

Article

# Chemical Composition of Lipophilic Bark Extracts from *Pinus pinaster* and *Pinus pinea* Cultivated in Portugal

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**Abstract:** The chemical composition of lipophilic bark extracts from *Pinus pinaster* and *Pinus pinea* cultivated in Portugal was evaluated using gas chromatography-mass spectrometry. Diterpenic resin acids were found to be the main components of these lipophilic extracts, ranging from 0.96 g kg<sup>-1</sup> dw in *P. pinea* bark to 2.35 g kg<sup>-1</sup> dw in *P. pinaster* bark. In particular, dehydroabietic acid (DHAA) is the major constituent of both *P. pinea* and *P. pinaster* lipophilic fractions, accounting for 0.45 g kg<sup>-1</sup> dw and 0.95 g kg<sup>-1</sup> dw, respectively. Interestingly, many oxidized compounds were identified in the studied lipophilic extracts, including DHAA-oxidized derivatives (7-oxo-DHAA, 7 $\alpha$ / $\beta$ -hydroxy-DHAA, and 15-hydroxy-DHAA, among others) and also terpin (an oxidized monoterpene). These compounds are not naturally occurring compounds, and their formation might occur by the exposure of the bark to light and oxygen from the air, and the action of micro-organisms. Some of these compounds have not been previously reported as lipophilic constituents of the bark of the referred pine species. Other constituents, such as aromatic compounds, fatty acids, fatty alcohols, and sterols, are also present in the studied extracts. These results can represent an opportunity to valorize *P. pinaster* and *P. pinea* by-products as a primary source of the bioactive resin acids that are integrated into the current uses of these species.

**Keywords:** bark; biorefinery; diterpenic resin acids; GC-MS analysis; *Pinus pinaster*; *Pinus pinea*

## 1. Introduction

*Pinus pinaster* and *Pinus pinea* are the two dominant pine species in Portugal, occupying ca. 714,000 and 176,000 ha, which represent 23% and 6% of the total forest area, respectively [1]. These conifers have an important impact on the national economy due to their vast applicability in timber and resin industries and also in pulp fibers production [2]. *P. pinea* is also cultivated because of the high commercial value of its edible seeds, which have high nutritional value and are a traditional component of the Mediterranean diet [2].

The bark of these two *Pinus* species consists in an interesting raw material, since it is produced in large amounts (6481 and 253 k ton year<sup>-1</sup> of *P. pinaster* and *P. pinea* bark, respectively [3]) as a by-product of the abovementioned industries. These bark residues are simply burnt to produce energy or used as an organic substrate for plant nurseries after composting. An interesting alternative is their exploitation as a source of added-value chemicals, such as bioactive ingredients with potential nutraceutical applications, which ultimately can be integrated into the referred traditional applications in a biorefinery perspective [4] in order to maximize the biomass value.

Resin acids (mainly abietane-type resin acids, including the abietic, neoabietic, palustric, and levopimaric acids, and minor amounts of pimarane-type resin acids, including the pimaric, isopimaric, and sandaracopimaric acids) are the major components of *Pinus* spp. wood resin [5]. Small amounts of dehydroabietic acid (DHAA), obtained by dehydrogenation of abietane-type resin acids, can also be found in wood resins. These compounds have been exploited as important commodities for the chemical industry [5]. In addition, they play an important role in the chemical defense of conifers. Many examples show a relationship between conifer diterpenic acid content and a tree's resistance to potential herbivores and pathogens [6]. Furthermore, resin acids display important beneficial properties for human health, being the antimicrobial, antiulcer, and cardiovascular activities the most representative ones [7,8].

Due to the mentioned significant biological properties, the search for new natural and synthetic resin acid derivatives has been an active research field. Most of these compounds have been tested for their cytotoxicity against cancer cells [9–14], and their antioxidant [9,15], antiviral [10,11,16], antimycotic [10], and gastroprotective [12] activities.

Resin acids can also be found in the lipophilic extracts of different morphological parts of several *Pinus* spp. [17–25], including the bark of *Pinus nigra* [21], the Turkish *P. pinea* L. [23], and the Pakistani *Pinus wallichiana*, *Pinus roxburghii*, and *Pinus gerardiana* [25]. Among the resin acids that have been identified in bark lipophilic extracts are the pimaric, sandaracopimaric, levopimaric, palustric, isopimaric, abietic, dehydroabietic, and neoabietic acids, and the 7-oxodehydroabietic, 16-hydroxydehydroabietic, and 8,15-isopimaridien-18-oic acids [21,23,25]. Additionally, other lipophilic constituents of *Pinus* spp. bark, such as several fatty acids, aliphatic alcohols, sterols (mainly  $\beta$ -sitosterol),  $\beta$ -caryophyllene, and some aromatic compounds (ferulic acid, vanillin, etc.), have also been detected [21,23,25].

Although there are some compositional studies of bark from *P. pinaster* [26,27] and *P. pinea* [28] cultivated in Portugal, a detailed chemical characterization of their lipophilic components is still missing. Therefore, in the present work, dichloromethane (DCM) extracts of bark from the referred *Pinus* spp. collected from the Centre of Portugal were prepared and analyzed by gas chromatography-mass spectrometry (GC-MS). The main aim was to evaluate the exploitation potential of these industries' by-products as an alternative source of bioactive compounds (e.g., resin acids) for further applications, such as nutraceutical and cosmetic usages. An interesting example of a possible application of resin-acids-rich natural extracts is Progres<sup>®</sup>, the only resin-acids-based product that is used for animal feed, which comes from the Finnish forest and has proven beneficial effects on the productivity of animals [29–31].

## 2. Materials and Methods

### 2.1. Chemicals

Dichloromethane (p.a.,  $\geq 99$  purity) was obtained from Fisher Chemicals (Pittsburgh, PA, USA). Pyridine (anhydrous, 99.8% purity), *N,O*-bis(trimethylsilyl)trifluoroacetamide (derivatization grade), chlorotrimethylsilane ( $\geq 99\%$  purity),  $\alpha$ -terpineol (90% purity), hexadecenoic acid (99% purity), nonadecan-1-ol (99% purity), stigmasterol (95% purity), tetracosane (99% purity), and vanillin (99% purity) were purchased from Sigma-Aldrich (Madrid, Spain). Betulinic acid (98% purity) was supplied by Chemos (Regenstauf, Germany). Dehydroabietic acid (DHAA) (99% purity) was obtained from Helix Biotech (Vancouver, BC, Canada).

### 2.2. Samples' Preparation and Extraction

*P. pinaster* and *P. pinea* bark samples were collected from standing trees cultivated in the Aveiro region, Portugal, in January 2017, and were air-dried for 2 weeks. Thereafter, the samples were ground and sieved to a particle size lower than 1 mm. Milled bark samples (7–10 g dry weight (dw)) were Soxhlet-extracted with dichloromethane (175 mL) for 8 h. The extraction yield was determined by

weighting the extract's mass and expressed as a percentage of dry biomass (% *w/w*). The extracts were prepared in duplicate.

### 2.3. The GC-MS Analysis

Before the GC-MS analysis, nearly 20 mg of each dried sample was trimethylsilylated in 250  $\mu$ L pyridine containing 0.3 mg of tetracosane (internal standard) through the addition of 250  $\mu$ L of *N,O*-bis(trimethylsilyl)trifluoroacetamide and 50  $\mu$ L of chlorotrimethylsilane. The mixture was kept at 70 °C for 30 min [32].

GC-MS analyses were carried out in a GC-MS-QP2010 Ultra (Shimadzu, Japan), and compounds were separated in a DB-1 J&W capillary column (30 m  $\times$  0.32 mm inner diameter, 0.25  $\mu$ m film thickness), using helium as the carrier gas (40 cm s<sup>-1</sup>). The chromatographic conditions were as follows: initial temperature, 80 °C for 5 min; temperature rate, 4 °C min<sup>-1</sup> up to 260 °C, 2 °C min<sup>-1</sup> up to 285 °C, which was maintained for 10 min, and 2 °C min<sup>-1</sup> up to 295 °C, which was maintained for 4 min; injector temperature, 250 °C; transfer-line temperature, 290 °C; split ratio, 1:60. The mass spectrometer was operated in the electron impact mode with an energy of 70 eV, and data were collected at a rate of 1 scan s<sup>-1</sup> over a range of *m/z* 33–700. The ion source was kept at 250 °C [33].

Compounds were identified by comparing their mass spectra (MS) with the equipment's mass spectral library (NIST Mass Spectral Library), with data from the literature [34–43], and by the co-injection of standards.

For the quantitative analysis, the GC-MS apparatus was calibrated with pure reference compounds that were representative of the major lipophilic extracts' components, namely  $\alpha$ -terpineol, dehydroabiatic acid, vanillin, hexadecanoic acid, nonadecan-1-ol, stigmasterol, and betulinic acid, in relation to tetracosane (the internal standard), which allowed us to determine the respective response factors. Compounds were quantified using tetracosane as an internal standard, and their abundance is expressed as mg kg<sup>-1</sup> dw of bark.

Two aliquots of each extract were analyzed, and the results are represented as the average of the concordant values that were obtained for the two aliquots of the two extracts in each sample (less than 5% variation between aliquots of the same sample, and between extracts of the same sample).

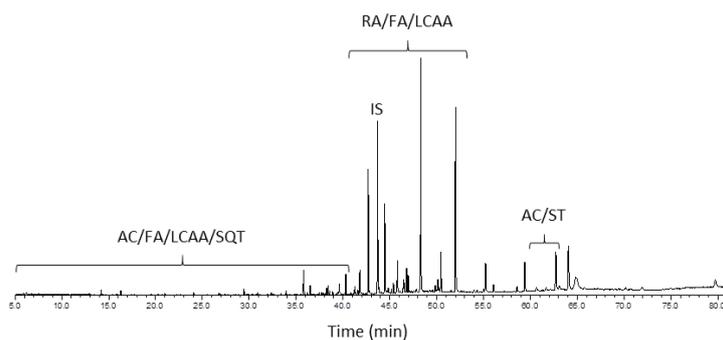
## 3. Results and Discussion

### 3.1. The Extraction Yield

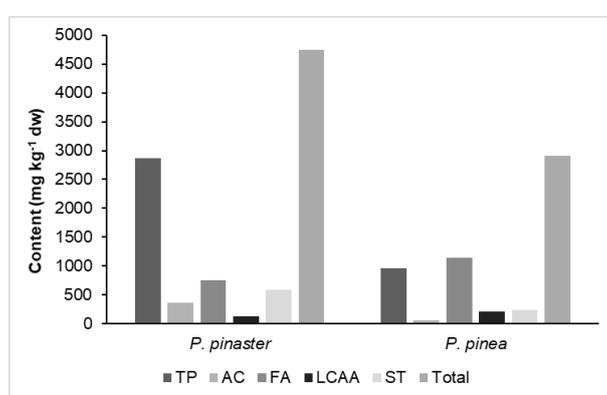
The dichloromethane extraction yield of *P. pinaster* bark ( $2.56 \pm 0.11\%$  *w/w*) was slightly higher than that of *P. pinea* bark ( $1.951 \pm 0.002\%$  *w/w*). Both values were within the lipophilic extracts yield range reported in earlier works for bark of *P. pinaster* (1.1–4.0% *w/w*) [26,27] and *P. pinea* (2.1% *w/w*) [28] grown in Portugal.

### 3.2. The Chemical Characterization of Lipophilic Fractions Derived from the *P. pinaster* and *P. pinea* Barks

Five major families of lipophilic components were identified and quantified in the lipophilic extracts from the bark of *P. pinaster* and *P. pinea* by GC-MS. These were terpenic compounds, aromatic compounds, fatty acids, long-chain aliphatic alcohols (LCAAs), and sterols, as demonstrated in Figures 1 and 2 and detailed in Table 1. The total content of identified compounds varied between 2910 mg kg<sup>-1</sup> dw in *P. pinea* bark and 4746 mg kg<sup>-1</sup> dw in *P. pinaster* bark. Terpenes are the main family present in these extracts, ranging from 965 mg kg<sup>-1</sup> dw in *P. pinea* bark to 2865 mg kg<sup>-1</sup> dw in *P. pinaster* bark, which accounted for 33% and 60% *w/w* of the total content of identified compounds, respectively.



**Figure 1.** A GC-MS chromatogram of a *Pinus pinea* bark trimethylsilyl-derivatized dichloromethane extract. Abbreviations: AC, aromatic compounds; FA, fatty acids; IS, internal standard; LCAA, long-chain aliphatic alcohols; RA, resin acids; SQT, sesquiterpenes; ST, sterols.



**Figure 2.** The major families of lipophilic components that were identified in the bark DCM extracts from Portuguese *P. pinaster* and *P. pinea*. Abbreviations: AC, aromatic compounds; FA, fatty acids; LCAA, long-chain aliphatic alcohols; ST, sterols; TP, terpenes.

### 3.2.1. Terpenic Compounds

Resin acids represent the major terpenic subclass present in the *P. pinaster* and *P. pinea* bark lipophilic fractions (Table 1), contributing 50% and 33% *w/w* of the total content of identified compounds, respectively. At a considerably lower abundance, betulin was the only pentacyclic triterpene identified in the *P. pinaster* bark extracts. Monoterpenes were also found in minor amounts, followed by sesquiterpenes.

Two groups of tricyclic diterpenic compounds were found in the studied *Pinus* spp. bark lipophilic extracts (Table 1), namely nine abietane-type and three pimarane-type resin acids (Figure 3). In fact, abietane-type resin acids were the most abundant components in the bark of the studied *Pinus* species, followed by pimarane-type resin acids.

DHAA (5) was found to be the most abundant component in bark of the studied *Pinus* species, representing 49% and 57% *w/w* of the total content of abietane-type resin acids in the *P. pinaster* and *P. pinea* bark samples, respectively (Table 1). Interestingly, this compound is only a minor constituent of fresh conifer resin; however, its abundance increases with aging, which results from the oxidation pathways of the abietadiene-type precursors (Scheme 1) [6,38,44]. Furthermore, the formation of DHAA might result from exposure to light and oxygen from the air. In addition, other DHAA oxidation derivatives were identified in the studied *Pinus* spp. extracts, namely 7 $\alpha$ / $\beta$ -hydroxy-DHAA (7 and 8), 7-oxo-DHAA (9), and other oxidation products (4 and 10–12) (Figure 3).

**Table 1.** The chemical composition of the lipophilic bark fractions from *P. pinaster* and *P. pinea* cultivated in Portugal (semi-quantitative data expressed in mg kg<sup>-1</sup> dw).<sup>1</sup>

RT (min)	No.	Compound	<i>P. pinaster</i>	<i>P. pinea</i>
		<b>Terpenic compounds</b>	<b>2865</b>	<b>965</b>
		<b>Diterpenic resin acids</b>	<b>2353</b>	<b>960</b>
		<i>Pimarane-type resin acids</i>	399	166
41.26	1	Pimaric acid	217	27
41.59	2	Sandaracopimaric acid	54	19
41.81	3	Isopimaric acid	128	120
		<i>Abietane-type resin acids</i>	1954	794
42.07	4	Di(dehydroabietic acid)	18	4
42.71	5	Dehydroabietic acid	954	452
43.41	6	Abietic acid	62	14
45.39	7	7β-Hydroxydehydroabietic acid	99	39
45.81	8	7α-Hydroxydehydroabietic acid	198	120
46.50	9	7-Oxodehydroabietic acid	179	57
46.98	10	15-Hydroxydehydroabietic acid	269	67
49.86	11	7,15-Dihydroxydehydroabietic acid	100	24
50.28	12	15-Hydroxy-7-oxodehydroabietic acid	75	17
		<b>Monoterpenes</b>	<b>55</b>	<b>n.d.</b>
7.86	13	Camphor	2	n.d.
8.46	14	Pinocarvone	6	n.d.
11.30	15	Geraniol	4	n.d.
11.74	16	Borneol	10	n.d.
14.08	17	Myrtenol	4	n.d.
15.06	18	α-Terpineol	13	n.d.
22.07	19	Terpin	16	n.d.
		<b>Sesquiterpenes</b>	<b>32</b>	<b>5</b>
17.47	20	Longifolene	21	2
22.67	21	Caryophyllene oxide	7	3
26.11	22	Isolongifolol	4	n.d.
		<b>Pentacyclic triterpenes</b>	<b>425</b>	<b>n.d.</b>
73.75	23	Betulin	425	n.d.
		<b>Aromatic compounds</b>	<b>366</b>	<b>53</b>
		<b>Aldehydes</b>	<b>37</b>	<b>9</b>
15.83	24	<i>p</i> -Hydroxybenzaldehyde	4	3
20.97	25	Vanillin	33	6
		<b>Acids</b>	<b>83</b>	<b>29</b>
28.39	26	Vanillic acid	6	n.d.
31.85	27	Syringic acid	1	n.d.
32.34	28	( <i>Z</i> )-Ferulic acid	3	n.d.
32.87	29	<i>p</i> -Coumaric acid	8	n.d.
36.51	30	( <i>E</i> )-Ferulic acid	58	29
38.07	31	Caffeic acid	7	n.d.
		<b>Resinols</b>	<b>246</b>	<b>15</b>
61.66	32	Pinoresinol	246	15
		<b>Fatty acids</b>	<b>749</b>	<b>1146</b>
		<b>Saturated fatty acids</b>	<b>670</b>	<b>1058</b>
6.22	33	Hexanoic acid	3	4
12.88	34	Octanoic acid	1	4
16.27	35	Nonanoic acid	1	11
19.51	36	Decanoic acid	tr	3
30.87	37	Tetradecanoic acid	1	4
33.38	38	Pentadecanoic acid	1	2
35.79	39	Hexadecanoic acid	55	50
37.46	40	Heptadecanoic acid	4	4
40.30	41	Octadecanoic acid	23	42
44.46	42	Eicosanoic acid	67	156
48.32	43	Docosanoic acid	251	401
50.14	44	Tricosanoic acid	30	36
52.00	45	Tetracosanoic acid	213	326
56.05	46	Hexacosanoic acid	20	15

Table 1. Cont.

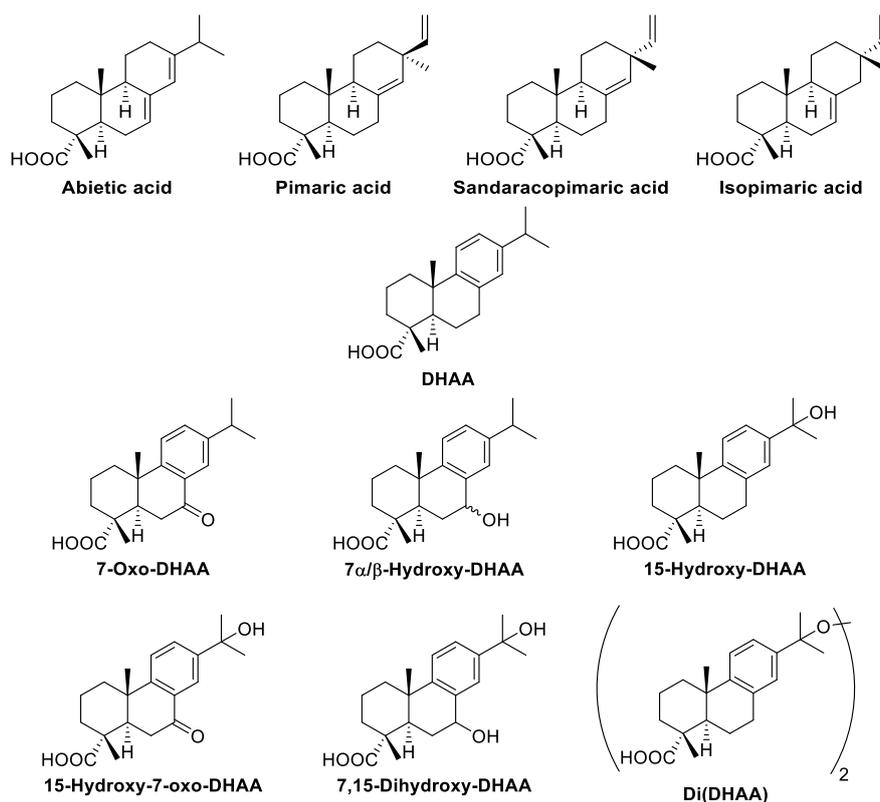
RT (min)	No.	Compound	<i>P. pinaster</i>	<i>P. pinea</i>
		<b>Unsaturated fatty acids</b>	<b>75</b>	<b>80</b>
39.38	47	(9Z,12Z)-Octadeca-9,12-dienoic acid	22	11
39.58	48	(9Z)-Octadec-9-enoic acid	48	39
39.79	49	(9E)-Octadec-9-enoic acid	5	6
43.83	50	(11Z)-Eicos-11-enoic acid	n.d.	24
		<b>Diacids</b>	<b>4</b>	<b>8</b>
29.42	51	Nonanedioic acid	4	8
		<b>Long-chain aliphatic alcohols</b>	<b>124</b>	<b>219</b>
33.94	52	Hexadecan-1-ol	11	8
37.86	53	Octadec-9-en-1-ol	11	8
38.57	54	Octadecan-1-ol	6	8
46.78	55	Docosan-1-ol	34	71
50.47	56	Tetracosan-1-ol	62	124
		<b>Sterols</b>	<b>587</b>	<b>242</b>
		<b><math>\Delta^5</math>-Sterols</b>	<b>467</b>	<b>242</b>
60.66	57	Campesterol	33	18
62.73	58	$\beta$ -Sitosterol	434	224
		<b><math>\Delta^4</math>-3-keto-steroids</b>	<b>120</b>	<b>n.d.</b>
64.08	59	Stigmast-4-en-3-one	120 <sup>2</sup>	n.d.
		<b>Others</b>	<b>55</b>	<b>285</b>
14.22	60	Glycerol	3	7
59.42	61	2,3-Dihydroxypropyl docosanoate	52	91
64.08	62	2,3-Dihydroxypropyl tetracosanoate	2	187
		<b>Total</b>	<b>4746</b>	<b>2910</b>

<sup>1</sup> The results represent the average of the concordant values obtained for the two aliquots of the two extracts in each sample (less than 5% variation between aliquots of the same sample, and between extracts of the same sample).

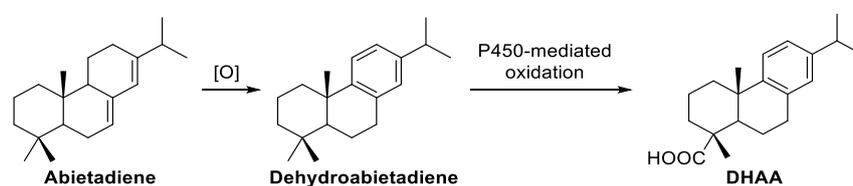
<sup>2</sup> The sum of the content of stigmast-4-en-3-one (59) and 2,3-dihydroxypropyl tetracosanoate (62). Abbreviations: n.d., not detected; tr, traces.

With respect to the identification of 7 $\beta$ -hydroxy-DHAA (7) and 7 $\alpha$ -hydroxy-DHAA (8), both TMS derivatives exhibit a small molecular ion at  $m/z$  460 and a fragment ion at  $m/z$  445 in their MS spectra, corresponding to the loss of a methyl radical from the ionized TMS groups. Further ions at  $m/z$  191, 234, 237, 252, 299, 370, and 417 are also observed. The relative abundances of these fragments differ strongly between the two isomers, which allows for the unambiguous assignment of their structures [38]. The chromatographic elution order was also crucial together with the comparison between the obtained MS spectra and the ones found in the literature [38].

Most of the identified diterpenic compounds, such as DHAA (5), abietic acid (6), 7-oxo-DHAA (9), 15-hydroxy-DHAA (10), pimaric acid (1), sandaracopimaric acid (2), and isopimaric acid (3), have already been reported to be components of *P. nigra* bark [21], Turkish *P. pinea* bark [23], and Pakistani *P. wallichiana*, *P. roxburghii*, and *P. gerardiana* bark [25]. However, to the best of our knowledge, their presence in Portuguese *P. pinaster* bark and *P. pinea* bark has not been previously reported.



**Figure 3.** The chemical structures of the diterpenic resin acids found in the Portuguese *P. pinaster* and *P. pinea* barks.



**Scheme 1.** The biosynthesis of DHAA.

The high abundance of DHAA and its oxidation derivatives adds value to these extracts, since they display important biological properties. For instance, DHAA (5) exhibits antiulcer, antimicrobial, antitumor, and anti-inflammatory effects [45]. Moreover, 7-oxo-DHAA (9) has shown contact allergenic properties, and 15-hydroxy-DHAA (10) and 15-hydroxy-7-oxo-DHAA (12) have shown anti-inflammatory activity.

Additionally, seven monoterpenes (13–19) and three sesquiterpenes (20–22) were detected at low abundances in the Portuguese *P. pinaster* bark, representing 1.9% and 1.1% *w/w* of the total terpene contents, respectively (Table 1). Terpin (19) was found to be the most abundant monoterpene (29% *w/w* of *P. pinaster* bark monoterpene content), whereas longifolene (20) was found to be the major sesquiterpene (66% *w/w* of total sesquiterpene content). The identified mono- and sesquiterpenes are reported in this study for the first time to be constituents of *P. pinaster* bark. Monoterpenes are common components of pine wood turpentine [46], and, in particular,  $\alpha$ -terpineol (18) has been identified in *P. pinea* needles [20], whereas longifolene (20) has been identified in *P. pinea* wood [23]. As in the case of DHAA and its oxidized derivatives, terpin is a dehydrated monoterpene that is not naturally found in wood. Therefore, its abundance in bark extracts should be associated with exposure to an external environment. Finally, the low abundance of monoterpenes that was observed in both studied *Pinus* spp. can be justified by their possible loss during the drying and grinding processes. The methodology

employed in the present work is not the most suitable for the characterization of their volatile fraction, which is beyond the scope of this work.

Betulin (**23**) was the single pentacyclic triterpene present in the *P. pinaster* bark lipophilic fraction, representing 15% *w/w* of the total terpene content (Table 1). Its identification was achieved through the detection of the molecular ion at *m/z* 586 from the corresponding TMS derivative, and the product ions common to the mass fragmentation of trimethylsilyl derivatives of lupane-type pentacyclic triterpenes, namely at *m/z* 571 ( $[M-CH_3]^+$ ), 496 ( $[M-TMSOH]^+$ ), 279, 190, and 189 as the base peak [47]. To the best of our knowledge, betulin (**23**) has been described herein for the first time in the bark of *P. pinaster*. Nevertheless, this compound has recently been detected in both the outer and inner bark of *Pinus merkusii* and the outer bark of *Pinus montezumae* [48]. The presence of this compound in *P. pinaster* bark can be an additional factor for exploiting this forest by-product, as it can be converted into betulinic acid, which exhibits several interesting biological properties, including Human Immunodeficiency Virus (HIV) inhibition, antimicrobial, and anti-inflammatory activity [49].

### 3.2.2. Fatty Acids

Nineteen fatty acids (**33–51**) were identified in the *P. pinaster* and *P. pinea* bark lipophilic extracts, accounting for 16% and 39% *w/w* of the total content of identified components, respectively (Table 1). Among the fatty acids, fourteen were saturated and four unsaturated, and their chain length varied between 6 and 26 carbon atoms. In both samples, the saturated fatty acids content was much higher than the unsaturated fatty acids content, ca. 8.9-fold and 13.2-fold for the *P. pinaster* and *P. pinea* bark lipophilic extracts, respectively. Indeed, docosanoic acid (**43**) was the major saturated fatty acid, whereas (9*Z*)-octadec-9-enoic acid (**48**) was the major unsaturated fatty acid. Considering the fatty acid composition of *P. pinaster* bark, all of the presented constituents of this family were herein identified for the first time, with the exception of tetradecanoic acid (**37**), hexadecanoic acid (**39**), and (9*Z*)-octadec-9-enoic acid (**48**) [50]. On the other hand, hexanoic acid (**33**), octanoic acid (**34**), nonanoic acid (**35**), decanoic acid (**36**), tricosanoic acid (**44**), hexacosanoic acid (**46**), and (11*Z*)-eicos-11-enoic acid (**50**) were identified for the first time in *P. pinea* bark in the present work.

### 3.2.3. Sterols

Two  $\Delta^5$ -sterols, namely campesterol (**57**) and  $\beta$ -sitosterol (**58**), and a  $\Delta^4$ -3-keto-steroid, named stigmast-4-en-3-one (**59**), were found, at lower abundances, in the *P. pinaster* and *P. pinea* bark lipophilic extracts (Table 1). Among these,  $\beta$ -sitosterol was found to be the major component in the studied *Pinus* bark samples. To the best of our knowledge, these sterols were also identified here for the first time in *P. pinaster* bark. On the other hand, campesterol (**57**) and  $\beta$ -sitosterol (**58**) have already been described as lipophilic components of *P. pinea* bark and wood [23].

### 3.2.4. Aromatic Compounds

Nine aromatic compounds (**24–32**) were analyzed in the *P. pinaster* and *P. pinea* bark lipophilic fractions, with concentrations ranging from 7.7% to 1.8% *w/w* of the total concentration of identified components (Table 1). Pinoresinol (**32**) was the major component of this class that was present in the *P. pinaster* bark. On the other hand, (*E*)-ferulic acid (**30**) was the major component in the *P. pinea* bark.

### 3.2.5. Long-Chain Aliphatic Alcohols and Minor Compounds

Low abundances of five LCAAs (**52–56**) were identified in the analyzed *P. pinaster* and *P. pinea* lipophilic extracts, representing 2.6% and 7.5% *w/w* of the total content of identified constituents, respectively (Table 1). The chain length of these compounds ranged from 16 to 24 carbon atoms, with octadec-9-en-1-ol (**53**) as the only unsaturated component of this subclass. Tetracosan-1-ol (**56**) was the major constituent of this lipophilic class in both of the *Pinus* lipophilic fractions. With the exception of hexadecan-1-ol (**52**) [50], the remaining LCAAs have been mentioned in the present work for the

first time as constituents of bark from the Portuguese *P. pinaster*. For the *P. pinea* bark, the LCAAs hexadecan-1-ol (52) and octadec-9-en-1-ol (53) were herein found for the first time.

Finally, glycerol (60) and two monoglycerides (61 and 62) were found in the *P. pinaster* and *P. pinea* bark samples, representing 1.2% and 9.8% *w/w* of the total content of identified compounds, respectively (Table 1).

#### 4. Conclusions

In conclusion, the Portuguese *P. pinaster* and *P. pinea* barks demonstrated a high abundance of diterpenic compounds, with abietic-type resin acids as the main constituents of this family. In particular, dehydroabietic acid was found to be the major resin acid component of these by-products, together with several oxidized DHAA derivatives, suggesting that extensive oxidation/dehydrogenation of naturally occurring abietane-type resin acids had taken place. In addition, aromatic compounds, long-chain aliphatic alcohols, fatty acids, and sterols were also identified at lower amounts. This study suggests that *P. pinaster* and *P. pinea* barks can be a good source of valuable diterpenic compounds for further nutraceutical applications. Finally, there is a commercially available bark extract from French maritime pine (Pycnogenol®) that is rich in polyphenolic compounds, such as catechin, taxifolin, procyanidins, and phenolic acids. Due to the importance of phenolic compounds, new studies involving polar extracts from Portuguese *P. pinaster* and *P. pinea* bark will be performed next.

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