not the only bark extract that has this band, as for example at
430 1234, 1156, 1078 and 1033 cm^{-1}. Those differences at bark extract structure make also
431 the difference at chemical properties of extracts.

432 In Figure 3, all the bark extract spectra are represented in order to compare them, and as
433 well as for the FT-IR of barks, the main differences are presented from 1720 to 700 cm^{-1}
434 even if some variances can be seen at the beginning of the spectra. For instance, bands at
435 2930 and 2850 cm^{-1} have very low relative intensity for Sweet chestnut and Common
436 oak. The band range 1705-1720 cm^{-1} is almost invisible for Black locust, and a bit
437 relatively more intense for Sweet chestnut and Northern red oak. Aromatic skeleton
438 vibrations identified at band 1605 cm^{-1} has relatively high intensity for all the bark
439 extracts but not for Common ash. Nevertheless, at 1515 cm^{-1} Common ash has the most
440 relatively intense band. Sweet chestnut and Common oak have a similar bands at 1308
441 and 1275 cm^{-1}, but the relative intensity is higher for the first one. Band range 1245 to
442 900 cm^{-1} identified for Northern red oak and Black locust are similar, as well as the band
443 at this range for Iberian white birch and Sweet chestnut, while the relative intensity of the
444 last one is bigger. Analysing the oaks spectra, it is noted that even though barks spectra

were similar, there are differences in the obtained bark extracts, which can confirm the differences at measured chemical properties.

4. Conclusions

Six different bark of the typical species of the mixed Atlantic forest of the Basque Country were characterised in order to consider their potential valorisation within a biorefinery approach. Taking into account all the different results obtained for the chemical composition of the studied raw material it is clear that the composition of bark depends mainly on the species. Identifying the chemical composition differences may affect the possible valorisation routes of barks. In this sense, Black locust would be very good for suberin extraction due to it high suberin concentration, and Sweet chestnut and Common ash would be very good for polar extractives due to their high content mainly on EtOH or water extractives. In general, the barks could be good for extractives valorisation due to their higher content on extractives and lower content on polysaccharides in comparison to wood.

The extraction yield with EtOH/H_2O using ultrasound bath were not close to the total extractives measured for the chemical characterisation of barks, so an optimisation of the extraction could be needed. Nevertheless, with those extracts analysis, it is understood that all of the studied barks can be considered as a source of polar extractives. These extractives are composed, among other things, by phenols and polyphenols that are important free radical scavenging antioxidants with interesting bioactivities. This property was measured in this study by phenolic contents and antioxidant capacities. All extracts have important phenolic content and good antioxidant capacities and even if the concentrations differ between species. Iberian birch bark is one with the highest antioxidant potential given by DPPH and Common oak has the higher antioxidant potential given by ABTS and FRAP.

For an integrated valorization strategy, the raw material from the wood-based industries is interesting sources of bioactive compounds or chemical intermediates due to their chemical functionalities and bioactivity. In this sense, bark can be considered as a source of bioactive compounds with a potential valorization as pharmaceuticals, additive in food, drugs, cosmetic industry or chemicals for bio-based materials and polymers.

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Table 1: Chemical composition (% of the total dry mass of bark) of the bark of six typical species of the mixed Atlantic forest of the Basque Country.

	Sweet chestnut	Northern red oak	Common oak	Black locust	Common ash	Iberian white birch
Ash	5.14 ± 0.02	6.23 ± 0.16	5.47 ± 0.02	6.22 ± 0.02	5.17 ± 0.08	3.39 ± 0.12
Extractives	31.89 ± 1.35	12.11 ± 0.36	22.99 ± 0.81	12.72 ± 0.74	29.44 ± 0.52	14.29 ± 0.48
Dichloromethane	1.95 ± 0.04	2.74 ± 0.13	1,09 ± 0.03	3,76 ± 0.07	4.30 ± 0.01	2.65 ± 0.01
Ethanol	9.52 ± 0.23	2.07 ± 0.11	7.41 ± 0.06	3.93 ± 0.23	18.49 ± 0.24	4.12 ± 0.22
Water	20.43 ± 1.08	7.30 ± 0.12	14.49 ± 0.72	5.04 ± 0.44	6.64 ± 0.27	7.52 ± 0.25
Suberin	4.02 ± 0.42	3.68 ± 0.17	3.93 ± 0.33	16.37 ± 0.28	3.01 ± 0.39	4.42 ± 0.25
Lignin	21.88 ± 0.39	32.75 ± 3.24	29.11 ± 0.93	27.38 ± 0.55	18.64 ± 1.14	36.42 ± 0.29
Klason	17.21 ± 0.34	26.63 ± 2.98	23.86 ± 0.72	22.63 ± 0.42	13.13 ± 0.61	30.82 ± 0.02
Acid soluble	4.67 ± 0.05	6.12 ± 0.26	5.25 ± 0.21	4.75 ± 0.13	5.51 ± 0.53	5.60 ± 0.27
Polysaccharides	34.56 ± 0.89	41.31 ± 0.71	35.61 ± 0.67	34.90 ± 1.53	41.90 ± 0.60	39.67 ± 0.25

Table 2: Bark extracts composition (TPC and TFC) and antioxidant capacity (analysed by the DPPH, ABTS and FRAP methods).

	Sweet chestnut	Northern red oak	Common oak	Black locust	Common ash	Iberian white birch
Extraction yield (%)	9.27 ± 0.18	3.20 ± 0.07	10.03 ± 0.31	3.08 ± 0.18	15.77 ± 0.14	5.09 ± 0.06
TPC (mg GAE/g dried bark extract)	635.08 ± 24.21	276.50 ± 3.23	610.63 ± 14.98	178.11 ± 5.79	316.47 ± 10.31	432.02 ± 3.00
TFC (mg CE/ g dried bark extract)	949.04 ± 39.17	650.43 ± 37.86	1021.78 ± 6.77	575.82 ± 21.37	439.19 ± 12.04	802.09 ± 28.51
DPPH (mg TE /g dried bark extract)	1217.18 ± 59.50	399.62 ± 8.79	1521.25 ± 56.27	167.23 ± 11.41	543.96 ± 14.08	1912.38 ± 25.04
ABTS (mg TE /g dried bark extract)	1413.40 ± 170.41	561.92 ± 98.48	1556.57 ± 74.66	584.85 ± 17.26	753.36 ± 14.92	1301.55 ± 55.99
FRAP (mg TE / g dried bark extract)	532.58 ± 3.29	194.13 ± 7.03	640.30 ± 22.03	146.11 ± 3.54	330.39 ± 12.53	410.14 ± 7.27

Table 3: Molecular weight of EtOH/H_2O bark extracts

					Global average		
	Percentage	Mw (g/mol)	Mn (g/mol)	Mw/Mn	Mw (g/mol)	Mn (g/mol)	Mw/Mn
Sweet chestnut	86.69	66134	9580	6.90	57387	2050	27.99
	7.49	499	460	1.08			
	5.81	249	248	1.01			
Northern red oak	58.54	28927	10290	2.81	17211	987	17.44
	13.66	1262	1145	1.10			
	16.97	458	428	1.07			
	9.07	243	243	1.00			
	1.76	264	262	1.01			
Common oak	76.76	26283	6504	4.04	20288	1376	14.74
	6.50	843	819	1.03			
	9.86	422	399	1.06			
	6.88	245	244	1.01			
Black locust	37.22	15661	8430	1.86	6334	696	9.11
	15.65	1859	1708	1.09			
	28.81	588	516	1.14			
	18.32	248	247	1.00			
Common ash	17.72	16982	10641	1.60	3682	556	6.62
	35.32	1429	1071	1.34			
	20.94	446	433	1.03			
	14.21	268	266	1.01			
	7.17	235	235	1.00			
	4.64	438	360	1.22			
Iberian white birch	82.42	37470	10045	3.73	30972	1914	16.18
	4.61	901	874	1.03			
	8.16	441	413	1.07			
	4.81	262	253	1.04			

Tabla 4: FT-IR spectra of MP

Wavenumber (cm-1)	Band assignment	Bark	Reference
3300	-OH stretch vibration in phenolic and aliphatic structures	IWB, SC, CA, NRO, CO, BL	a, j
2925-2930	-CH stretch vibration in aromatic methoxy groups and in methyl and methylene groups of side chains	IWB; SC; CA; NRO; CO; BL	a, j
2850	-CH stretch vibration in aromatic methoxy groups and in methyl and methylene groups of side chains	IWB; SC; CA; NRO; CO; BL	a, j
1730-1736	C=O stretch of acetyl and carbonyl groups	IWB; SC; CA; NRO; CO; BL	b, d
1635	Absorbed O–H and conjugated C–O in polysaccharides	BL	f, g, h
1603-1610	Aromatic skeletal and C=O stretch vibration	IWB; SC; CA; NRO; CO	b, d
1508-1510	C=C stretching of the aromatic ring, C=O bond vibrations in extractive	IWB; SC; CA; NRO; CO; BL	e, f, i
1440	aromatic skeleton vibrations and to CH deformation	IWB; SC; CA; NRO; CO; BL	a, j
1420-1425	C–H asymmetric deformation in methoxyl, aromatic skeletal vibrations,	CA; NRO; BL	e, f
1369-1372	C-H deformation vibration	IWB; SC; CA; NRO; CO; BL	b, c, l
1315-1317	CH2 rocking vibration	IWB; SC; CA; NRO; CO; BL	b, c, l
1264	G-ring plus C=O stretch	IWB; CA; BL	b, c, l
1224	Syringyl ring and C-O stretch	SC; NRO; CO	b, d, l
1152-1159	C-O-C symmetric stretching	IWB; SC; CA; NRO; CO; BL	b, c
1100-1103	Ring asymmetric valence vibration	IWB; SC; CA; NRO; CO; BL	b, c, l
1020-1029	C–O stretching in primary alcohols in cellulose	IWB; SC; CA; NRO; CO; BL	e, f
890-893	Aromatic C-H out of plane deformation	IWB; SC; NRO	b, c, d, l
825	CH out of plane bending in guaiacyl units	IWB; SC; NRO; BL	f, k

a: (Chupin et al., 2015) b: (Özgenç et al., 2017b) c: (Özgenç et al., 2017a) d: (Naumann et al., 2005) e: (Popescu et al., 2010) f: (Traoré et al., 2018) g: (Genest et al., 2013) h: (Karunakaran et al., 2015) i: (Zhou et al., 2015) j: (Boeriu et al., 2004) k: (Faix, 1991) l: (Durmaz et al., 2016)

Tabla 5: FT-IR spectra of bark extracts

Wavenumber (cm-1)	Band assignment	Bark extracts	References
3300	-OH stretch vibration in phenolic and aliphatic structures	IWB; SC; CA; NRO; CO; BL	a, b, c, d, e
2925-2930	-CH stretch vibration in aromatic methoxy groups and in methyl and methylene groups of side chains	IWB; SC; CA; NRO; CO; BL	a, b, d, e, f
2850	-CH stretch vibration in aromatic methoxy groups and in methyl and methylene groups of side chains	IWB; SC; CA; NRO; CO; BL	a, d, e
1705-1720	conjugated carbonyl-carbonyl stretching	IWB; SC; CA; NRO; CO; BL	a, d, f
1605	aromatic skeleton vibrations	IWB; SC; CA; NRO; CO; BL	a, b, d, f
1515	aromatic skeleton vibrations	IWB; SC; CA; NRO; CO; BL	a, d, e, f
1440	aromatic skeleton vibrations/ -CH deformation	IWB; SC; CA; NRO; CO; BL	a, b, d, e, f
1412	Aromatic vibration	CA	b, d
1370-1380	phenolic stretch vibration of -OH and aliphatic -CH deformation in methyl groups	IWB; SC; CA; NRO; BL	a, d, e
1308	C-C frame stretchuing (C-CHR-C)	SC; CA; NRO; CO; BL	b, d
1275	C-O C asymmetric stretch vibration	IWB; NRO; CO	C, d, e
1260	C-O stretch vibration	CA; CO	d, e, g
1245	C-O-C asymmetric stretch vibration	IWB; NRO; CA; BL	c, d
1200	C-O stretching vibration	IWB; SC	a, d, e
1155	aromatic CH in-plane bending vibration	IWB; CO; CA; BL	c, d
1105-1115	aromatic -CH bending in-plane vibration	IWB; SC; CA; NRO; CO; BL	b, d, e
1040-1050	C-O stretching vibration	IWB; NRO; CA; BL	b, d, e
1035	C-O stretching or aromatic C-H deformation associated with the C-O, C-C stretching and C-OH bending in polysaccharides	SC; CA; NRO; CO; BL	a, d
921	Aromatic -CH out of plane bending vibration	CA	b, d
<900	Aromatic -CH stretch vibrations	IWB; SC; CA; NRO; CO; BL	a, c, d, e

a: (Boeriu et al., 2004) b: (Ping et al., 2012) c: (Soto et al., 2005) d: (Chupin et al., 2015) e: (Chupin et al., 2013) f: (Vázquez et al., 2008) g: (Vázquez et al., 2000)

Figure captions

Figure 1: GPC chromatogram of EtOH/H_2O bark extracts of the six raw materials.

Figure 2: FT-IR spectra of six different barks of hardwoods: a) Iberian white birch b) Sweet chestnut c) Common ash d) Northern red oak e) Common oak f) Black locust.

Figure 3: FTIR spectra of EtOH/H_2O extracts of the six barks: a) Iberian white birch b) Sweet chestnut c) Common ash d) Northern red oak e) Common oak f) Black locust.

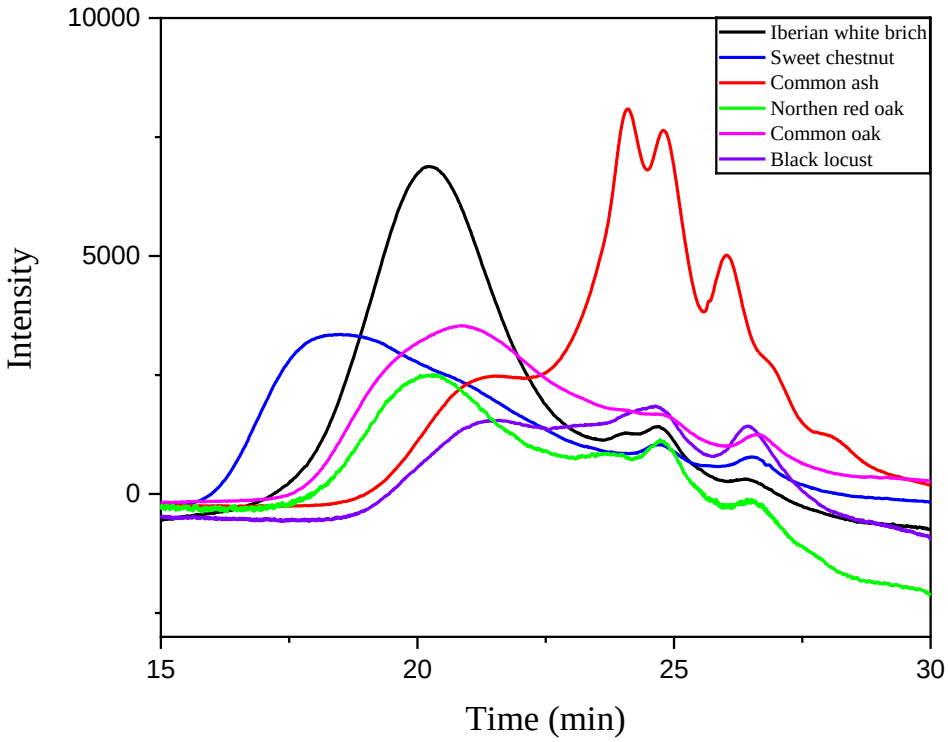
10000
5000
0
Intensity
15
20
25
30
Time (min)
Iberian white brich
Sweet chestnut
Common ash
Northen red oak
Common oak
Black locust

Figure 1.

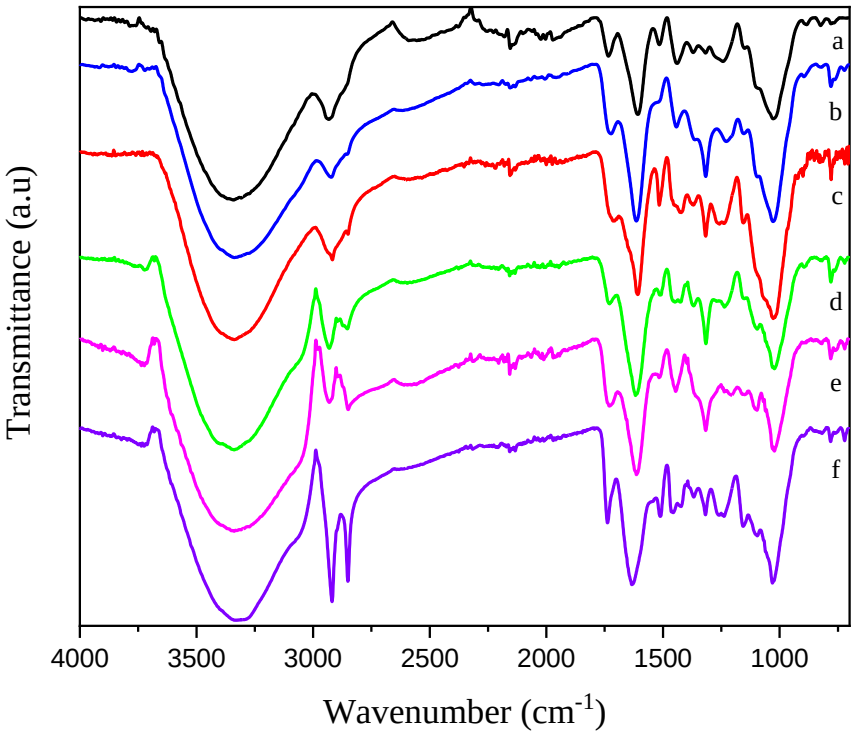
Transmittance (a.u)
a
b
c
d
e
f
4000
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)

Figure 2.

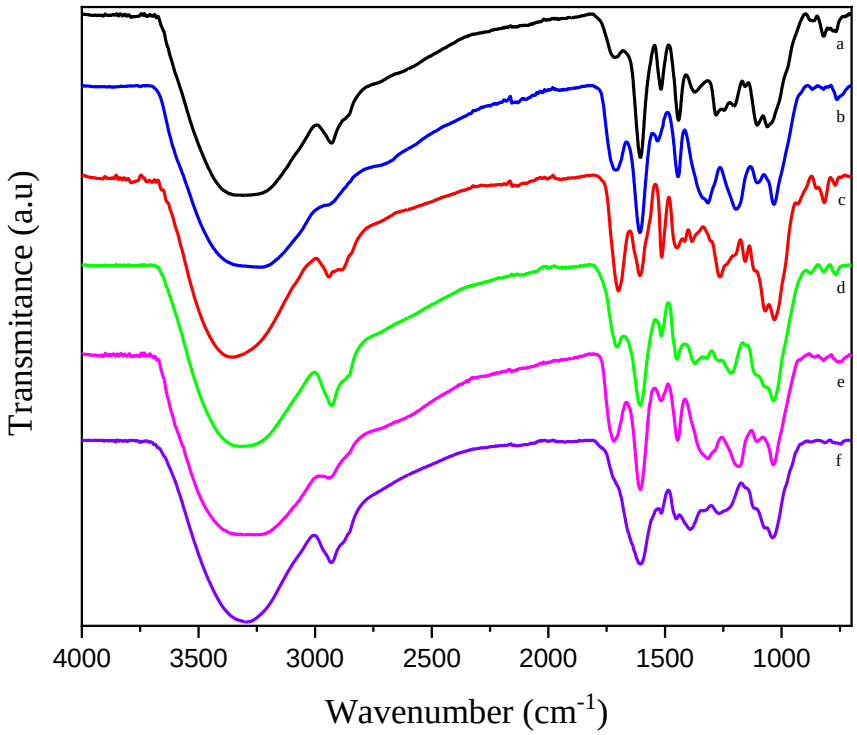
Transmitance (a.u)
a
b
c
d
e
f
4000
3500
3000
2500
2000
1500
1000
Wavenumber (cm-1)

Figure 3.