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Biochemical Characterization and Fuel Properties of Endemic Taurus Flowering Ash (*Fraxinus ornus* subsp. *cilicica*) Bark from Turkey

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Abstract: Taurus flowering ash (*Fraxinus ornus* subsp. *cilicica*) is an endemic tree species in Turkey. The bark of the species was characterized for summative chemical composition, the monomeric composition of polysaccharides, phenolic content, in vitro and ex vivo antioxidant properties of hydrophilic extracts, the composition of lipophilic extractives and suberin, and thermal degradation. The bark has an elevated ash content (17%), primarily composed of calcium, and a noteworthy extractive content (38.9%), predominantly of hydrophilic compounds. The antioxidant activity of the bark extracts is moderate, with an IC50 value of 40 µg/mL and an EC_{50} value of 230 µg/mL by DPPH and TBARS methods. The lipophilic extractives principally contain fatty acids and diterpenoids. The suberin content is low (1%) and composed primarily of ω-hydroxy acids with 9,10,18 trihydroxyoctadecanoic acid as the major suberin monomer. The lignin content is low (9.8%), and polysaccharides represent 33%. The ignition temperature of the bark is 190 °C, the burnout temperature is 653 °C, and the activation energy in combustion is 29 kJ mol^{-1}. A biorefinery concept was developed considering the bark's chemical and thermal characteristics to convert approximately 90% of the bark mass into valuable chemicals, extracts, functional materials, and additives.

Keywords: calcium; ω-hydroxy acids; suberin; antioxidant; ignition temperature

Citation: Şen, A.U.; Simões, R.; Yücedağ, C.; Miranda, I.; Fernandes, Â.; Pereira, H. Biochemical Characterization and Fuel Properties of Endemic Taurus Flowering Ash (*Fraxinus ornus* subsp. *cilicica*) Bark from Turkey. *Processes* **2023**, *11*, 2774. https://doi.org/10.3390/pr11092774

Academic Editor: Albert Ratner

Received: 21 August 2023
Revised: 5 September 2023
Accepted: 14 September 2023
Published: 16 September 2023

1. Introduction

Ash trees are important members of Turkey forests where a total of four different ash species grow naturally, including *Fraxinus excelsior*, *Fraxinus ornus*, *Fraxinus oxycarpa*, and *Fraxinus numidica* [1]. The species *Fraxinus oxycarpa* and *F. ornus* are also present as subspecies, such as *F. oxycarpa* subsp. *oxycarpa*, *F. oxycarpa* subsp. *parvifolia*, *F. oxycarpa* subsp. *syriaca*, *F. ornus* subsp *ornus*, and *F. ornus* subsp. *cilicica* is endemic to Turkey [1,2].

Flowering or manna ash (*Fraxinus ornus*) is a medium-sized deciduous tree that belongs to the *Oleaceae* family and is primarily used as an ornamental plant due to the aesthetic characteristics of its flowers. The endemic subspecies Taurus flowering ash is distributed in the southwest and south of Turkey, with the major presence being in the Taurus mountains [1,2]

Flowering ash is an interesting tree for bark-based biorefineries because a number of chemicals may be produced from its bark. For instance, bark tapping results in the formation of a yellow gum called manna, which is composed of polysaccharides [3]. It

has been reported that the names mannose and mannitol derive from this extract [4]. The gums are functional solid materials because they have gelling, emulsification, thickening, moisture retention, and stabilization properties that allow them to be used as an additive or as an ingredient in the food industry [5]. Moreover, the bark contains a high amount of extractives, and five different groups of hydrophilic extractives, including coumarins, flavonoids, lignans, secoiridoids, and phenylethanoids, were reported to be present in the bark [6–9]. Coumarins are fragrant compounds typically found in cassia cinnamon, green tea, and olive oil and exhibit antimicrobial properties [10]. Flavonoids are a wide group of polyphenols found in plants with bioactive properties such as anti-cancer and anti-aging [11]. Lignans are phenolic compounds composed of two phenyl propane units, found particularly in heartwood extracts of softwoods such as pine and spruce [12], that possess anti-tumor and anti-viral properties [13]. Secoridoids are derivatives of cyclopentane [c] pyran monoterpenoids and are distinctive extracts of the *Oleaceae* family with pharmacological effects such as anti-diabetic, antioxidant, anti-inflammatory, immunosuppressive, neuroprotective, and anti-cancer [14]. Phenylethanoids are also polyphenols usually found in olive oils and show strong antioxidant properties [15]. *F. ornus* bark is thus an interesting source for obtaining bioactive extracts in addition to manna gums. In addition to these properties, the bark of *F. ornus* was also a source of a natural blue dye for coloring carpets in Turkey [16]. Interestingly, a similar blue dye use was reported for the bark of European or common ash and blue ash (*F. excelsior* and *F. quadrangulata*) [17–19].

The chemical composition of the flowering ash bark regarding its structural macro constituents, inorganic elemental and lipophilic compositions, and ex vivo antioxidant properties were not studied before, to the best of our knowledge. Thermochemical conversion of lignocellulosic biomass is a fast and effective biorefinery process and may be broadly grouped as combustion, dry and wet pyrolysis, as well as gasification [20]. Combustion and gasification properties indicate the potential of a lignocellulosic material for fuel uses. The combustion properties may be evaluated through analyses of activation energy, ignition, and burnout temperatures, with lower values desired for co-combustion applications. Pyrolysis properties indicate the potential of lignocellulosic material for charcoal or bio-oil production. These properties are important for closing the material loops in a bark-based biorefinery system, leading to a circular economy. This study explores the biorefinery-relevant properties of the endemic Taurus flowering ash for the first time, aiming to contribute to its valorization and protection.

2. Materials and Methods

2.1. Materials

Bark specimens of *Fraxinus ornus* subsp. *cilicica* were obtained from the Kovada Lake National Park (Figures 1 and 2), approximately 30 km air distance from Isparta, southwest of Turkey, between elevations of 946 and 1100 m and exposure to the south (37°39′36″ N–30°51′24″ E). A total of five bark specimens (approximately 200 g each) from five different trees (approximately 20 years old and with an average diameter of 30 cm) were collected. According to Köppen and Geiger classification, the climate of the sampling area is classified as Csa. The average annual temperature is 14.0 °C, and the average annual rainfall is approximately 968 mm.

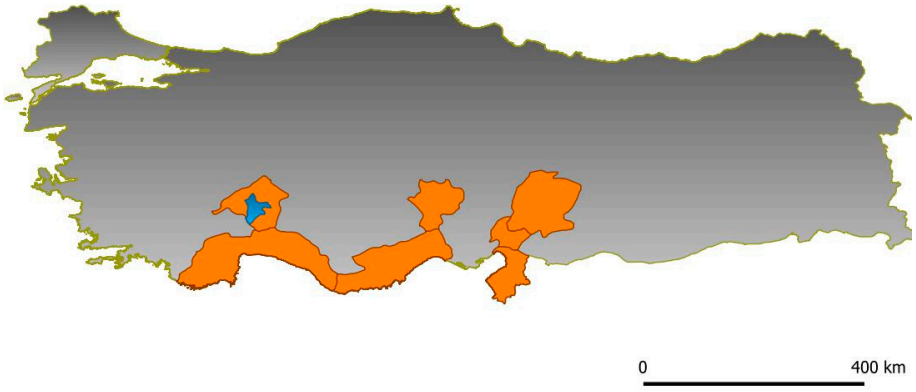

Figure 1. Distribution of *Fraxinus ornus* subsp. *cilicica* (orange) in the administrative zones of Turkey and the sampling area (blue). Adapted from Flora of Turkey and the East Aegean Islands vol. VI [21].

2.2. Summative Chemical Composition

The analysis of the overall chemical composition of *F. ornus* bark, which includes extractives, lignin, and suberin contents, as well as the monomeric composition of polysaccharides, was conducted on samples that had been previously dried. These samples were subjected to a drying process, initially at 60 °C for approximately 16 ± 1 h, followed by an additional drying phase at 100 °C for 2 h. The complete extractive content was assessed through a series of three consecutive Soxhlet extractions following the guidelines outlined in TAPPI Standards (T 204 om-88 and T 207 om-93) [22,23]. These extractions involved the use of three different solvents: dichloromethane (CH_2Cl_2/DCM), ethanol (C_2H_5OH/EtOH), and water (H_2O). Each solvent extraction was conducted over specific durations, with a 6-hour extraction for dichloromethane, an 18 h extraction for ethanol, and another 18 h extraction for water.

Figure 2. Bark of *Fraxinus ornus* subsp. *cilicica*.

The suberin content was determined as a percentage of the dry mass through a methnolysis procedure performed on the extractive-free material. Approximately 1.5 g extractive-free material was subjected to an alcoholysis process where it was refluxed with 100 mL of a 3% methanolic solution containing $NaOCH_3$ in CH_3OH for a duration of 3 h. Subsequently, the sample was filtered, rinsed with methanol, and then refluxed again with 100 milliliters of CH_3OH for a 15 min period, followed by filtration. The filtered liquids were merged and adjusted to a pH of 6 by adding 2 M H_2SO_4, after which they were evaporated until complete dryness. The residue was dissolved in 50 mL of water, and the products resulting from the alcoholysis reaction were retrieved through three consecutive liquid-liquid extractions, each employing 50 mL of CH_2Cl_2. The extracts were dehydrated using anhydrous Na_2SO_4, and then the solvent was removed through evaporation, leaving a concentrated sample for compositional analysis. The aqueous phase was kept for analysis of glycerol.

Klason lignin and acid-soluble lignin contents were assessed following the TAPPI T 222 om-88 and TAPPI UM 250 Standards [24,25] on the extractive and suberin-free (desuberinised) bark specimens. A total of 3.0 mL H_2SO_4 (72%) was introduced to 0.35 g of extracted and desuberinised material, and the mixture was reacted in a water bath at 30 °C for a period of 1 h, after which it was diluted to a concentration of 4% H_2SO_4 and hydrolyzed for 1 h at 120 °C [26]. The sugar monomers were determined using high-performance anion-exchange chromatography (HPAEC) with Aminotrap plus CarboPac SA10 anion exchange columns. In the conditions used, mannose was eluted partially with xylose and was not singled out. All chemical experiments were conducted with four repetitions.

2.3. Inorganic Elemental Composition

The elemental composition of the inorganic (ash) fraction of *F. ornus* bark was analyzed by molecular absorption or atomic absorption spectrometry after a hydrochloric digestion of the ash. Phosphorus was quantified by molecular absorption spectrometry in a Hitachi U-2000 Vis/UV equipment, and the Ca, Mg, Na, K, Cu, Mn, Zn, S, Fe, and B contents were assessed by atomic absorption spectrophotometer in a Pye Unicam SP-9 apparatus (Cambridge, UK) equipped with a GF95 graphite furnace.

2.4. FT-IR Analysis

The bark samples (60–80 mesh particles) of *F. ornus* bark were positioned on the diamond for ATR-FTIR analysis. The transmittance spectra were acquired with a Perkin Elmer Spectrum Two mid-infrared FT-IR instrument in the range of 4000–400 cm^{-1} with a spectral resolution of 8 cm^{-1}.

2.5. Phenolic Content

The determination of phenolics comprises the contents of total phenolics (TPC), flavonoids, and condensed tannins. Approximately 1 g of the ground *F. ornus* bark sample was extracted with ethanol-water (50/50, *v*/*v*) with a solid–liquid ratio of 1:10 (*m*/*v*) for 60 min at 50 °C using an ultrasonic bath. The insoluble materials were removed by filtration, and the supernatant extract was stored at 4 °C.

The assessment of total phenolic content was conducted by the Folin–Ciocalteu colorimetric method and the results were expressed as milligrams of gallic acid equivalents (GAE)/gram of the dry extract. The quantification of total flavonoid content was performed by aluminum chloride ($AlCl_3$) colorimetric assay, and the findings were expressed as milligrams of (+)−catechin equivalents (CE)/gram of the dry extract. The condensed tannin content was quantified using the vanillin-H_2SO_4 method and expressed as milligrams of (+)−catechin equivalents (CE)/gram of the dry extract.

2.6. In Vitro and Ex Vivo Antioxidant Activity

The in vitro antioxidant activity of the *F. ornus* bark extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) method. The DPPH antioxidant activity was expressed as the amount of extract required to reduce by 50% the DPPH concentration (IC_{50}) and as Trolox equivalents (TEAC) on a dry extract base (mg Trolox/g dry extract). The FRAP (ferric reducing antioxidant power) assay followed the Benzie and Strain method [27] with modifications. Aqueous solutions of known Fe (II) concentrations in the range of 0–1500 µmol/L ($FeSO_4.7H_2O$) were used for the calibration curve, and the findings were expressed as millimoles Fe (II)/g extract. Trolox and catechin were used as reference compounds.

The ex vivo antioxidant activity of the hydroethanolic extracts of the *F. ornus* bark samples was evaluated through a cell-based thiobarbituric acid reactive substances (TBARS) assay [28]. The granulated bark samples (~5 g, 250–420 µm) were stirred in 30 mL of ethanol/water (80:20, *v*/*v*) at 25 °C for 1 h and filtered through a Whatman No. 4 paper. The residue was re-extracted with an additional 30 mL of the hydroethanolic mixture. The combined extracts were concentrated at 40 °C under reduced pressure using a rotary evaporator (Büchi R-210, Flawil, Switzerland) and subsequently lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA).

For the TBARS assay, the hydroethanolic extracts were first dissolved in water and subjected to dilutions ranging from 10 mg/mL to 0.3125 mg/mL. The inhibition of lipid peroxidation in porcine (*Sus scrofa*) brain cell homogenates was evaluated by measuring the decrease in TBARS and the color intensity of the malondialdehyde–thiobarbituric acid (MDA–TBA) through the absorption measurement at 532 nm.

The lipid peroxidation inhibition ratio (%) was calculated using the Formula (1):

$$(A - B)/A \times 100 \quad (1)$$

where A and B represent the absorbance of the control and extract samples, respectively; the results were expressed as EC_{50} values (µg/mL), which indicates the sample concentration providing 50% antioxidant activity and were calculated from the graph showing the percentage inhibition against extract concentration. Trolox (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control.

2.7. Composition of Lipophilic Extractives

Aliquots of the dichloromethane extracts (5 mL) were evaporated under N_2 flow and dried at room temperature under vacuum overnight (16 ± 1 h). The samples were derivatized in 100 µL of pyridine by adding 100 µL of bis(trimethylsilyl)-trifluoroacetamide (BSTFA), by which all the compounds with hydroxyl and carboxyl groups were trimethylsilylated into trimethylsilyl (TMS) ethers and esters, respectively. The reaction mixture was heated at 60 °C for a duration of 30 min in an oven. The derivatized extracts were analyzed by GC–MS [EMIS, Agilent 5973 MSD, 70 eV, MS source 220 °C] under the following GC conditions: Zebron 7HGG015-02 Phenomenex column (30 m, 0.25 mm; ID, 0.1 µm film thickness), injector 280 °C, oven temperature program, 100 °C (1 min), →150 °C (10 °C min^{-1}), →300 °C (4 °C min^{-1}), →370 °C (5 °C min^{-1}), →380 °C (8 °C min^{-1}), holding time 5 min.

The lipophilic compounds were identified as TMS derivatives by comparing their mass spectra with a GC–MS spectral library (Wiley, NIST) and by evaluating their fragmentation profiles. The peak areas in the TIC were determined and expressed as normalized relative percentages. Triplicate injections were performed for each aliquot.

2.8. Suberin Monomeric Composition

The composition of suberin was quantified in aliquots taken from the methanolic extracts obtained after the suberin depolymerization. The extracts were evaporated, derivatized by trimethysilylation, and immediately analyzed by GC-MS, with the fol-

lowing conditions: Zebron 7HGG015-02 column (Phenomenex, Torrance, CA, USA) (30 m, 0.25 mm; ID, 0.1 µm flm thickness), injector 400 °C, oven temperature program: 50 °C (held 1 min), 10 °C min^{-1} to 150 °C, 5 °C min^{-1} to 200 °C, 4 °C min^{-1} to 300 °C, 10 °C min^{-1} to 380 °C (held 5min). The MS source was held at 220 °C, and the electron impact mass spectra (EIMS) were at 70 eV of energy. The glycerol released by suberin depolymerization was assessed in the aqueous layer obtained from the liquid–liquid separation of the solubilized compounds by suberin depolymerization applying high-performance liquid chromatography (HPLC). The HPLC analysis was performed with a Dionex ICS-3000 system equipped with an electrochemical detector (Sunnyvale, CA, USA), with Aminotrap plus CarboPac SA10 anion exchange columns (Thermo Scientific, Waltham, MA, USA). A mobile phase of an aqueous 2 nm sodium hydroxide (NaOH) solution at a flow rate of 1.0 mL min^{-1} at 25 °C was applied.

2.9. Thermogravimetric Analysis

The thermogravimetric analysis (TGA) of the *F. ornus* bark was carried out with TA Instruments SDT 2960 simultaneous DSC-TGA analyzer using alumina pans under air or nitrogen flow rates of 50 mL min^{-1}. For the thermogravimetric analyses, a linear heating program was applied between 30 °C and 800 °C. Approximately 5 mg of bark samples and a heating rate of 10 °C min^{-1} were used with three replicates.

The ignition (T_i) and the burnout temperatures (T_b) were assessed by analyzing thermogravimetric (TG) curves employing the intersection and the conversion method at 99%, respectively [29]. The temperature at maximum degradation rate (t_m) was obtained from the differential thermogravimetric (DTG) curves.

2.10. Kinetic Analysis of Combustion

The reaction order models are frequently employed for the solid-state reaction model f(α), where α is conversion. In the present study, the Coats-Redfern method with the first-order kinetic model was used [30]. The kinetic parameters were derived from the plot of Equation (2) [31].

$$\ln\left(-\frac{ln(1-\alpha)}{T^2}\right) vs \frac{1}{T} \quad (2)$$

In this equation, T represents the temperature (K).

3. Results

3.1. Chemical Composition

The chemical analysis of *Fraxinus ornus* bark showed significant features: an extraordinarily high extractive content (39%), a high ash content (17%), and a low lignin content (9.8%) (Table 1). Approximately 94% of the extractives are hydrophilic (EtOH and H_2O soluble).

The *F. ornus* bark contains a significant amount of ash, and its inorganic composition showed that approximately 91% of the detected minerals are calcium, with potassium (5.1%), magnesium (1.7%), and sulfur (1.3%) also detected in important amounts (Table 1). *F. ornus* bark also contains oligoelements such as iron, zinc, copper, and manganese.

The monomeric composition of the bark polysaccharides is primarily composed of glucose (59%) and xylose (26%). The bark polysaccharides are also contained in smaller amounts, arabinose (8%), galactose (5%), and rhamnose (1.5%), as well as acetyl groups (0.3%) (Figure 3). The xylose content possibly contains residual mannose polysaccharides because, under the applied HPAEC conditions, they are co-eluted.

3.2. FT-IR Analysis

The FT-IR spectrum of *F. ornus* bark is shown in Figure 4. A total of 11 prominent peaks were detected and assigned to the bark chemical components (Table 2). The spectrum shows typical bands of calcium oxalate at 514, 780, 1316, and 1609 cm^{-1} [32], which is in agreement with the elemental analysis (Table 1).

Table 1. Chemical composition of *Fraxinus ornus* bark.

Component	% Dry Weight	% Ash-Free Weight
Ash	17.1 ± 3.2	-
Extractives-DCM	2.5 ± 0.3	3.0
Extractives-EtOH	29.4 ± 3.4	35.5
Extractives-H_2O	7.0 ± 0.3	8.5
Total extractives	38.9 ± 3.3	46.9
Suberin	1.0 ± 0.2	1.2
Klason lignin	6.7 ± 1.1	8.1
Acid-soluble lignin	3.1 ± 0.7	3.8
Total lignin	9.8 ± 1.8	11.8
* Polysaccharides	33.2 ± 9.2	40.1
Major elements	mg kg^{-1} bark	% Elements
Na	25.3	0.1
K	2448.8	5.1
Ca	43,356.7	90.7
Mg	813.6	1.7
P	274.3	0.6
S	636.6	1.3
Fe	228.9	0.5
Oligoelements	mg kg^{-1} bark	% Oligoelements
Cu	6.1	15.2
Zn	7.6	18.9
Mn	13.8	34.3
B	12.7	31.6
Total	47,824.4	100.0

* Calculated by difference.

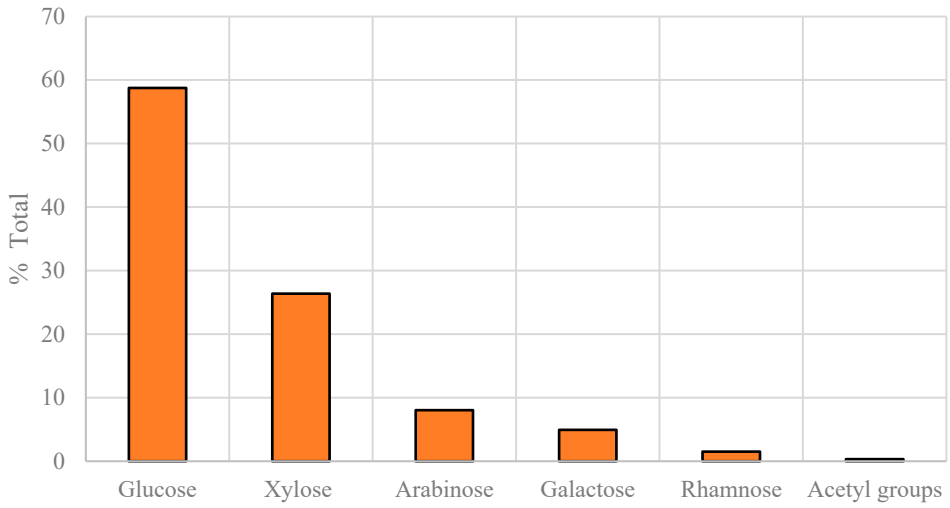

Figure 3. Monomeric composition of polysaccharides of *F. ornus* bark.

The peaks at 1016 cm^{-1} and 1332 cm^{-1} are assigned to O-H and C-O stretching from polysaccharides [33,34], the peak at 1332 cm^{-1} may also be assigned to the breathing vibrations of syringyl lignin [35] while the peak at 1016 cm^{-1} is attributed to C-O stretching in lignin [36]. The peak at 1419 cm^{-1} is assigned to aromatic skeletal vibrations combined with CH deformation of lignin [36,37], and the peak at 1263 cm^{-1} is assigned to guaiacyl ring breathing, or C-O stretches in lignin [36]. The peak at 2934 cm^{-1} is attributed to C-H stretching from suberin, while the peak at 3327 cm^{-1} is assigned to O-H stretching from moisture or polysaccharides.

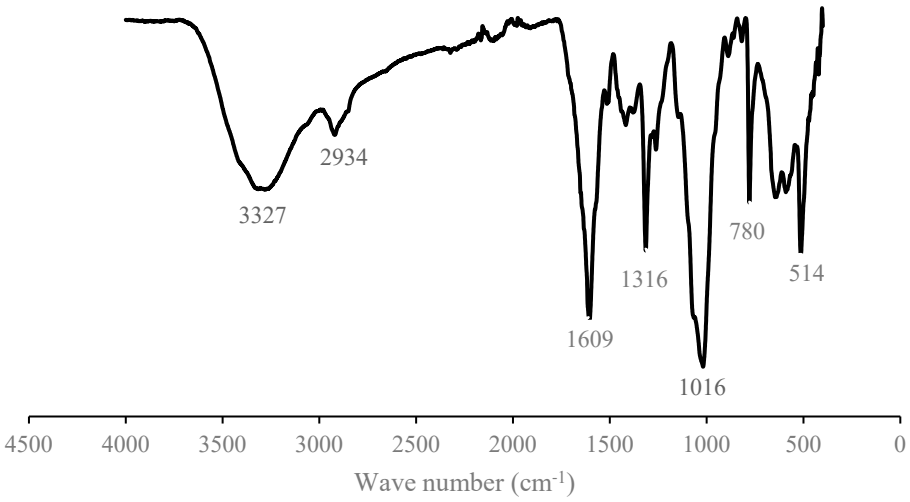

Figure 4. Transmittance FT-IR spectra of *Fraxinus ornus* bark.

Table 2. Assignment of major peaks of FT-IR spectra of *Fraxinus ornus* bark.

Signal (cm^{-1})	Functional Group	Assignment to Chemical Components	Reference
512	O-C-O	CaOxa	-
780	O-C-O	CaOxa	[32]
1016	C-O	Cellulose or lignin	[36]
1316	O-C-O	CaOxa	[32]
1419	C-H	Lignin	[36]
1609	C=O	CaOxa	[32]
2934	C-H	Suberin	-
3227	O-H	Moisture or phenolic structures	-

3.3. Phenolic Content and Antioxidant Properties

The phenolic content and in vitro antioxidant properties of *F. ornus* bark are shown in Table 3. *F. ornus* bark contains approximately 242 mg GAE/g extract total phenolic content, 172 mg CE/g extract flavonoids, and 107 mgCE/g extract condensed tannins. The in vitro antioxidant activity of the bark is moderate, with a 40 µg/mL IC_{50} value or a 0.3 mM FRAP value.

Table 3. The phenolic content and in vitro antioxidant properties (DPPH and FRAP) of *F. ornus* bark.

Extract Yield (%)	TPC (mg GAE/g Extract)	Flavonoids (mg CE/g Extract)	Condensed Tannins (mg CE/g Extract)	DPPH (mg TE/g Extract)	DPPH IC_{50} (µg/mL)	FRAP (mM)
35.5 ± 2.1	241.8 ± 3.2	171.6 ± 13.6	106.6 ± 3.8	113.4 ± 44.5	40.0 ± 20.9	0.3 ± 0.0

The results of the ex vivo antioxidant properties of *F. ornus* bark are shown in Table 4. The hydroethanolic extracts of *F. ornus* bark also showed weak antioxidant activity according to the TBARS method with an EC_{50} value of 230 µg/mL.

Table 4. Ex vivo antioxidant properties (TBARS) of *F. ornus* bark.

Extract yield (%)	24.9
EC_{50} (µg/mL)	230 ± 10
Trolox (µg/mL)	5.4 ± 0.3

3.4. Lipophilic Extractives

The lipophilic composition of *F. ornus* bark is shown in Table 5. Simple fatty acids are the principal extractives, followed by ω-hydroxy fatty acids, together making up almost half of the detected compounds. Hexadecanoic acid was the primary fatty acid (15.1% of all compounds), followed by octadecanoic acid (8.8%). Terpenoids were also an important fraction of the lipophilic extractives, with approximately 21% of the detected compounds.

3.5. Suberin Composition

The suberin composition of *F. ornus* bark was analyzed, and the results are shown in Figure 5, grouped by chemical family. Suberin included 9.6% glycerol and glycerol derivatives, 3.9% aromatic compounds, 20.5% dicarboxylic acids, 55.6% ω-hydroxyalkanoic acids, 10.1% alkanoic acids, and 0.5% alkanols.

Table 5. Composition of the lipophilic extractives (dichloromethane solubles) of *F. ornus* bark expressed in % of the chromatographic peak areas.

Compound Families	Compounds	% Total Peak Area
Alcohols	Total	1.43
	Dodecanol	0.12
	Hexadecanol	0.07
	Docosanol	0.48
	Hexacosanol	0.11
	Octacosanol	0.65
Fatty acids	Total	42.99
	Octanoic acid	2.55
	Nonanoic acid	0.46
	Decanoic acid	0.16
	2-Ethyl hexanoic acid	6.86
	Tetradecanoic acid	0.14
	Pentadecanoic acid	0.17
	Hexadecanoic acid	15.13
	Heptadecanoic acid	0.69
	9,12-Octadecadienoic acid	0.32
	9-Octadecenoic acid	2.46
	Octadecanoic acid	1.99
	Eicosanoic acid	1.85
	Heneicosanoic acid	0.98
	Docosanoic acid	3.63
	Tricosanoic acid	0.43
	Tetracosanoic acid	1.42
	Pentacosanoic acid	0.10
	Hexacosanoic acid	1.32
	Octacosanoic acid	1.79
	Triacontanoic acid	0.54
Diacids	Total	7.45
	Oct-2-ene-1,8 dioc acid	2.13
	Nonanedioic acid	3.56
	Octadecanedioic acid	1.79
ω-hydroxy fatty acids	Total	8.79
	9,10-Epoxy-18 hydroxy octadecanoic acid	8.79

Table 5. *Cont.*

Compound Families	Compounds	% Total Peak Area
Phenolics	Total	0.49
	Homovanillyl alcohol	0.49
Steroids	Total	0.79
	β-sitosterol	0.67
	Stigmast-4-en-3-one	0.11
Terpenoids	Total	20.89
	Dehydroabietic acid	4.53
	Abietic acid	16.18
	Betulinic acid	0.18
Sugars	Total	0.15
	Sitosteryl-3β-D-glucopyranoside	0.15

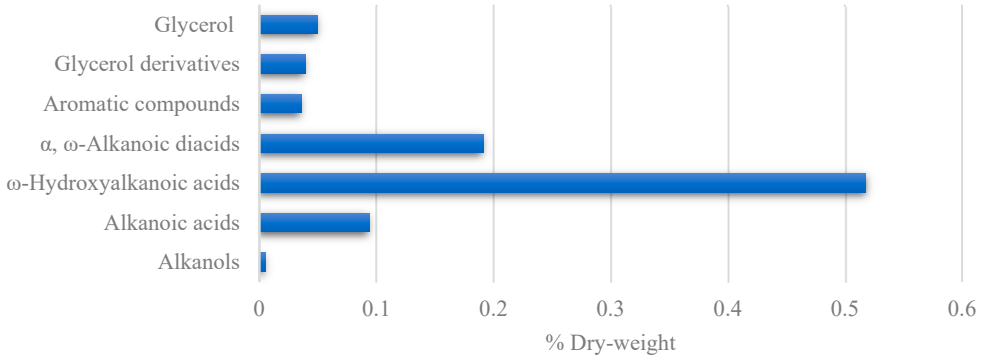

Figure 5. Suberin monomers of *Fraxinus ornus* bark grouped by chemical families (% dry-weight).

Table 6 summarises the composition of the lipophilic monomers of suberin. The suberin of *F. ornus* bark is predominantly composed of ω-hydroxy acids (47.5% of the total long-chain compounds) followed by α-ω-dicarboxylic acids (19.4%). The major suberin monomer was 9,10,18 trihydroxyoctadecanoic acid (14.6%), followed by 9,10-epoxy-18-hydroxy octadecanoic acid (12.8%) and 18-hydroxy octadecanoic acid (12.8%) which make up approximately 40.2% of total suberin monomers. The eicosanedioic acid was the principal α-ω-dicarboxylic acid (14.2%), comprising approximately 73% of dicarboxylic acid monomers.

3.6. Thermogravimetric Analysis

The thermal degradation of *F. ornus* bark performed under oxygen (combustion) and nitrogen (pyrolysis) environments is shown in Figure 6.

The thermal degradation starts at temperatures below 200 °C and proceeds fast between 200 and 380 °C until 60% mass loss (Figure 6). The initiation of the thermal degradation around 122 °C possibly arises due to its low suberin and lignin contents, which is a distinct feature of the *F. ornus* bark (Table 7). Thermal degradation of the bark is limited to 200 °C (8.2%) and occurs largely due to moisture loss and, to a lesser extent, loss of bark macro components (Table 7). The main mass loss region is found between 200 °C and 450 °C, where approximately half the bark weight is devolatilized.

During thermal degradation, at least two independent char oxidation reactions occurred at approximately 440 °C and 667 °C, respectively. The ignition temperature was 190 °C, the burnout temperature was 653 °C, and the activation energy and pre-exponential factor during combustion were 29 kJ mol^{-1} and 19.15 min^{-1}, respectively (R^2 = 0.987).

Table 6. Suberin monomeric composition of *F. ornus* bark expressed in % of the chromatographic peak areas.

Compound Families	Compounds	% Total Peak Area
Alcohols	Total	0.46
	Octadecanol	0.17
	Eicosanol	0.22
	1,22-Docosanediol	0.07
Fatty acids	Total	14.93
	Hexadecanoic acid, methyl ester	0.14
	Octadecanoic acid, TMS	0.05
	Octadecanoic acid, 9,10-dihydroxy-, methyl ester	5.37
	Methyl 2-hydroxytetradecanoate, TMS	0.19
	15-Tetracosenoic acid, TMS	9.19
α-ω-dicarboxylic acids	Total	19.37
	Hexadecanedioic acid, dimethyl ester,	0.54
	Octadec-9-enedioic acid, dimethyl ester	1.19
	Octadecanedioic acid, dimethyl ester	0.39
	Eicosanedioic acid, dimethyl ester	14.16
	Docosanedioic acid, dimethyl ester	0.69
	Hexadecanedioic acid, 2TMS	0.05
	Octadec-9-enedioic acid, (18-trimethylsiloxy)-, methyl ester	0.23
	Octadecanedioic acid 9,10-dihydroxy(di-TMS), 1,18-(dimethylsilyl)	2.12
ω-hydroxy acids	Total	47.52
	Hexadecanoic acid, (16-trimetilsiloxy)-, methyl ester	0.30
	Methyl 18-hydroxy-9-octadecenoate, TMS	12.83
	Octadecanoic acid, 9,10-epoxy-18-trimethylsiloxy)-, methyl ester cis	12.84
	Eicosanoic acid, (20-trimethylsiloxy)-, methyl ester	0.41
	9,10,18 Trihydroxyoctadecanoic acid, methyl ester, threo, 3TMS	14.60
	Docosanoic acid, (22-trimethylsiloxy)-, methyl ester	5.84
	22-hydroxy docosanoic acid, (bis-methylsilyl)-, derivative	0.12
	Tetracosanoic acid, (24-trimethylsiloxy)-, methyl ester	0.52
	Hexacosanoic acid, (26--trimethylsiloxy)-, methyl ester	0.06
Phenolics	Total	3.94
	Vanillin	0.10
	Methyl p-coumarate, TMS derivative	0.22
	Methyl isoferulate, TMS	0.31
	Methyl ferulate, TMS	3.29
	Ferulate Ac.C25:0, TMS	0.02
	Ferulate Ac.C27:0, TMS	0.01
Glycerol derivatives	Total	3.63
	Docosyl glycerol (C22:0)	3.63
Diacids	Total	0.08
	Octanedioic acid, dimethyl ester	0.02
	Nonanedioic acid, dimethyl ester	0.06

Table 7. Thermogravimetric parameters of *Fraxinus ornus* bark in pyrolysis.

	Moisture Mass Loss Region (40–120 °C)	**Low-Temperature Mass Loss Region (40–200 °C)**	**Main Mass Loss Region (200–450 °C)**	**Residual Char (%)**
Mass loss (%)	4.2 ± 0.7	8.2 ± 1.5	51.7 ± 1.4	17.3 ± 0.4
Tmax (°C)	76.9 ± 11.7	148.5 ± 8.1	345.3 ± 21.4	-
Onset (°C)	-	121.9 ± 9.6	197.1 ± 4.2	-

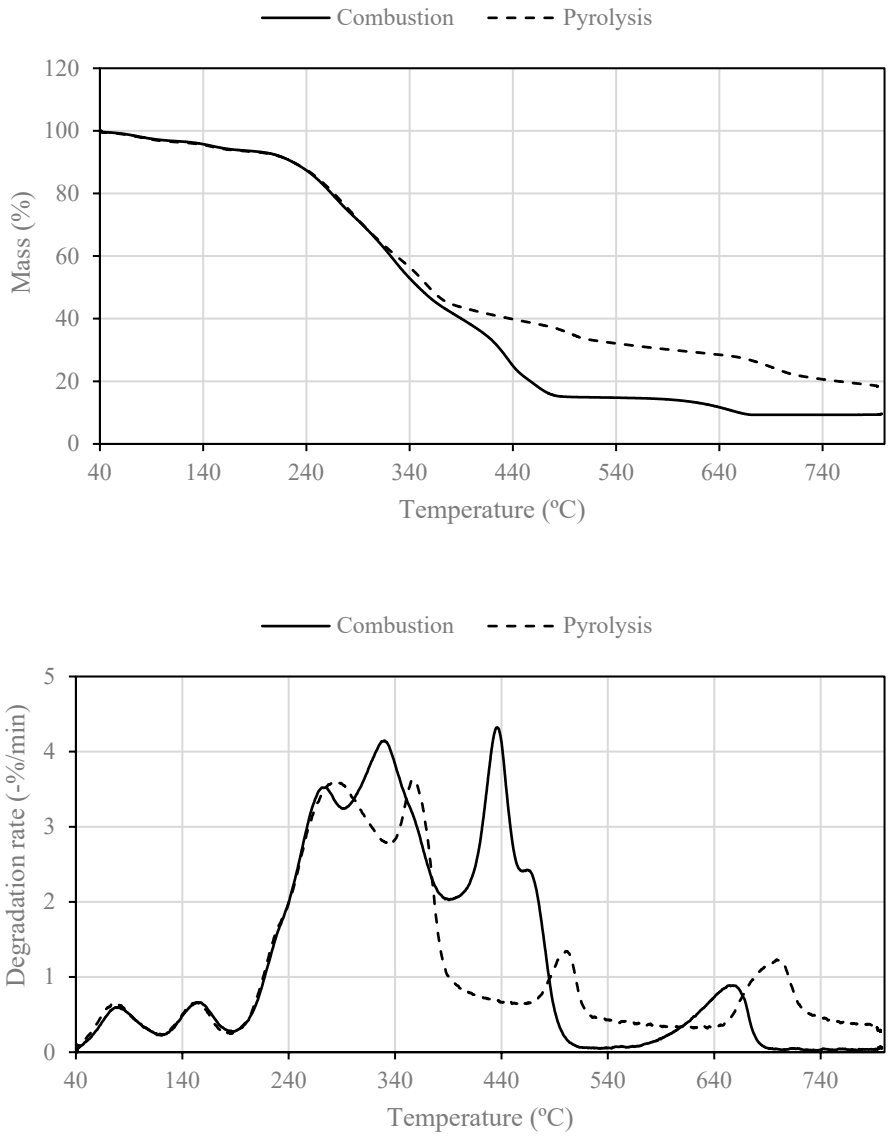

Figure 6. Thermal decomposition of *Fraxinus ornus* bark: Mass loss (TGA-**above**) and degradation rate (DTG-**below**).

4. Discussion

Data on the chemical composition of *F. ornus* bark are scarce to the best of our knowledge. Previous studies on the bark of the European or common ash *Fraxinus excelsior* reported its chemical composition as 5.1% ash, 29.4% extractives (25% of which are hydrophilic extracts, and 85% are hydrophilic), 3% suberin, 18.6% lignin, and 42% polysaccharides [38]. In comparison, our results showed that *F. ornus* bark contains higher ash and extractive contents and lower lignin content than *F. excelsior* (Table 1). Hydrophilic compounds dominate the extract profile, similar to the *F. excelsior* bark.

Our study reports for the first time the inorganic composition of *F. ornus* bark. According to Holdheide (1951), the inorganic ash fraction of *Fraxinus* barks (the species is not clear) may contain over 80% calcium [39]. Calcium is usually the principal element in wood and bark (50% of the total elements and 82–95%, respectively), usually present as calcium oxalate (CaC_2O_4) crystals [12]. In fact, the presence of calcium oxalate crystals in *Fraxinus* barks in the shape of crystal sand (microcrystals) [39] is a specific feature and has a diagnostic value [40]. The results of the FT-IR analysis (Figure 3) confirm the presence of calcium oxalate in *F. ornus* bark with distinct peaks at 512 cm^{-1}, 780 cm^{-1}, 1316 cm^{-1}, and 1609 cm^{-1}.

The notable high extractive content is a striking feature of *F. ornus* bark, and the high proportion of ethanol and water-soluble extractives suggests that these fractions could be the target for bark valorization. Previous studies have shown that *F. ornus* bark contains a high amount of hydrophilic extractives, and four compounds are frequently cited, including hydroxycoumarins, lignans, phenylethanoids, and secoiridoids [6–9]. These compounds are found in different ash species [41] and are reported to exhibit bioactive properties, in particular, antimicrobial properties [42]. Thus, the extractive composition of *F. ornus* bark explains its use as folk medicine in Bulgaria and Poland [9].

Our results showed that although *F. ornus* bark extracts exhibit some antioxidant activity, determined by in vitro DPPH, FRAP, and ex vivo TBARS methods, the effect was comparatively weaker than that of other bark and lignocellulosic biomass types (Table 8); this shows that a high extractive content (36% in DPPH, FRAP methods, and 25% in TBARS method) of the bark does not necessarily correlate with antioxidant activity. The results of the TBARS method agree with the results of the DPPH method. Several bark types, including eucalypt and Douglas fir barks, showed much higher antioxidant activity with lower IC_{50} values (Table 8). Cork and phloem fractions of oak and pine bark, as well as pine nut shells and walnut husks, also had a higher antioxidant activity than *F. ornus* bark (Table 8).

Table 8. Comparison of the phenolic content and in vitro antioxidant properties of different lignocellulosic biomass.

Biomass	Extract Yield (%)	TPC (mg GAE/g Extract)	Flavonoids (mg CE/g Extract)	Tannins (mg CE/g Extract)	TEAC (mg TE/g Extract)	IC_{50} (μg/mL)	FRAP (mM)	Reference
F. ornus bark	35.5	241.8	171.6	106.6	113.4	40	0.3	This work
Eucalypt bark	12.6	332.5	141.6	138.3	338.4	39.7	9.6	[43]
Douglas fir bark	11	674	429	141	2554	2	12	[44]
Waste oak cork	6.9	733.8	306.6	419.4	729.5	8.8	3.3	[45]
Waste oak phloem	4.9	255.2	190.0	131.4	435.8	7.3	1.8	[45]
Pine phloem	-	439.8	86.6	101.6	-	1.5	3.5	[46]
Pine nutshell (Turkey)	-	455	-	-	825	-	9.5	[47]
Pine nutshell (Portugal)	3.9	303.1	114.6	30.4	934.9	8.2	5.2	[48]
Almond shell	3.4	188.6	99.4	34.6	646.0	7.9	3.1	[48]
Walnut shell	7.3	317.9	197.6	60.1	188.6	15.2	5.1	[48]

An explanation for the moderate antioxidant properties of *F. ornus* bark may derive from the relatively low phenolic content (242 mg GAE/g extract) and average flavonoid and condensed tannin contents (172 mg CE/g extract and 107 mg CE/g extract) compared to other barks and lignocellulosic residues (Table 8).

Additionally, the chemical composition of the extractives possibly plays a role in the antioxidant activity. According to Kostava and Iossifova (2002), coumarin glycosides exhibit significantly lower antioxidant activity than aglycones (coumarins) due to the blocking of the active phenol group by the sugar moiety [7]. The results of the antioxidant assays suggest that coumarins of *F. ornus* bark are composed predominantly of glycosides. The results also suggest a high antimicrobial activity for the extracts since the presence of sugar moiety enhances the water solubility and transport of the coumarins [49].

The lipophilic composition of *F. ornus* bark is also reported here for the first time. The yield is low (2.5%), but the fatty acid percentage is remarkable (51.6% of the detected compounds), with hexadecanoic (palmitic) acid as the principal fatty acid. Terpenoids are the second most important group of lipophilic extracts, including mostly abietic acid and dehydroabietic acid (99% of the terpenoids). Abietane diterpenoids are interesting compounds that exhibit biological activity and were already isolated from other ash barks, such as *Fraxinus sieboldiana* [50].

The suberin content of *F. ornus* bark is low (1%), which is indicative of a diminutive formation of cork in the bark periderms. This is also highlighted by the FTIR spectrum (Figure 4) that lacks the suberin fingerprint peak at 1740 cm^{-1} due to the C=O stretch of the carbonyl group and the low intensity of the CH_2 peaks. The analyzed *F. ornus* bark samples have a thin bark with a limited amount of cork tissues (Figure 2), which is similar to *Fraxinus angustifólia* [51]. Similarly, the Sudan IV staining of *Fraxinus americana* bark was weak, suggesting the low suberin content [52]. The suberin of *F. ornus* bark (Table 6) is primarily composed of ω-hydroxy acids (47.5%) followed by α,ω-dicarboxylic acids (19.4%), and the proportion of the various long-chain families, and glycerol (Figure 5) is in line with what is known for suberin in cork-rich barks. However, their proportion and the predominance of single compounds varies in different species, namely in the ratio ω-hydroxy acids/α,ω-diacids, or in the mid-chain functionalization of the compounds. Although the chemical macromolecular structure of suberin is important to define the properties of the cork cells [53], this aspect is not relevant here due to the diminutive amount of cork in *F. ornus* bark. However, the data is important to strengthen the knowledge of the chemical variability of suberin in different species.

The burnout temperature of *F. ornus* bark was 653 °C (Table 5), which is in the higher range of the lignocellulosic materials, implying that the bark is stable in combustion. On the other hand, the ignition temperature was slightly lower than that of other lignocellulosic biomass [54], and the activation energy during the main devolatilization region (between 200 and 400 °C) was 29 kJ mol^{-1}. The complex anatomical and chemical composition of the bark possibly plays a role in the combustion characteristics of the bark. A higher activation energy is expected in pyrolysis, where a higher energy barrier exists to reach the transition state. Our results are in agreement with the pyrolysis kinetics of *Jasminum nudiflorum* bark from the *Oleaceae* family, for which the activation energy was calculated as 52 kJ mol^{-1} by applying the Coats-Redfern method [55].

The chemical composition of *F. ornus* bark indicates that non-fuel applications should be the target since it is likely that the elevated ash content will be unfavorable for combustion applications, while its low polysaccharide and lignin contents imply low bio-oil and biochar yields in pyrolysis [56,57]. An integrated biorefinery scheme was developed for *F. ornus* bark, taking into consideration its chemical characteristics (Figure 7). According to the biorefinery concept, it is possible to fractionate approximately 90% of the *F. ornus* bark to produce fatty acids, terpenoids, antimicrobial agents, suberin monomers, functional cellulosic materials, and calcium. The remaining lignin may be used as a fuel or pyrolyzed to produce biochar.

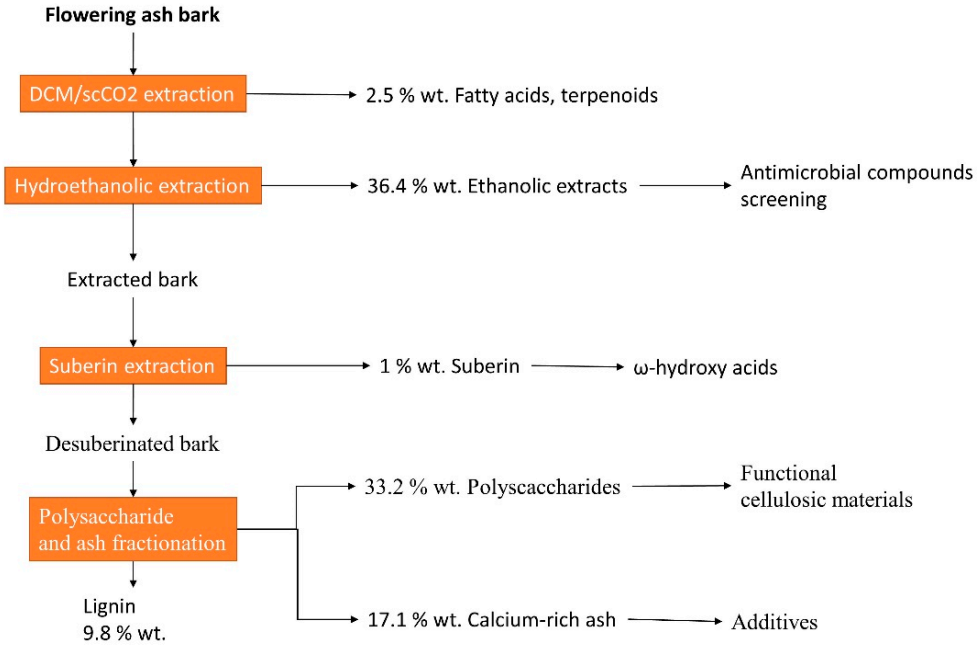

Figure 7. Biorefinery concept for *Fraxinus ornus* bark.

The most important bark fraction is hydrophilic extractives, frequently reported to exhibit bioactive properties [7,42]. The antimicrobial properties of endemic Taurus flowering ash are currently not known, and therefore, its bioactive properties should be determined and tested for the production of antimicrobial compounds. The lipophilic extractive content of the bark is low, as well as that of suberin, but they include interesting compounds such as palmitic acid, diterpenoids, and ω-hydroxy acids that may be valorized in an integrated biorefinery.

Polysaccharides are the second most important fraction of the bark. They may be extracted by acid hydrolysis or alkaline treatment and be used to produce functional materials such as cellulose nanocrystals (CNC). The CNCs have excellent mechanical and barrier properties as well as biodegradability and may be incorporated into composites or packaging materials to improve their performance [58].

Calcium is the third important bark fraction. This fraction may be used for partial lime replacement in cement production [59], which not only reduces the accumulation of bark ash but also contributes to the reduction of CO_2 emissions. Cement production is one of the major contributors to CO_2 emissions released during the calcination of limestone. In order to reduce these CO_2 emissions, clinkers are partially replaced with calcium-containing supplementary cementitious materials (SCMs) such as steel slag and limestone fillers [60]. Therefore, calcium fractions of *F. ornus* bark may be valorized as a cement additive. Another possible application of the calcium fraction is soil application as a liming agent to improve soil fertility [61].

5. Conclusions

The chemical composition, phenolic extractives, antioxidant properties, and thermal behavior of the endemic *Fraxinus ornus* subsp. *cilicica* bark is here reported for the first time and used to design a potential valorization of the species. The following key conclusions may be derived from this study:

1. *Fraxinus ornus* subsp. *cilicica* bark contains a notable amount of extractives (39%) that are predominantly polar compounds;
2. The inorganic content of the bark is elevated (17%), comprising primarily calcium;
3. The polar bark extractives contain a moderate phenolic content as well as moderate in vitro and ex vivo antioxidant properties;

4. The lipophilic extractives are rich in fatty acids and abietane diterpenoids;
5. Suberin content is low and primarily composed of ω-hydroxy acids. The current study contributes to the ongoing research to enlarge the data on the monomeric composition of suberins of different barks;
6. The ignition temperature of the bark is 190 °C, and the burnout temperature is 653 °C;
7. A biorefinery concept was developed for the fractionation and valorization of *F. ornus* bark to produce fatty acids, terpenoids, hydrophilic extracts, suberin monomers, cellulose nanocrystals, and cement-replacement materials.

Author Contributions: Conceptualization, A.U.Ş. and H.P.; methodology, A.U.Ş. and H.P.; investigation, A.U.Ş., R.S., Â.F. and I.M.; resources, C.Y.; data curation, A.U.Ş. and H.P.; writing—original draft preparation, A.U.Ş., Â.F. and I.M.; writing—review and editing, A.U.Ş., C.Y., Â.F. and H.P.; visualization, H.P.; supervision, H.P. All authors have read and agreed to the published version of the manuscript.

Funding: This work has been supported by FCT–Fundação para a Ciência e Tecnologia within the R&D Unit Forest Research Centre, CEF (UIDB/00239/2020) and also from the FCT/MCTES (PIDDAC) national funds to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021). U. Şen acknowledges support from FCT through research contracts (DL 57/2016), R. Simões by a Ph.D. scholarship (PD/BD/128259/2016), and Â. Fernandes by the national funding by FCT and P.I. in the form of institutional scientific employment program.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank Joaquina Silva from Instituto Superior de Agronomia for chemical analysis, Francisco Lemos, José Condeço, and Marta Martins from Instituto Superior Técnico for TGA and FT-IR analyses.

Conflicts of Interest: The authors declare no conflict of interest.

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