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Low-Molecular-Weight Phenols Recovery by Eco-Friendly Extraction from *Quercus Spp.* Wastes: An Analytical and Biomass-Sustainability Evaluation

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Abstract: In this work, chemical–physical protocols aimed at the implementation of eco-friendly and biomass-sustainable recovery processes of useful compounds from forestry and/or wood industry wastes were evaluated. Four species of interest in industrial and environmental fields (*Quercus cerris*, *Quercus ilex*, and *Robinia pseudoacacia* from Central Italy, *Quercus petraea* from France) were submitted to neutral extraction and analyzed by gas chromatography, with mass spectrometry identification of low-molecular-weight phenols. Moreover, *Quercus petraea* heartwood samples were submitted to three extraction/hydrolysis protocols in an alkaline environment, and the byproducts from the lignin degradation were identified and evaluated. The recovery of bioactive phenols from forestry wastes by applying eco-friendly extractive protocols may reveal a precious strategy for rethinking the management of such wastes, in line with the fundamentals of “circular economy”.

Keywords: wood waste; mild extraction/hydrolysis protocols; lignin; phenolic compounds recovery; circular economy

1. Introduction

Oak wood, widely used by a variety of industries, is characterized by the presence of high levels of tannins of considerable scientific interest, besides their well-known practical value [1–6]. Phenolic compounds such as gallic acid, catechin, and epicatechin are notoriously endowed with antioxidants, astringents, and antimicrobial activity. Therefore, such species are subject of increasing interest in various fields such as pharmaceuticals, leather tanning, cosmetics, and the food industry [7–11]. Low-molecular-weight hydroxybenzoic acids and some of their derivatives can be obtained from wood extractives and via induced lignin degradation [12,13]. Lignin is a phenolic high-molecular-mass biopolymer, composed of a highly branched phenylpropanoid framework based on the three monomers coumaryl, coniferyl, and sinapyl alcohol. A number of other high-value fine chemicals such as vanillin, syringaldehyde, and *p*-coumaric, ferulic, *p*-hydroxybenzoic, syringic, and vanillic acids [14,15] can be obtained among the various products of wood lignin degradation following de-polymerization processes (e.g., alkaline oxidation).

The recovery of molecules endowed with a biofunctional role from wood wastes, and their reuse and application in several fields, is one of the most challenging and promising actions in the circular economy domain. Based on the above facts, the possibility to improve (or implement new) standard

chemical extraction/hydrolysis protocols to obtain organic compounds within a timber recycling context represents an issue of practical interest. The final goal is the individuation of mild, eco-friendly chemical and physical reaction conditions to achieve the compounds of interest [16]. A proper understanding of the distribution and structural features of extractives between bark, sapwood, and heartwood, as well as their variability in relative abundance between species, may have a significant impact on wood utilization [17,18].

Sawdust wastes deriving from the *Quercus petraea* industrial manufacturing, and wooden wastes of the forestry activities derived from *Quercus cerris*, *Quercus ilex*, *Quercus petraea*, and *Robinia pseudoacacia* could be exploited as a source of useful compounds. Oak bark is the wood tissue richest in tannins, and *Q. petraea* bark is a source of gallic and ellagic acids as well as catechin and other tannin derivatives [19,20]. Typically, bark represents only the 10%–20% of the volume of a log, so the sawdust-like wastes from heartwood manufacturing processes, combined with the woody wastes from forestry activities, could potentially represent a greater source for the recovery of the above-mentioned compounds. Moreover, sawdust produced by industrial heartwood treatment (sawmill processes) is much more suitable for extraction/hydrolysis processes compared to the byproducts of crude mechanical debarking. In addition, the lignin content of hardwoods like oaks amounts to about 25%–30% in weight [21], and represents the main potential source of useful organic compounds.

Heartwood from *Q. petraea* (Matt.) Liebl. (common name: French oak) is an interesting raw material, widely used in the industrial production of parquet, furniture, barrels for wine, and as a structural material in the construction industry, due to its excellent mechanical characteristics. It is also used as fuel for heating [22].

Q. cerris L. (common name: Turkey/Austrian oak) is not particularly valuable compared to the other *Quercus* species, due to its lack of tannins which makes it less durable. It is hard but not very resistant, and is used mainly as fuel.

Q. ilex L. (common name: Holm oak) has plenty of tannins and is a very resistant wood, but is difficult to work and to season. Therefore, it is primarily used for heating. Due to the richness of extractives, its bark is employed as a source of tannins for leather tanning [22]. Consequently, forestry activities wastes from these two “cheap” oak woods could become an important source of valuable chemical compounds.

R. pseudoacacia L. (common name: Black locust) is widely cultivated as a wood and honey-producing tree, with a high rate of development. This wood is used as a structural material as a substitute for some types of tropical wood in the production of parquet and, to some extent, of furniture [22]. Furthermore, it represents a substitute for oak wood in making containers in the cooperage industries [5,6,23,24].

As previously outlined, the recovery and identification of such extractives currently represents an area of great interest for the development of renewable-resource-based systems. In this work, we focused on the chemical eco-friendly extraction, identification, and recovery of low-molecular-weight phenols obtainable from three species of oak heartwood: *Q. cerris*, *Q. ilex* (from Umbria region, Italy), and especially *Q. petraea* (from Fontaines, France). Moreover, the extractive analysis of *R. pseudoacacia* (from Umbria region, Italy) fresh bark/sapwood was also performed. The identification of low-molecular-weight phenols and the extract composition was assessed using a gas chromatography–mass spectrometry (GC-MS) system. Identification of trimethylsilyl derivatives (TMS derivatives) phenols from extracts was done by comparing the GC-MS profiles, retention times, and fragmentation patterns with those produced by the analysis of certified standards.

A further goal of the study was the evaluation of coniferyl and sinapyl alcohols, which are nominally the constituent monomers (monolignols) of the lignin structure in angiosperm species, as lignin degradation indicators.

2. Materials and Methods

2.1. Wood Sample Collection

Heartwood from *Q. cerris* and *Q. ilex* trees, grown in the Umbria region (Central Italy), were taken after one year of natural seasoning. The *R. pseudoacacia* juvenile sample came from the same location (43°6'43" N; 12°23'19" E). The 1 year naturally seasoned heartwood samples of *Q. petraea* came from Fontaines forest (Chalon-sur-Saône, France: 46°46'59" N; 4°51'0" E).

2.2. Reagents and Standard Compounds

Reference compounds used as standards are listed in Table 1. Coniferyl and sinapyl alcohols were synthesized according to the procedures reported in the literature [25]. Pure water was obtained using a Milli-Q Plus185 system from Millipore (Milford, MA, USA). Methanol (MeOH, ≥99.9%), ethyl acetate (EtOAc, ≥99.9%), diethyl ether (Et₂O, ≥99.0%), tetrahydrofuran (THF, ≥99.0%), hydrochloric acid (HCl; w/v 37%), sodium hydroxide (NaOH, ≥98.0%), anhydrous sodium sulphate (Na₂SO₄, ≥99.0%), pyridine (≥99.8%), and the silylation agent N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, ≥99.0%), as well as reference phenolic standards (vanillin ≥ 99.0%; *p*-hydroxybenzoic acid ≥ 99.0%; syringaldehyde ≥ 98.0%; vanillic acid ≥ 97.0%; protocatechuic acid ≥ 97.0%; syringic acid ≥ 95.0%; gallic acid ≥ 99.0%; ferulic acid ≥ 99.0%; caffeic acid ≥ 98.0%; (–)-epicatechin ≥ 98.0%; (+)-catechin ≥ 98.0%), were purchased from Sigma-Aldrich (Milan, Italy). All the reagents and standards used were of analytical grade.

Table 1. Characteristics MS fragments of phenols as TMS derivatives.

Identified Compounds	Peak Number	Retention Time (min)	Molecular Weight (g/mol)	<i>m/z</i> Values
Vanillin	1	12.65	224	193, 194, 209
<i>p</i> -Hydroxybenzoic acid	2	14.33	282	193, 223, 267
Syringaldehyde	3	15.91	254	195, 224, 239
Vanillic acid	4	16.84	312	149, 165, 223, 253, 267, 282, 297
Protocatechuic acid	5	17.87	370	193, 223, 267, 311, 355
(<i>Z</i>)-Coniferyl alcohol	6	18.6	324	204, 235, 293, 309
Syringic acid	7	19.2	342	253, 297, 312, 327
(<i>E</i>)-Coniferyl alcohol	8	20.3	324	204, 235, 293, 309
Gallic acid	9	20.22	458	147, 178, 281, 443, 444
Dihydrosinapyl alcohol	10	20.47	356	210, 240, 341
(<i>Z</i>)-Sinapyl alcohol	11	20.6	354	204, 323, 339
Ferulic acid	12	22.22	338	219, 249, 293, 279, 308, 323
Caffeic acid	13	22.85	396	179, 191, 219, 381
(<i>E</i>)-Sinapyl alcohol	14	22.47	354	204, 323, 339
(–)-Epicatechin	15	29.94	650	147, 267, 355, 357, 368, 369, 370
(+)-Catechin	16	30.04	650	147, 267, 355, 368

MS, mass spectrometry; TMS, trimethylsilyl.

2.3. Applied Extraction Protocols

Based on previously optimized extraction/hydrolysis protocols [16] and experimental results, several parameters were properly tuned in order to maximize the yield of phenol extractions. This step was carried out by taking into account the polarity and the weak acid behavior of the investigated molecules. The use of high pH values proved to be suitable to increase compound solubility, while precipitation occurred at low values (pH < 4–5). Other variables were then screened, such as extraction time and temperature and the fractionation solvent (diethyl ether vs. ethyl acetate). The obtained results allowed the following experimental conditions to be selected. From each sample, 10 g of sawdust (particle dimension ≤ 3 mm) were mechanically derived. Two types of processes were performed: a NEP (Neutral Extraction Protocol) on all the four species, and EHPs (Extraction-Hydrolysis Protocols) in an alkaline environment, only applied on *Q. petraea* samples. Details are reported as follows.

2.3.1. NEP

Samples were extracted via 300 mL of a MeOH/water (1:1, v/v) neutral solution for 24 h at 25 °C, in darkness condition. Extracts were then filtered using a Büchner funnel (Whatman® grade 42 filter paper, from Sigma-Aldrich (Milan, Italy) and MeOH was removed at 35 °C. Next, the aqueous solutions were fractionated by liquid–liquid extractions with Et₂O or EtOAc (30 mL × 4) [26–28].

2.3.2. EHPs

Three samples (10g) of *Q. petraea* (denoted as A, B, and C) were treated with 200 mL of a 1.5 M MeOH/NaOH (1:1, v/v) solution under mechanical mixing at 50 °C, as follows:

EHP-A: Sample A was extracted and hydrolyzed for 72 h. The extracts were filtered using a Büchner funnel and centrifuged to remove woody particles, then concentrated through a rotary evaporator at 35 °C for MeOH removal. The aqueous solution (pH 12.7) was neutralized by adding EtOAc (60 mL) and then acidified up to pH 1.0 by HCl (12 mL). After filtration and centrifugation for sodium acetate removal, the obtained acid solution was fractionated *via* liquid–liquid extractions with EtOAc (25 mL × 4);

EHP-B: Sample B was extracted and hydrolyzed for 24 h. After the woody particle and MeOH removal, the aqueous phase was acidified up to pH 1.0 by HCl (10 mL), and then fractionated with EtOAc (25 mL × 4);

EHP-C: Sample C was extracted and hydrolyzed for 72 h. After the woody particle and MeOH removal, the aqueous solution was acidified up to pH 1.0 by HCl (10 mL). Acid phase was fractionated with Et₂O (25 mL × 4).

2.4. GC-MS Apparatus

GC analyses were performed on an Agilent 6850 Series gas chromatograph apparatus fitted with a splitless injector for a low background HP-5MS fused silica capillary column (60 m × 0.25 mm i.d., 0.25 µm f.t.). Detection was achieved by a 5975B Mass single quadrupole spectrometer and the data were elaborated using a Chem Station software package (Agilent). The injector and detector temperatures were 250 and of 280 °C, respectively. The oven temperature gradient program was as follows: initial temperature of 90 °C held for 1 min; raised to 220 °C (at 6 °C/min); raised to 290 °C (at 10 °C/min) and held for 1.23 min; and finally raised to 310 °C (at 40 °C/min) held for 7.5 min. Helium was used as the carrier gas (1.0 mL/min flow rate). The injection volume was 1.0 µL. Electron impact ionization energy was 70 eV and the system was scanned in a 140–465 *m/z* mass range.

2.5. Sample Preparation

The organic fractions, dried at low pressure at 35 °C, were re-dissolved in a known quantity (1.0 mL) of the respective fractionation solvent by ultrasonic mixing. Finally, the compounds were submitted to a silylation procedure [29,30]. All reagents used in silylation were previously dehydrated by anhydrous Na₂SO₄, to avoid moisture interference with respect to the phenolic hydroxyl groups. Each 1 mL sample was transferred into a micro-vial and dried. Consequently, the residue was re-dissolved in 200 µL of anhydrous EtOAc. A volume of 50 µL of the silylation mixture (BSTFA-pyridine-EtOAc, 4:1:5, v/v/v) was added and the whole solution was mechanically shaken for 1 min at room temperature. Before injection, samples were further diluted with 250 µL of EtOAc.

2.6. GC-MS Analysis

Each extract was prepared in triplicate before being submitted to GC-MS determination. The obtained values were then used to calculate the global amount extracted from the initial 10 g samples according to Equation (1).

$$(1/3) \times (f.V.) \times \sum df_i [X]_i, i = 1, 2, 3 \quad (1)$$

where (f.V.) is the final volume of the re-dissolved extracts, df_i is the dilution factors, and $[X]_i$ is the concentrations in mg/mL of the three 1 mL portions that underwent the silylation procedure.

Low-molecular-weight phenols were identified using certified standards and treated to give the TMS derivatives according to References [29,30], as described above. The fragmentation pattern obtained for each derivatized standard was first compared with data available in the literature [29–36]. The fragment ions of the selected peaks from the analysis of real samples were then identified and confirmed, based on the retention times and profile matching with signals from the corresponding standard (Table 1). Only the species that resulted pure through the “peak purity” response provided by the GC-MS software from the analysis of the real samples were submitted to quantitative evaluations. The quantitation was performed by injecting 1.0 μ L of the respective TMS standard solution at concentrations ranging from 0.02 to 0.50 mg/mL for ferulic acid and (–)-epicatechin, 0.04 to 1.00 mg/mL for syringaldehyde, vanillic acid, gallic acid, (E)-coniferyl alcohol, and (+)-catechin, and 0.2 to 5.0 mg/mL for vanillin. The method produced significantly linear (as indicated by the elevated R^2 values > 0.99) results in the explored ranges, and appreciably low LOD (limit of detection) and LOQ (limit of quantification) values were calculated for the investigated phenols (see Table S2 in Supporting Information for details).

3. Results

3.1. Compounds Identified in the Investigated Arboreal Species

Table 1 lists the identified compounds, while the mass spectral library matching is reported in Table S1 (Supporting Information). Tables 2 and 3 show the reproducibility of the NEP-EHPs peak areas for the quantified compounds (expressed as mean value ($n = 3$) \pm percentage standard deviation, %SD). NEP extractions using EtOAc produced the best results in terms of number of identified species and peak purity, with respect to the EHPs. Hence, only the chromatographic data obtained using EtOAc as the fractionation solvent were considered (Figure 1).

Table 2. Peak areas (mean value \pm %SD) for the NEP-quantified compounds ($n = 3$).

TMS Derivatives	Peak Number	<i>Q. cerris</i>	<i>Q. ilex</i>	<i>R. pseudoacacia</i>	<i>Q. petraea</i>
Syringaldehyde	3	9.53×10^5 ($\pm 3.59\%$)	-	-	1.56×10^7 ($\pm 15.81\%$)
Vanillic acid	4	1.18×10^7 ($\pm 10.77\%$)	9.45×10^6 ($\pm 9.29\%$)	1.16×10^7 ($\pm 22.12\%$)	-
(E)-Coniferyl alcohol	8	1.43×10^8 ($\pm 37.01\%$)	4.78×10^7 ($\pm 5.69\%$)	7.68×10^7 ($\pm 21.96\%$)	2.18×10^8 ($\pm 18.36\%$)
Gallic acid	9	2.70×10^7 ($\pm 35.32\%$)	3.21×10^7 ($\pm 16.25\%$)	-	5.60×10^8 ($\pm 35.99\%$)
(–)-Epicatechin	15	1.10×10^6 (u)	3.81×10^6 ($\pm 23.84\%$)	6.51×10^6 ($\pm 50.04\%$)	-
(+)-Catechin	16	7.31×10^6 (u)	3.62×10^8 ($\pm 5.97\%$)	4.27×10^7 ($\pm 51.17\%$)	-

u: unambiguously detected only in one chromatogram; -: not detected.

Table 3. Peak areas (mean value \pm %SD) for the EHP-quantified compounds ($n = 3$).

TMS Derivatives	Peak Number	EHP-A	EHP-B	EHP-C
Vanillin	1	-	-	9.81×10^7 ($\pm 29.30\%$)
Syringaldehyde	3	1.06×10^7 ($\pm 53.35\%$)	-	6.95×10^8 ($\pm 7.74\%$)
Vanillic acid	4	3.12×10^8 ($\pm 8.26\%$)	-	2.48×10^8 ($\pm 38.01\%$)
(E)-Coniferyl alcohol	8	5.94×10^7 ($\pm 17.76\%$)	1.52×10^8 ($\pm 55.13\%$)	1.95×10^7 ($\pm 33.29\%$)
Ferulic acid	12	7.90×10^8 ($\pm 3.55\%$)	-	-

-: not detected.

NEP-extracted samples from *Q. petraea* were characterized by a substantial presence of gallic acid, which was also sufficiently abundant in *Q. ilex*, followed by *Q. cerris* (Table 2, Figure 1). In contrast, *R. pseudoacacia* NEP and *Q. petraea* EHP extracts displayed no trace of this phenol compound.

Vichi et al. [32] reported the volatile components of oak wood chips analyzed by GC–MS without derivatization, as also reported for the analysis of other volatiles [37,38]. According to the literature [26,39], *p*-hydroxybenzoic acid, syringaldehyde, vanillic acid, protocatechuic acid, syringic

acid, (*E*)-coniferyl alcohol, and (*E*)-sinapyl alcohol were identified in *Q. petraea* (Table S1, Supporting Information). The conspicuous amounts of (*E*)-coniferyl alcohol (Table 4) obtained by *NEP* and *EHP* could be plausibly attributed to the ageing and to the induced process of lignin degradation, respectively (see Table S3 in Supporting Information for *NEP/EHP* yield comparisons with data reported in literature, and Figure S1 for synthesized coniferyl alcohol identification) [40].

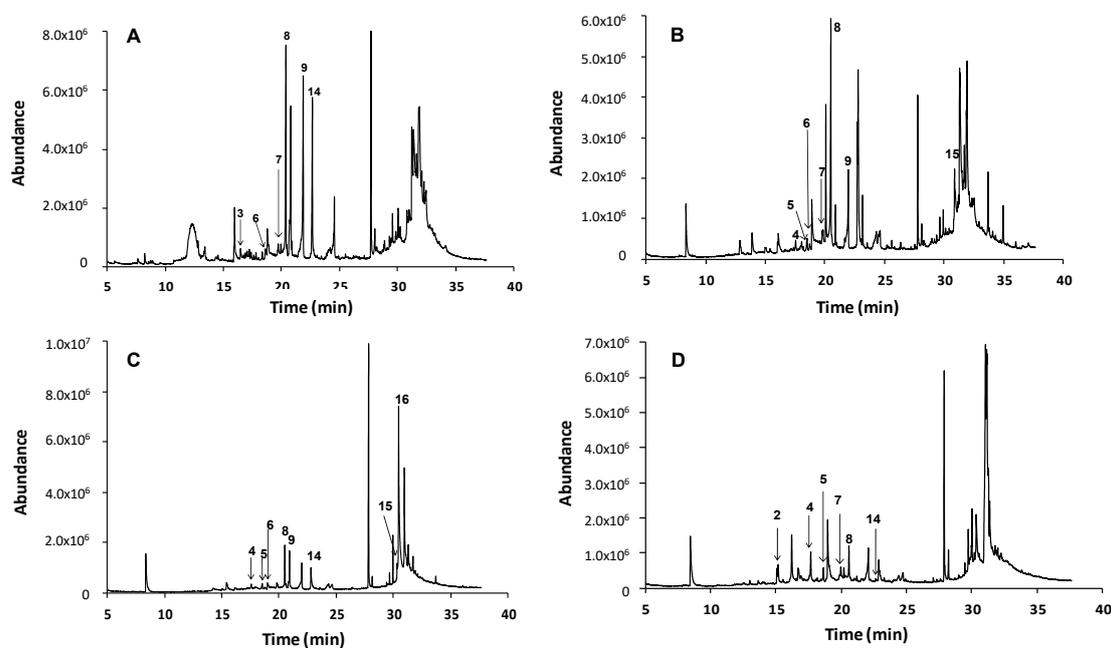


Figure 1. Profile according to *NEP* of (A) *Q. petraea*; (B) *Q. cerris*; (C) *Q. ilex*; (D) *R. pseudoacacia*. Peak numbers identify the compounds as reported in Table 1.

Table 4. TMS compound amounts (mg) calculated with respect to the extracted mass (10 g).

TMS Derivatives	Amount in Wood Samples (mg/10 g)						
	<i>Q. cerris</i> *	<i>Q. ilex</i> *	<i>R. pseudoacacia</i> *	<i>Q. petraea</i> *	<i>Q. petraea</i> ^a	<i>Q. petraea</i> ^b	<i>Q. petraea</i> ^c
(+)-Catechin	0.19	9.92	2.19	-	-	-	-
(<i>E</i>)-Coniferyl alcohol	0.61	0.22	0.19	0.20	0.42	0.99	0.13
(-)-Epicatechin	-	0.24	0.53	-	-	-	-
Ferulic acid	-	-	-	-	15.96	-	-
Gallic acid	0.09	0.26	-	1.14	-	-	-
Syringaldehyde	0.01	-	-	0.02	0.17	-	7.40
Vanillic acid	0.06	-	0.04	-	3.27	-	1.70
Vanillin	-	-	-	-	-	-	0.55

* by *NEP*; ^a by *EHP-A*; ^b by *EHP-B*; ^c by *EHP-C*; -: not detected.

Low levels of gallic and vanillic acids were found in *Q. cerris* heartwood, while the highest concentration of (*E*)-coniferyl alcohol was found in this wood. *Q. ilex* was found to be rich in gallic acid, (+)-catechin, and (-)-epicatechin, as expected based on the literature data [41] (Table 2).

Qualitative identification of (*E*)-sinapyl alcohol was possible for *Q. petraea* and *Q. cerris* samples extracted by *NEP*. Moreover, this compound was identified in the *Q. petraea* extracts obtained according to protocols *EHP-B* and *EHP-C* (Table S1, Supporting Information). However, no quantitative evaluations of the sinapyl alcohol isomers were possible, due to the poor standard purity grade. Figure 2 shows typical chromatograms obtained from *EHP* from *Q. petraea* heartwood. Only the (*E*)-coniferyl alcohol was quantitatively determined on the extracts submitted to *EHP-B* (time of reaction: 24 h). *EHP-C*, based on the use of Et₂O, was the only protocol that allowed vanillin quantitation, while *EHP-A* allowed the evaluation of ferulic acid. By comparing *EHP-A* and *EHP-C* with *EHP-B* results, the key

role of the reaction time (72 vs. 24 h) was clearly evident (Table 4). It is noteworthy that (*E*)-coniferyl alcohol was unambiguously identified and quantified in all the examined extract solutions.

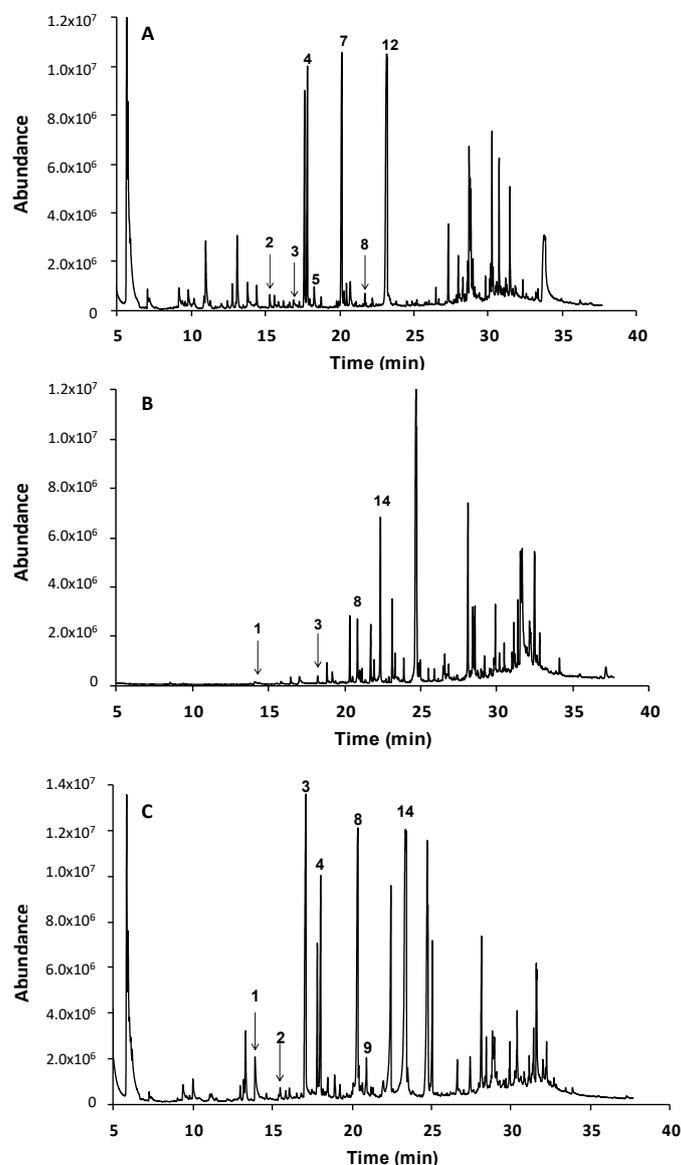


Figure 2. Profile of *Q. petraea* according to (A) EHP-A; (B) EHP-B; (C) EHP-C. Peak numbers identify the compounds as reported in Table 1.

The abundance of (+)-catechin and (–)-epicatechin in the younger tissues of *R. pseudoacacia* highlighted the potential for their recovery from forestry-activity-derived wastes. Moreover, the presence of quite a high level of (*E*)-coniferyl alcohol confirmed its biosynthetic origin. Accordingly, lignin biosynthesis implies the co-polymerization of the coniferyl and sinapyl alcohols [14,40]. In *R. pseudoacacia* extracts, caffeic, ferulic, protocatechuic, and vanillic acids were also detected, but only the last one was quantitatively evaluated (Table 4; Figure 1).

3.2. Implementation of Eco-Friendly Extraction/Hydrolysis Protocols in the Forestry Waste Management Field

Quantitative recovery of several useful compounds from *Q. petraea* lignin hydrolysis was achieved under mild chemical and physical conditions, with low concentrations of NaOH, low reaction temperature (50 °C), and using solvents with a negligible toxic/environmental impact, except for MeOH

which could be reasonably substituted by EtOH [42]. Moreover, air oxygen was the oxidizing agent used, and the prolonged reaction time, with strong mechanical mixing of the reaction solutions, was able to replace harsher reaction conditions. This approach was more ecofriendly than standard industrial processes carried out for the lignin treatment. Strong acid conditions used in *EHP-A* promoted significant formation of the ultimate oxidation products of coniferyl alcohol (ferulic and vanillic acids), whereas a weaker acidification, as in *EHP-C*, led to the prevalent formation of aldehydes (vanillin and syringaldehyde). As reported in Table 4, the *EHP* yields were influenced by the fractionation solvent. In fact, for instance, vanillin was freely soluble in Et₂O (according to the *EHP-C* protocol), thus enabling its quantification.

The use of EtOAc in *EHP-A* to lower the pH caused its hydrolysis to EtOH and NaOAc, which precipitates and hence could be easily separated. Interestingly, recovered EtOH could be reused as primary extraction solvent or, combined with the AcOH obtained via HCl acidification and NaOAc, could regenerate EtOAc. The formation of EtOH during the EtOAc hydrolysis in an alkaline environment allows a solvent endowed with a middle polarity ($\epsilon = 26.0$) to be used, on a scale where water is the most polar ($\epsilon = 80.1$), and AcOH ($\epsilon = 6.2$) with EtOAc ($\epsilon = 6.0$) represent the lower dielectric constants. Water/EtOH mixtures are commonly used to extract the most polar tannins, namely those with two or more hydroxyl groups and with a middle/high molecular weight [42,43]. EtOAc is suitable for phenols with a low polarity (generally with low molecular weight) and for huge nonpolar compounds.

Although mild lignin degradation conditions imply low yields, the target compounds were achieved in a more ecofriendly and energy-saving way, with respect to the traditional methods. Moreover, the use of wastes from forestry activities and of eco-friendly solvents allowed products not contaminated by unwanted/toxic chemicals to be obtained, in contrast to the byproducts from the Kraft and sulfite processes [15]. Additionally, the variety of obtainable substances could compensate their lower yields. Some simple forest resource planning could provide an overview of the potential exploitable biomasses achievable from the evaluated oaks' woods. For each species, the biomass obtainable from forestry management can be evaluated by the following relationship (Equation (2)).

$$ABM = SW \times AABI \times AFWC \quad (2)$$

where ABM (kg/(ha \times y)) is the available biomass (kg) from a forest area of one hectare per year, SW (kg/m³) is the specific weight of the wood with a 12% content in weight of moisture, AABI (m³/(ha \times y)) is the average annual biomass increase, and AFWC (%) is the average forestry waste coefficient, which indicates the percent rate of exploitable annual biomass. This parameter depends on factors such as forest type and management, age and type of logging, and especially on the final main use of the woody biomass.

In Central Italy's forestry management, *Q. cerris*, *Q. ilex*, and *R. pseudoacacia* are mainly exploited as firewood. This feature justifies the possible use of their biomasses for extractions. For *Q. cerris* and *Q. ilex*, Central Italian forest estimations provide an AFWC value of 17.5%. The same value can be assumed for *Q. petraea*. *Q. ilex*'s AABI is 3.5 m³/ha, while a value of 3.0 m³/ha is the lower limit for the other species. Considering the woody mass as heartwood, a first evaluation for ABMs and the achievable compounds is given in Table 5.

A possible industrial recovery cycle based on *EHP-A* could be implemented to obtain phenols (low and high molecular weight) from lignin hydrolysis. By using EtOH instead of MeOH and AcOH instead of HCl, the only net inputs would be NaOH and the energy required for the process. This energy could be obtained from the biomass itself, and the extracted wastes could be reused as energetic material. In addition, EtOH can be obtained by biomass fermentation. It is noteworthy that NaCO₃/KCO₃ "lye" solutions can be obtained (with low environmental impact) by settling or thermal treatment of woody ashes with water [40], and carbonate solutions could replace NaOH in the hydrolysis process.

Table 5. Compounds yields (grams per hectare per year) and available biomass (ABM) values.

TMS Derivatives	<i>Q. cerris</i> ^h	<i>Q. ilex</i> ^h	<i>Q. petraea</i>	<i>R. pseudoacacia</i> ^s
(+)-Catechin	7.5	431.4	-	86.2
(E)-Coniferylalcohol	43.9	9.6	44.2 ^b	7.5
(-)-Epicatechin	-	10.4	-	20.9
Ferulic acid	-	-	712.2 ^a	-
Gallic acid	3.5	11.3	50.9 ^h	-
Syringaldehyde	0.4	-	330.3 ^c	-
Vanillic acid	2.4	-	145.9 ^a	1.6
Vanillin	-	-	24.5 ^c	-
Specific weight (SW, kg/m ³)	750	710	850	750
ABM, kg/[ha × y]	393.7	434.9	446.3	393.8

^h Heartwood by NEP, ^s sapwood-bark by NEP; ^a heartwood by EHP-A; ^b heartwood by EHP-B; ^c heartwood by EHP-C; -: not detected.

With regard to NEP conditions, it should be underlined that “cheap” woods such as *Q. ilex* and *R. pseudoacacia* produced notable quantities of (+)-catechin and (-)-epicatechin under very mild conditions. The evaluations for *R. pseudoacacia* were based on a NEP sapwood/bark analysis, but young, branch-type woody wastes are also easily available as byproducts of the forestry activities. Moreover, the NEP quantifications did not account for high-molecular-weight phenols soluble in water/MeOH, which could be recovered by a global industrial process.

4. Conclusions

The main goal was the identification of the most suitable extraction conditions in terms of efficiency, eco-friendliness, and possibly low cost. The applied methods could potentially be extended to large-scale applications. This work showed the usefulness of NEP and especially of EHP in the treatment of woody wastes which, by mild and eco-friendly conditions, allowed

- (i) the quantitative recovery by NEP of several naturally occurring valuable compounds ((+)-catechin, (E)-coniferyl alcohol, (-)-epicatechin, gallic acid, syringaldehyde, and vanillic acid);
- (ii) the possible implementation of an industrial cycle, particularly for the EHP-A protocol, justified by the estimated yields of (E)-coniferyl alcohol, ferulic acid, syringaldehyde, vanillic acid, and vanillin obtained from *Quercus petraea* heartwood samples.

The protocols described in the study allowed an analytical characterization of the bioactive compounds attainable from oak waste. The unambiguous GC-MS detection of (E)-coniferyl alcohol in all the analyzed samples provided the basis for implementation of a valuable method for the quantitative appraisal of the natural and alkali-induced lignin degradation processes.

This study lays the basis for future investigations aimed at the individuation of optimal and sustainable conditions for oak waste valorization, addressed at the recovery of precious bioactive molecules in the perspective of their applications in multiple industrial fields (pharmaceutical, textile, cosmetic, and food industries).

Supplementary Materials: The following are available online at <http://www.mdpi.com/2227-9717/8/4/387/s1>, Figure S1: GC-MS chromatogram and mass spectrum profile in EI mode at 70 eV for the identification of the synthesized coniferyl alcohol (as TMS-derivative) [3,4]. Table S1: Mass spectral matching (%) between the selected TMS-standards and the identified phenols in the extracts; Table S2: Explored linearity ranges, LOD and LOQ values for the investigated and identified phenols; Table S3: NEP/EHPs yields (mg/10g) comparison with data reported in literature for *Quercus petraea*.

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