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Exploring the Potential of Residues from Industrial Tannins Extraction through Comprehensive Characterization

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Abstract

The industrial extraction of tannins from maritime pine (Pinus pinaster) bark generates multiple solid by-products at different stages, including the raw bark, partially extracted material, and final residue, whose valorization remains largely unexplored. This study presents a comprehensive chemical and structural characterization of these three materials using a multi-analytical approach, with the aim of assessing their potential for material recovery and sustainable reuse. Fourier-transform infrared spectroscopy (FTIR) revealed depletion of hydroxyl-rich phenolics and enrichment in aliphatic associated structures. Proximate analysis demonstrated increased volatile content and fixed carbon in the final residue, indicating improved suitability for thermochemical conversion. Despite successive extractions, the final residue retained significant levels of total phenolics (373.86 mg GAE/g), condensed tannins (116.8 mg CE/g), and strong antioxidant activity (up to 425 µg AAE/mg), confirming the presence of bioactive compounds. However, the Stiasny number remained below 40%, suggesting limited reactivity with formaldehyde. In terms of structural composition, the final residue was enriched in acid-insoluble lignin (≈80%) and showed a dramatic loss in cellulose and hemicellulose content (<7%), highlighting extensive structural degradation. Solvent extractive tests (Soxhlet, TAPPI T 264) revealed unusually high solubility (up to 85%), linked to depolymerized fragments. Size exclusion chromatography confirmed a decrease in molecular weight, suggesting breakdown of macromolecules during processing. Overall, these residues exhibit promising characteristics for energy recovery and extraction of phenolic-rich fractions, supporting their integration into circular bioeconomy strategies.

Keywords: maritime pine bark, tannin extraction, industrial residues, waste valorization, circular bioeconomy

1. Introduction

In 2020, the area covered by forests and woodlands in Europe was estimated at 180 million hectares, 45% of its total area. Given the magnitude of this valuable natural resource, the wood industry significantly impacts the European economy. In 2020, four hundred thousand companies worked in wood processing across Europe, representing one-fifth of the total manufacturing sector [1]. The European wood industry has many uses, including construction and building materials, furniture, pulp and paper, energy production, and wood-based chemicals [2,3]. However, the wood industry also generates large quantities of solid residues, prompting ongoing studies to explore ways to extract value from this waste. One area of focus involves using polyphenols, such as lignin and tannins, which are being developed for various applications such as additive for resins [4], eco-friendly adhesives [5], bioactive compounds in food and biomedicine [6], dentistry [7], copolymers, 3D-printing [8], biodegradable composite films [9][10], biodiesel [11,12], and many other applications. Lignin is the second most abundant natural polymer after cellulose, making up to 15–30 % wt of lignocellulosic biomass [13]. In contrast, tannins, which are secondary metabolites involved in plant defense, can reach up to 20% of bark dry weight, particularly in species like maritime pine and mimosa [14,15]. Nowadays, tannins are increasingly extracted for use in various industrial sectors, including the leather and textile industry [16], wood-based panels and construction [17], food packaging, [18,19], cosmetics and pharmaceuticals [20,21], and in environmental technologies such as water purification [22]. The global tannin was valued at USD 2.98 billion in 2025 and is projected to reach USD 4.14 billion by 2030, expanding at a compound annual growth rate (CAGR) of 6.80% during the forecast period [23]. This upswing is largely fueled by a rising inclination towards natural inputs in sectors like leather, wine, wood composites, and specialty nutrition. The valorization of this type of product promotes the development of regional bioeconomies within forested regions [24].

Tannins have been divided into two classes condensed tannins (CTs) and hydrolysable tannins (HTs) [25]. CTs, also named proanthocyanins, are oligomers and polymers of flavonoids without a sugar core, while HTs consist of a polyol core, often glucose, esterified with phenolic acids like gallic or ellagic acid, forming gallotannins or ellagitannins, and can be readily hydrolyzed to release phenolic acids [26]. Their high hydroxyl content gives them strong antioxidant properties. A more recent and minor class of tannins has come into consideration: complex tannins, which are rare hybrids of CTs and HTs. They are composed of an ellagitannin unit and a flavonoid unit, combining one monomeric unit of HTs and one unit of CTs [27,28].

Tannins are found naturally in food and beverages such as tea, coffee, grapes, and wine, where they contribute to taste, astringency, and antioxidant properties [29]. Their composition and reactivity are influenced by botanical origin, tree species, age, and environmental factors [30]. The tannins of maritime pine bark, for instance, are known for their anti-radical, antioxidant, and anti-inflammatory properties [31]. These bioactive extracts are commercially available under the name Pycnogenol®, as a nutritional supplement and used in the treatment of cardiovascular, inflammatory, and metabolic disorders [32]. Pycnogenol® is rich in flavonoids, mainly procyanidins, catechins, and taxifolins, which contribute to its health-promoting properties [33,34]. In the oenological sector, tannins extracted from the skins and seeds of grapes are employed to enhance aroma stability, color fixation, and oxidative resistance during wine aging. These oenological tannins, often added to improve mouthfeel and aging potential, are selectively extracted for their color and structural properties, depending on grape variety and processing methods [35,36]. Industrial extraction of tannins typically relies on solvent-based processes, which strongly influence yield, molecular weight distribution, and bioactivity of the final tannin extracts [37]. At large scale, boiling water is most common, combined with other solvents such as ethanol [38], acetone [39], methanol [40,41], ethyl acetate [42], or a mixture of these to improve extraction efficiency. Another method for improving extraction efficiency is to use an alkaline solution [43,44]. Despite their efficiency, these methods are time- and solvent-intensive. New techniques such as ultrasound-assisted and microwave-assisted extraction (UAE, MAE) reduce processing time and solvent use, though parameters must be optimized for each feedstock due to the chemical diversity of tannins [45].

Bark is the second most significant tissue in the tree trunk after wood, accounting for approximately 10%–20% of its mass, depending on the species and environmental conditions [46,47]. Beyond its traditional uses, pine bark offers promising opportunities for value-added applications, thanks to its high content of extractable substances. It is a chemically complex tissue composed of lignin, cellulose, hemicellulose, and various extractives [48]. Compared to wood, it contains a higher proportion of polyphenols and suberin, including compounds like tannins, flavonoids, lignans, and stilbenes [49]. However, during industrial extraction processes, tannins are selectively solubilized, while the remaining compounds are left behind in the solid residue, which is often underutilized or discarded. Considering the large amounts generated, understanding the composition of these residues is essential to identify valorization routes beyond waste disposal.

This study aims to characterize residues obtained after tannin extraction from maritime pine bark, a side stream that has received limited attention. While some research has explored similar residues, such as those from larch bark, no in-depth analysis has been published since 2012 [50], leaving a knowledge gap in understanding the composition, structure, and potential uses of these byproducts. By identifying the remaining functional molecules and their properties, this work provides a foundation for their potential use in bio-based materials and energy recovery, contributing to sustainable waste management and circular bioeconomy development.

2. Materials and methods

2.1 Chemicals

Folin Ciocalteu reagent, sodium carbonate (CAS No. 497-19-8), formaldehyde 37% (CAS No. 50 00 0), methanol (CAS No. 67 56 1), acetone (CAS No. 67-64-1), petroleum ether (CAS No. 64742-49-0), ethanol (CAS No. 67 56 1) were obtained from Fisher Scientific. Hydrochloric acid 37% (CAS No. 7647-01-0), vanillin (CAS No. 121 33 5), sulfuric acid (CAS No. 7664 93 9), potassium iodate (CAS No. 7758 05 6), tannic acid (CAS No. 1401 55 4), ascorbic acid (CAS No. (CAS No. 50-81-7), glacial acetic acid (CAS No. 64-19-7), sodium chlorite (CAS No. 7681-52-9) , sodium hydroxide (CAS No. 1310-73-2), sodium sulfate (anhydrous) (CAS No. 7757-82-6) , ethylene glycol (CAS No. 107-21-1) , polystyrene sulfonate standards (CAS No.9080-79-9), and dichloromethane (CAS No. 75-09-2) were purchased from Acros Organics. Toluene (CAS No. 108-88-3), butylated hydroxytoluene (BHT) (CAS No. 128-37-0), sodium methoxide (CAS No. 124-41-4), chloroform (CAS No. 67-66-3) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (CAS No. 1898 66 4) were obtained from Alfa Aesar. Catechin (CAS No. 154-23-4) and pyridine were products of Sigma Aldrich and gallic acid (CAS No. 149-91-7) of VWR. Finally, bis(trimethylsilyl)trifluoroacetamide (BSTFA) (with 1% of trimethylchlorosilane) was purchased from Thermo Scientific**.**

2.2 Maritime Pine Tannin Extraction Residue

Maritime pine bark (MPB), the residue after the first stage of extraction (MPTER-1E), and the final residue after tannin extraction (MPTER-FR) were gently supplied by a local company (Biolandes). This company conducts the extraction of tannins by treating the bark with a solvent, removing the undissolved solid fraction, and concentrating the liquid extract. MPTER corresponds to the precipitate obtained after filtering this concentrated solution. The sample was received as a red-brown solid and was ground with a pestle and mortar to obtain a fine powder, dried at 50 °C until a low moisture content was reached, and stored for further analysis.

2.3 Chemical characterization

2.3.1 Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR)

ATR-IR spectroscopy was used to analyze the chemical composition of the samples. Spectra were recorded using a Jasco FTIR-4700 spectrometer equipped with a Jasco ATR unit (Jasco, Japan). All spectra were recorded over a 4000-400 cm-1 range using 64 scans and 2 cm-1 resolution.

2.3.2 Total Polyphenolic Content (TPC)

The total phenolic content (TPC) of the three samples MPB, MPTER-1E, and MPTER-FR, was determined using the Folin–Ciocalteu method, with slight modifications. This colorimetric assay estimates the amount of phenolic compounds based on their reducing capacity, with results expressed in milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g) Eq. (1). [51].

$$\text{mg } GAE/g\ dry\ matter\ = (C_{GAE} * V * D)/m \quad \text{(Eq. 1)}$$

With:

- C_{GAe}: Concentration of gallic acid equivalent obtained by the calibration curve (g/L)
- V: volume - D: dilution factor - m: initial mass of residue

A standard curve was established using gallic acid dissolved in methanol at 5 mg/mL as mother standard solution to prepare dilutions of 0, 30, 100, 130, and 160 µg/mL. For each dilution, 150 µL of the standard mother solution was mixed with 0.6 mL of Folin–Ciocalteu reagent (pre-diluted 1:10, v/v, with distilled water), agitated for 4 seconds, and kept in the dark for 3 min. Then, 1.2 mL of 7.5% sodium carbonate solution was added to each dilution, vortexed for 1 minute, and the mixture was incubated for 1 h at room temperature in the dark. The absorbance was measured at 760 nm using a UV-visible spectrophotometer (SHIMADZU UV-VIS) against a blank (methanol).

2.3.3 Condensed Tannin Content (CTC)

Condensed tannins content of the three samples MPB, MPTER-1E and MPTER-FR was measured using the vanillin method described by Broadhurst and Jones [52]. A total of 3 mL of vanillin solution (4%, w/v in methanol) was mixed with 0.5 mL of the sample solution. The sample solution was prepared by dissolving 300 mg of dry MPTER in 25 mL of 70% aqueous acetone, stirred for 2 h at room temperature, and then filtered to remove insoluble solids. The average solubility percentage was calculated. After mixing with vanillin, 1.5 mL of concentrated hydrochloric acid (37%) was added. The mixture was kept in the dark at 20 °C for

15 min. Absorbance was measured at 500 nm using a UV-Visible spectrophotometer (SHIMADZU UV-VIS). A calibration curve was prepared using catechin at 5 g/mL as mother standard solution to prepare dilutions of 0, 50, 100, 150, 200, 240, 270, 300 mL/L. Results were expressed in milligrams of catechin equivalent per gram dry matter (mg CE/g matter) Eq. (2).

$$\text{mg } CE/g\ dry\ matter = (C_{CE} * V * D)/m \quad \text{(Eq. 2)}$$

With:

- C_{CE}: Concentration of catechin equivalent obtained by the calibration curve (g/L)
- V: volume - D: dilution factor - m: initial mass of residue

2.3.4 Hydrolysable Tannin Content (HTC)

The hydrolysable tannin content in the MPB sample was determined by the method described by Bossu et al. [53]. A total of 0.25 g of dry MPB was extracted with 50 mL of 80% methanol (v/v in water). The mixture was stirred for 6 h at room temperature, followed by centrifugation at 2000 rpm for 10 min. The supernatant was collected and diluted five times with distilled water. For the colorimetric assay, 5 mL of potassium iodate solution (2.5%, w/v) was preheated to 30°C for 7 min. Then, 1 mL of the diluted sample extract was added, and the mixture was incubated in a water bath at 30 °C for 2 min. The absorbance was measured at 550 nm using a UV-visible spectrophotometer (Secomam, France). A calibration curve was prepared using a tannic acid dissolved in methanol (5000 mg/L). Standard solutions were obtained by serial dilution in water. The results were expressed in milligrams of tannic acid equivalent per gram of dry matter (mg TAE/g matter) Eq. (3).

$$\text{mg } TAE/g\ dry\ matter = (C_{TAE} \times V \times D)/m \text{ (Eq. 3)}$$

With:

- C_{TAE} : concentration of gallic acid obtained by the calibration curve (g/L)
- V: volume - D: dilution factor - m: initial mass of residue

2.3.5 Reactivity with Formaldehyde (Stiasny Number)

The reactivity of the MTER-FR sample with formaldehyde was determined by the Stiasny number as described by Voulgaris et al. [54] . A 50 ml aqueous solution (0.4%, w/w) was poured into a round bottom flask. Then, 5 mL of formaldehyde (37%) and 5 mL of hydrochloric acid (10 M) were added. The mixture was heated under reflux for 30 min. After cooling, the resulting precipitate was filtered using a sintered glass Gooch filter (P3), washed, and dried in an oven at 105 °C until a constant weight was achieved. The Stiasny number (SI, %) was

calculated as the ratio of the dry weight of the precipitate (m_p, g) to the initial dry sample weight (m_0, g) Eq. (4):

$$SI\ (\%) = (m_1/m_0)\ \times 100 \text{ (Eq. 4)}$$

2.3.6 Antioxidant Activity (DDPH Method)

The antioxidant activity of the MPTER was determined using the DPPH radical scavenging method, following the procedure described by Maitane Maisterra Udri [55] . Ascorbic acid was used as the standard antioxidant at (60 mg/L in methanol) as mother standard solution to prepare dilutions of 0, 1.7, 3.5, 7.5, 15, and 30 mg/L. For each dilution, 0.5 mL of the solution was mixed with 1.5 mL of freshly prepared DPPH reagent (4.5 mg of DPPH dissolved in 50 mL of methanol). The mixtures were kept in the dark for 30 min. The absorbance was measured at 517 nm using a UV-visible spectrophotometer (SHIMADZU UV-VIS spectrophotometer), with methanol as a blank. The antioxidant activity of MPTER-FR was measured using the same protocol. A concentration of 60 mg/L was prepared by dissolving 6 mg MPTER-FR in 100 mL of methanol–acetone (50:50, v/v) for 2 hours at room temperature with moderate agitation, followed by filtration to remove insoluble solids. The absorbance of the resulting extract was measured, and the antioxidant activity was expressed in micrograms of ascorbic acid equivalent per milligram of dry residue (µg AAE/mg residue).

2.3.7 Molecular Weight Distribution (SEC)

The weight-average molar mass (Mw), number-average molar mass (Mn), and polydispersity index (Đ) of the MPTER samples were determined using Size Exclusion Chromatography (SEC) with NaOH solution as the eluent. Measurements in NaOH solution were performed on a Thermo Scientific Ultimate 3000 system (USA) equipped with a Diode Array Detector (DAD), a Multi-Angle Light Scattering (MALS) detector, and a Differential Refractive Index (DRI) detector. Polymers were separated using three Tosoh (G4000PW-2*G3000PW (7.8x300mm)) columns (exclusion limits from 200 Da to 300 000 Da) at a flow rate of 1 mL/min. The temperature of the column was held at 25 °C. Ethylene glycol was used as the flow marker, and polystyrene sulfonate was used as the calibration standard.

2.4 Proximate analysis

The physicochemical properties of the MPTER were analysed to determine the moisture content, volatile matter, ash content, fixed carbon, and the theoretical heating value.

2.4.1 Moisture Content

To determine the moisture content, approximately 2 g of the MPTER sample (initial weight mi) was oven-dried at 105 °C for 24 h, cooled in a desiccator, and reweighed (final dry weight mf). The moisture content (%) was then calculated as:

$$\text{Moisture content (\%)} = (m_i - m_f/m_i) \times 100 \text{ (Eq. 5)}$$

2.4.2 Volatile matter

A dry MPTER sample (m_{dry}= 0.5 g) was weighed into a moisture-free, tared ceramic crucible with a lid. The crucible was placed in a muffle furnace (Carbolite, Newtown, PA, USA) and heated to 575 °C for 30 min under an inert atmosphere. After heating, the crucible was cooled in a desiccator to room temperature and reweighed (m_f, final mass after volatilization). The volatile content was calculated as:

$$\text{Volatile content (\%)} = (m_{dry} - m_f/m_{dry}) \times 100 \text{ (Eq. 6)}$$

2.4.3 Ash Content

After determining the volatile matter, the same crucible (without lid) was reheated at 575 °C for an additional 3.5 h to oxidize and remove all organic matter. The remaining residue, corresponding to the ash mass (*mf*), was then cooled in a desiccator and weighed. The ash content was calculated based on the initial dry sample weight (*mi*) used in Section 2.4.2:

$$\text{Ash content (\%)} = (m_f/m_i) \times 100 \text{ (Eq.7)}$$

2.4.4 Fixed Carbon Content

The fixed carbon (FC) content was calculated indirectly by subtracting the sum of the moisture content (Mc), volatile content (Vc), and ash content (Ac)—previously determined in Sections 2.4.1 to 2.4.3—from 100:

$$\text{FC (\%)} = 100 - (M_c + V_c + A_c) \text{ (Eq. 8)}$$

2.4.5 Theoretical Heating Value

Various theoretical models have been developed to estimate heating values based on proximate analysis data. Among them, one study demonstrates a linear correlation between the heating value (hv, in MJ/kg) and the contents of fixed carbon (FC), volatile matter (Vc), and ash (Ac) [56]. The following equation (Eq.9), valid within specific constituent ranges (0.92–90.6% for Vc, 1.0–91.5% for FC, and 0.12–77.7% for Ac), was used:

$$\text{hv (MJ/kg)} = 0.3536 \cdot F_C + 0.1559 \cdot V_c - 0.0078 \cdot A_c \text{ (Eq. 9)}$$

2.5 sugar and lignin measurement

2.5.1 Solvent Extractive Content (Toluene/Ethanol Soxhlet)

The solvent extractive content of MPTER was determined using a Soxhlet extraction. In brief, 5 g of the dry MPTER was placed in a cellulose cartridge and inserted into the Soxhlet apparatus. A mixture of 187.5 mL of toluene/ethanol (2:1, v/v) was added to the round-bottom flask. The extraction was carried out by refluxing at 150 °C for up to 6 h, equivalent to 24 extraction cycles. This extraction was performed in order to remove extractives (waxes, resins, tannins, phenolics, etc.), which could otherwise interfere with the accuracy of carbohydrate and lignin determinations.

2.5.2 Acid-Insoluble Lignin Content

The acid-insoluble lignin content was determined using a two-stage acid hydrolysis method. 1 g of extract-free MPTER (m_0) was mixed with 15 mL of 72% sulfuric acid and left to hydrolyze at room temperature for 12 h. Then, 260 mL of distilled water was added, and the mixture was refluxed for 4 h. The hydrolysate was filtered (filter No. 3), and a portion of the first filtrate was reserved for future analysis. The solid residue was washed with hot distilled water until a neutral pH, dried at 105 °C for 24 h, cooled in a desiccator, and weighed (*mf).* The percentage of removable materials (R) was determined from the extractable content (section 2.5.1).

$$\text{Lignin (\%)} = (m_f/m_0) \times (100 - R) \quad \text{(Eq. 10)}$$

2.5.3 Holocellulose Content :

To determine the holocellulose content, 2 g of extract-free MPTER (*m0*) was mixed with 80 mL of distilled water in a covered Erlenmeyer flask with a watch glass and heated at 70 °C in a water bath. Using two syringes, 0,5 ml of glacial acetic acid and 2,6 ml of 25% sodium chlorite were added every hour for 6-8 h, after which it was kept in the bath for a further 12 h. The content was filtered (filter No. 2), washed with hot water until neutral pH, dried at 105 °C for 24 h, cooled in a desiccator, and weighed (*mf*). The percentage of removable materials (R) was determined from the extractable content (section 2.5.1).

$$\text{Holocellulose (\%)} = (m_f/m_0) * (100 - R) \quad \text{(Eq. 11)}$$

2.5.4 Cellulose Content:

Cellulose was isolated from holocellulose by alkaline extraction. A total of 0.6 g of dried holocellulose (*m0*) was mixed with 3 mL of 17.5% NaOH. Three additional 1.5 mL portions of NaOH were added at 5-minute intervals (total 7.5 mL). After 30 min, 9.9 mL of distilled water was added, stirred, and allowed to stand for 1 h. The mixture was filtered, and the residue was washed with 30 mL of 8.3% NaOH, followed by several washes with hot distilled water. The fibers were then treated with 4.5 mL of 10% acetic acid for 3 min (vacuum was stopped during

this step) and washed with distilled water until neutral pH. The final residue was dried at 105 °C for 24 h, cooled in a desiccator, and weighed (*mf*).

$$\text{Cellulose (\%)} = (m_f/m_0) \times \%Holocelluse \quad \text{(Eq. 12)}$$

2.5.5 Hemicellulose Content:

The hemicellulose content was calculated as the difference between the holocellulose and cellulose contents:

$$\text{Hemicellulose (\%)} = \%\ \text{Holocellulose} - \%\ \text{Cellulose} \quad \text{(Eq. 13)}$$

2.6 Sequential Solvent Extraction (TAPPI T 264 Standard)

Sequential extraction was performed according to the TAPPI T 264 standard, using three solvents of increasing polarity: petroleum ether, toluene/ethanol (2:1 v/v), and distilled water, for each extraction cycle, we start with petroleum ether, then toluene/ethanol, and finish with water. Cellulose cartridges were used to perform the extraction study. Each of the three materials (MPB, MPTER-1E, and MPTER-FR) was placed in a separate cartridge, which was filled while leaving approximately 1 cm of free space at the top. The cellulose cartridges filled with matter was placed in a Soxhlet apparatus for 6-8 hours using petroleum ether, 4-5 hours using toluene/ethanol and 12 hours using water as solvent. Each extraction was carried out under continuous reflux to ensure complete solubilization of extractable fractions. After each solvent cycle, the samples were dried at 105 °C until constant weight, and the extractive content was calculated gravimetrically by the difference in weight before and after extraction. The three extractive fractions were expressed as percentages relative to the dry mass. To further characterize the extracted materials, Fourier Transform Infrared (FTIR) spectra were recorded for each sample (before and after extraction). The ATR-FTIR analysis was performed as described in Section 2.3.1 to monitor the evolution of chemical functional groups and identify compositional changes in the biomass as a function of extraction solvent and processing stage. This method provided a deeper insight into the solubility and polarity-based distribution of extractives and degradation products across the three processing stages.

3 Results and discussion

3.1 Chemical characterization

3.1.1 Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR)

Figure 1 shows the ATR-FTIR spectra of the raw maritime pine bark (MPB), the insoluble bark obtained after tannin extraction (MPTER-1E), and the final solid residue recovered from the liquid extract (MPTER-FR). All spectra were normalized to the absorption band at 2919.7 cm^{-1}, corresponding to the C–H stretching vibration of aliphatic groups, in order to facilitate comparative analysis. The spectrum of the insoluble bark after the tannin extraction shows no significant change in major absorption bands compared to the raw bark, suggesting that the solvent treatment primarily targeted soluble components and did not strongly modify the structural backbone of the material. The peaks observed between 400 and 600 cm^{-1} are attributed to out-of-plane deformations of the aromatic rings or C–C bending vibrations within the rings found in tannins in bark before extraction [57] . There are also notable bands between 1000 and 1300 cm^{-1}, which correspond to C–O stretching, particularly associated with phenolic and ether groups, alongside the C=C stretching vibrations from the aromatic rings in the range of 1500-1600 cm^{-1} [58]. The presence of an alcoholic -OH (hydroxyl) group is established at peak formation of 3348 cm^{-1}.

On the other hand, the final residue (precipitate recovered from the extract) exhibits notable differences. The lower bands in the range 1035 cm^{-1} can be due to an opening of the cyclic ether structure of polyflavonoids [59] . The C=C stretching vibrations from the aromatic rings in the range of 1500-1600 cm^{-1} confirm the presence of polyphenolic content. The band near 1695 cm-1 typically associated with C=O stretching suggest the presence of hydrolysable tannins, basically constituted of gallic acid and derivatives [57]. Small peaks at 2918 and 2851 cm^{-1} originate from CH stretch vibration in aromatic methoxyl groups and in methyl and methylene groups of side chains, can be more prominent due to less interference from polar functional groups. The broad band at 3385 cm^{-1}, attributed to O–H stretching in hydroxyl groups (abundant in tannins and other polyphenols), shows a pronounced decrease in intensity, reflecting the depletion of phenolic compounds.

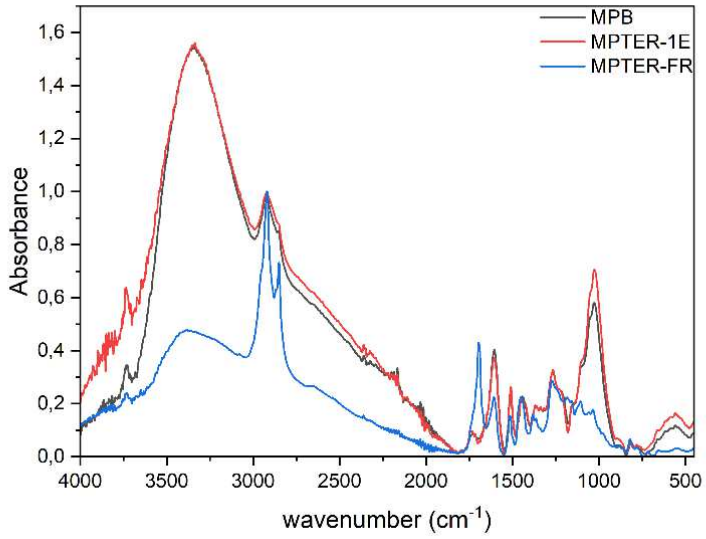


Figure 1: FTIR spectra of MPB, MPTER-1E and MPTER-FR

3.1.2 Total Polyphenol Content (TPC):

The total phenolic content (TPC) of each of the three samples MPB, MPTER-1E, and MPTER-FR is shown in Table 1. The obtained values can offer an indication of the efficacy of the extraction procedure of tannins and the richness of phenol in the materials. The calibration curve showed good linearity with a determination coefficient (R^2) of 0.9779. From the absorbance values and calculated concentrations, the TPC values were calculated using Equation (1) in section 2.3.2.

Table 1: TPC of each type of material

matter	TPC ($mg\ GAE\ g^{-1}\ dry\ matter$)
MPB	4,6799
MPTER-1E	2,4537
MPTER-FR	373,8588

The TPC results presented in Table 1 show a decrease in the TPC of the MPB sample from 4.68 mg GAE/g to 2.45 mg GAE/g for MPTER-1E, indicating partial removal of soluble tannins. In contrast, the MPTER-FR sample, obtained from precipitation of the liquid extract, displayed a remarkably high TPC of 373.86 mg GAE/g, suggesting a concentrated presence of phenolic compounds.

This significant variation in the final residue may be attributed to the nature of the extraction process. The raw and first-extracted materials contain a high proportion of insoluble lignocellulosic content, structural polysaccharides, and intact biomass limiting the solubility to 25%, and hence the release of phenolics into the acetone–water extraction medium (70:30 v/v). On the other hand, the final residue, being a precipitate from the already soluble extract, is largely free of bulk lignocellulosic matter and thus allows a more complete solubilization and quantification of phenolics during analysis. This observation agrees with the results reported by Vieito et al. [60] and Seabra et al. [61], who demonstrated the influence of solvent polarity and biomass solubility on the efficiency of phenolic compound recovery from pine bark.

3.1.3 Condensed tannin content:

The condensed tannin content (CTC) of the three samples MPB, MPTER-1E, and MPTER-FR, was quantified using the vanillin assay. A calibration curve was established at 500 nm using catechin as standard, with a regression coefficient of $R^2 = 0.9922$.

The absorbance values were converted to concentrations, and the condensed tannin content was expressed in mg catechin equivalents per g of dry matter (mg CE/g) as shown in table 2 , using equation 2 in section 2.3.3.

Table 2: CTC of each type of material

matter	CTC (mg CE g^{-1} dry matter)
MPB	11,4272
MPTER-1E	6,5187
MPTER-FR	116.8

The CTC of MPB was found to be 11.43 mg CE/g, indicating a relatively modest condensed tannin content in the native bark. On the other hand, MPTER-1E showed a reduced value of 6.52 mg CE/g, confirming partial removal of extractable condensed tannins. In contrast, MPBTER-FR showed a high value of 116.8 mg CE/g, despite being the residual solid after tannin extraction. This elevated value is explained by the solubility behavior of the samples. While MPB and MPTER-1E were only ~25% soluble in the acetone–water (70:30 v/v) solvent system due to their content of structural lignin and polysaccharides, MPTER-FR was almost entirely soluble. The estimation was performed on total dry matter, because only soluble component was assessed, which results in the apparent overestimation. This trend reflects both: the more exhaustive extraction of remaining condensed phenolics in the fully soluble MPTER,

and a concentration effect, as earlier extractions may have selectively removed non-phenolic or non-condensed tannin components. These findings agree with those of Muhayyidin et al., [62] acetone-to-water ratio significantly influences tannin yield.

3.1.4 Hydrolysable tannins

Hydrolysable tannins in MPB were quantified at 25.1 mg TAE/g of dry extract. This value aligns closely with previous reports, notably that of Ferreira et al [63]. who reported a similar concentration of 26.3 mg TAE/g in softwood bark extracted using hot water. This result suggests that a significant fraction of phenolic compounds in MPB are present in a hydrolysable form.

3.1.5 Stiasny number

The Stiasny number is a useful indicator for evaluating the reactivity of tannins with formaldehyde, thus predicting their suitability for the formulation of phenol-formaldehyde adhesives. The results of this test indicate a Stiasny number of 36.9 ± 5.8%. According to a previous study, a Stiasny number of 65% is required to have good reactivity with formaldehyde and thus to produce suitable adhesives [64]. However, the extracts studied here show Stiasny numbers below the limit, indicating poor formaldehyde reactivity. This relatively low value suggests that the phenolic compounds present in the extract are not highly reactive toward formaldehyde. This outcome is coherent with the nature of the samples studied, which correspond to residues obtained after prior tannin extraction. As such, these residues are expected to contain a lower proportion of reactive phenolic structures, particularly those involved in methylene bridge formation typical of condensed tannins. The results are thus in line with the measured tannin content.

3.1.6 Antioxidant Activity

The antioxidant activity was calculated only for the final residue samples from maritime pine bark (MPTER-FR) because MPB and MPTER-1E are insoluble in methanol-acetone. The antioxidant activity of MPTER-FR was evaluated using the DPPH radical scavenging assay, with results expressed in ascorbic acid equivalents (AAe). The calibration curve built with ascorbic acid demonstrates high linearity (R^2 = 0.9942), confirming the reliability of the quantification method. The AAe content is 411,1997 µg AAe/mg. This antioxidant capacity is significant and correlates well with the previously measured high condensed tannin content in the final residue (116.8 mg/g). Condensed tannins, also known as proanthocyanidins, are

recognized for their strong radical scavenging ability due to the presence of multiple aromatic hydroxyl groups capable of donating electrons or hydrogen atoms to neutralize free radicals. These results are supported by literature: Wei et al. [65] demonstrated potent DPPH radical scavenging activity in condensed tannins from Acacia confusa, while Zhang and Lin [66] found similar activity in Canarium album tannins, establishing a direct correlation between tannin concentration and antioxidant potential.

3.1.7 Molecular Weight Distribution (SEC)

The molecular weight characteristics, including number-average molecular weight (Mn), weight-average molecular weight (Mw), and polydispersity index (PDI), are presented in Table 3. These parameters are important indicators of the structural complexity of the extract and can significantly influence the physicochemical behavior of the compounds [67]. This aspect is crucial when evaluating the potential applications of the extract, especially in formulations requiring defined reactivity or film-forming properties. In general, a higher molecular weight is often associated with lower reactivity, due to steric hindrance that can limit accessibility to reactive sites [68], [69]. In the case of this study, the values of Mn and Mw were relatively low, suggesting degradation or oxidation of polyphenols and other compounds during the tannin extraction process, which involved the use of solvents. The role of solvents in altering molecular structures has been widely reported, particularly in biomass fractionation studies where cleavage of chemical bonds and breakdown of macromolecules can occur [70].

Table 3: Molecular weight distribution of MPTER-FR

Mn (g/mol)	3226
Mw (g/mol)	559
PDI	5,77

3.2 Proximate analysis

Figure 2 illustrates the proximate composition of the three samples analyzed: MPB, MPTER-1E, and MPTER-FR. The four standard parameters evaluated were: moisture content, volatile matter, ash content and fixed carbon, each expressed as a percentage of dry weight. In addition to the theoretical heating value.

The raw material (MPB) showed a moisture content of 30%, volatile matter of 64%, ash content of 0,15%, and a fixed carbon content of 6%. These values indicate a high proportion of easily degradable organic compounds and a low mineral load. A slight increase in moisture (34%)

was observed in MPTER-1E, along with a marginal decrease in fixed carbon (2%) and stable volatile matter (63%). This suggests that the first solvent extraction removed mainly thermally stable compounds, possibly polyphenolic in nature, leaving behind materials with altered combustibility. In contrast, MPTER-FR displayed a sharp decrease in moisture to just 8%, a significant rise in volatile matter to 79%, and a marked increase in fixed carbon to 13%. These changes reflect a transformation of the biomass composition due to successive extractions, leading to a residue enriched in volatile and carbon-rich components, and thus more amenable to thermochemical conversion processes such as pyrolysis or gasification. These findings are in alignment with the work of Xavier et al, who demonstrated that Pinus taeda bark residues obtained after tannin extraction exhibited enhanced calorific properties due to the concentration of energetic compounds [71].

The three materials—MPB (6.19 MJ/kg), MPTER-1E (10.25 MJ/kg), and MPTER-FR (16.89 MJ/kg), show a significant rise in energy content after every step of processing. In particular, the heating value rises by around 66% in the first tannin extraction and by about 173% in the last residue relative to the raw material. This pattern implies that the extraction method successfully eliminates low-energy chemicals, therefore producing a relative enrichment of high-energy components like lignin and, most significantly, fixed carbon. The fixed carbon and acid-insoluble lignin analysis corroborate this view as MPTER-FR has the most fixed carbon content and acid-insoluble lignin of the three materials, in straight coherence with its high heating value.

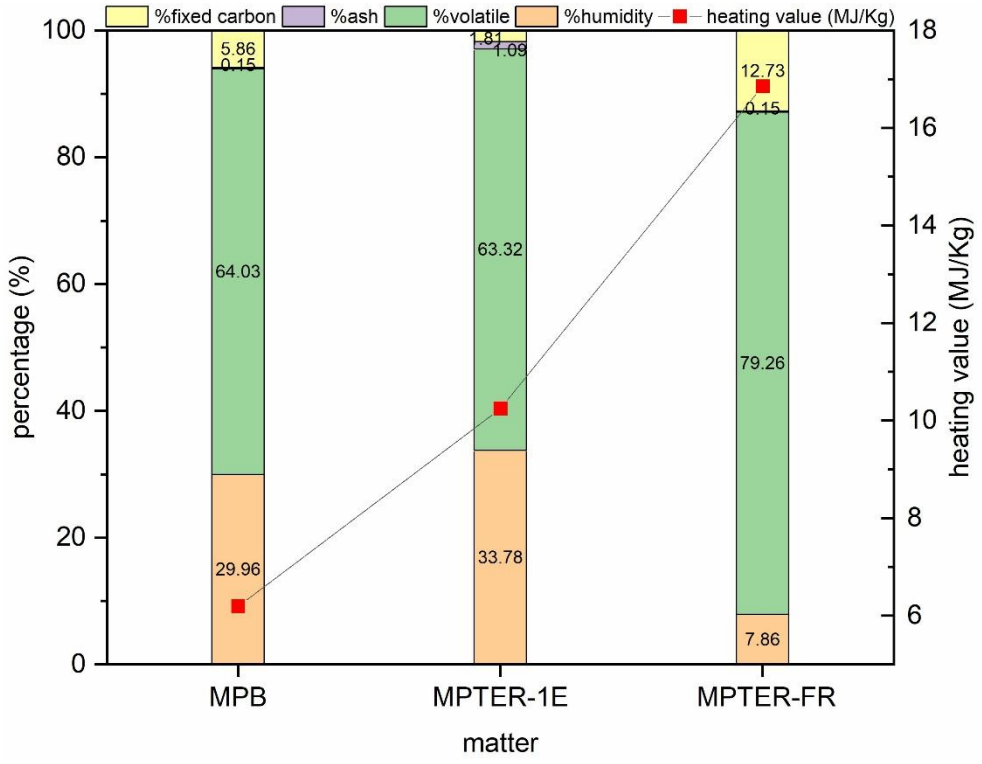


Figure 2: proximate analysis and heating value of: MPB, MPTER-1E and MPTER-FR

3.3 Sugar and lignin measurement

3.3.1 Solvent extractive content (toluene-ethanol)

The extractable content of the three samples MPB, MPTER-1E, and MPTER-FR, was determined using Soxhlet extraction with a toluene/ethanol mixture (2:1 v/v), as shown in Figure 3. This method targets non-structural, lipophilic components such as waxes, resins, and free phenolics, allowing insight into the impact of sequential extraction steps on biomass composition.

In the untreated MPB, the average extractable content was 3.05%, which showed that a significant amount of the easily soluble compounds was not present. After the initial extraction stage (MPTER-1E), the extractive yield dropped even more to 2.02% relating to an extractive removal of a considerable part of the compounds, among which tannins, by industrial extraction. On the other hand, the last deposit (MPTER-F) raised noticeably the extractive content to 83.23% indicating the high concentration of the extractable compounds that remained in the fraction lignocellulosic matrix, possibly due to depolymerization or cleavage of polysaccharide chains, rendering the material largely soluble. This high solubility correlates with the earlier findings on total phenolic content and supports the notion that the residue no

longer retains the structural integrity typical of lignified biomass. Such behavior is consistent with the literature Seabra et al. [61]demonstrated that intensive extraction conditions can lead to near-complete solubilization of biomass residues. Hence, the change in the amount of extractable material not only reflects the efficiency of the applied treatments but also emphasizes the progressive chemical alteration of the biomass matrix which should be taken into consideration in the further analysis.

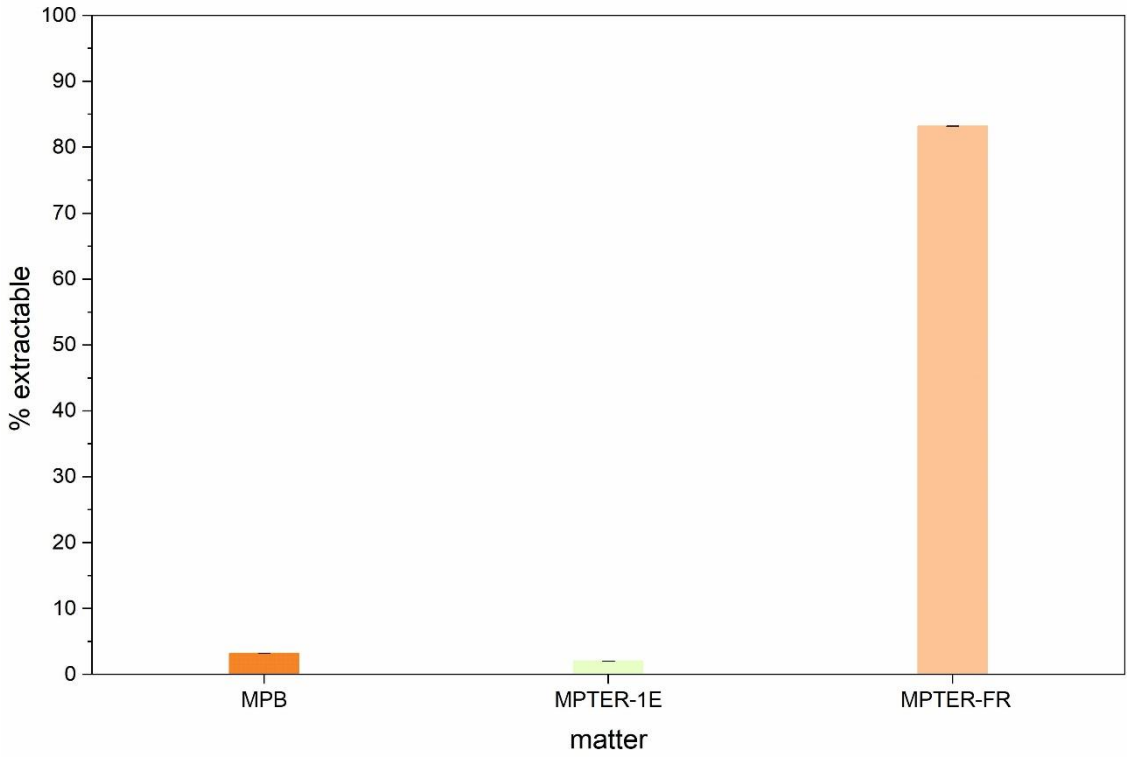


Figure 3: Percentage of Extractable Compounds from Different Material Types Using Toluene-Ethanol Mixture

3.3.2 Acid-insoluble lignin content

The acid-insoluble lignin content was evaluated for the three sample MPB, MPTER-1E, and MPTER-FR. This analysis aims to assess the structural lignin fraction that remains after the exhaustive removal of soluble and hydrolysable constituents, thus reflecting the chemical stability and resistance of the biomass matrix throughout the extraction process. As shown in Figure 4a, the acid-insoluble lignin content in the raw bark MPB was approximately 57%, slightly decreasing to 54% in MPTER-E1. This modest decline may be attributed to the removal of loosely bound phenolic compounds or small lignin oligomers, suggesting minor disruption of the lignin network during the first stage of extraction. On the other hand, a substantial increase in lignin content was observed in MPTER-FR, reaching nearly 80%. This sharp rise is

not due to lignin formation but rather to the relative enrichment of the remaining matrix following successive removal of non-lignin constituents, including extractives, hemicelluloses, and partially hydrolyzed cellulose. As more labile fractions are depleted, the residue becomes essentially recalcitrant lignin rich. These findings align with those reported by Fradinho et al. [72], who observed that multiple extraction steps on pine bark increased the relative lignin content, despite minimal changes in absolute lignin amount. Similarly, Monties [73] emphasized the structural robustness and acid resistance of Klason lignin in bark matrices, especially after the removal of extractives. Together, these results highlight the chemical resilience of lignin and its dominance in highly purified residues following solvent-based fractionation processes.

3.3.2 Holocellulose content

The holocellulose content, representing the combined fraction of cellulose and hemicellulose, was measured in three samples MPB, MPTER-1E, and MPTER-FR. This analysis gives information on the evolution of structural polysaccharides during sequential extractions and the extent of their preservation or degradation.

Figure 4b illustrates that the average total holocellulose content of the raw material was 31.70%, which is a normal value for bark biomass because of its very high lignin and extractives from which carbohydrates are limited in relative terms [74]. The average total holocellulose content after the first stage of extraction (MPTER-E1) turned out to be a bit higher, namely 33.42%. The main reason for this seeming enrichment is probably the removal of non-structural compounds such as tannins and lipophilic substances, which cause the relative proportion of the structural carbohydrates to increase in the remaining dry matter. At the opposite end of the spectrum, the final residue (MPTER-F) showed a decrease so large that the average total holocellulose content was only 5.60%. This drop is indicative of the sequential extraction procedure as a method for the degradation or solubilization of structural carbohydrates, thus, the first fractions for extractives and lignin were not the only ones impacted, but also those of cellulose and hemicellulose, most probably due to their partial hydrolysis or oxidation.

3.3.3 Cellulose content

These data from Figure 4c highlight that the cellulose content trend over the three samples (MPB, MPTER-1E, and MPTER-FR) is consistent with the stepwise disappearance of one of the main polysaccharides of wood through their extraction. In the untreated bark (MPB), the

532 average cellulose content was 18.82%, which is quite normal for pine bark if we consider that
533 it is a composite material made of lignin, hemicellulose, and extractives. Once the first stage of
534 tannin extraction has been realized, the average cellulose content goes up slightly to 20.02%,
535 which is typical of a concentration effect generated by the partial removal of tannins and
536 lipophilic extractives that are components free of cellulose. Probably, this step leaves the
537 crystalline cellulose structure unaltered, thus explaining the relative enrichment. Nevertheless,
538 in the last residue, the average cellulose content plunged to 0.12%. The significant decrease in
539 the average value is indicative of the sequential extraction process that has led to the almost
540 total breakdown and solubilization of structural cellulose. This substantial decline clearly
541 signals a degradation or solubilization of cellulose resulting from the combined effect of
542 prolonged exposure to solvents and harsh chemical conditions used in the successive
543 extractions. These findings underscore the vulnerability of cellulose to depolymerization and
544 chemical attack under extraction conditions. This aligns with the review by Knill and Kennedy
545 [75], which documented how various forms of cellulose, especially amorphous or less
546 crystalline regions can be hydrolyzed or fragmented during chemical pretreatments. Therefore,
547 the final residue can be considered nearly cellulose-free, indicating a deep alteration in the
548 carbohydrate structure of the bark biomass after extraction process.

549 **3.3.4 Hemicellulose content**

550 Figure 4d shows the hemicellulose content calculated as the difference between holocellulose
551 and cellulose for the samples MPB, MPTER-1E, and MPTER-FR. MPB had a 12.87% average
552 hemicellulose content, which is a significant fraction and is within a range of values although
553 softwood barks are amorphous polysaccharides-rich like xylans, arabinogalactans, and
554 mannans that are the source of the biomass matrix's flexibility and porosity. After the first tannin
555 extraction stage in MPTER-1E, the average hemicellulose content was 13.34%, which indicates
556 a slight increase. This is because some extractives or low-molecular-weight compounds were
557 removed partially, thus the relative percentage of structural carbohydrates retained like
558 hemicelluloses increased. Besides, it confirms that the first extraction stage did not break down
559 the polysaccharide matrix significantly, so hemicelluloses present here in large amounts. Yet
560 the average hemicellulose content was 5.33% in MPTER-FR, which was so much lower than
561 other samples that this decrease was highly significant indicating that these polysaccharides had
562 been degraded or dissolved during the sequential extraction process. This sharp decrease
563 suggests that the prolonged or harsh chemical conditions used in the latter extraction stages

(e.g., oxidative or acid-based treatment) led to depolymerization or hydrolysis of these branched and chemically labile carbohydrates. Hemicelluloses are known to be more susceptible than cellulose to acid and alkaline hydrolysis, solvent attack, and thermal degradation. These results are consistent with findings by Santos et al. [76], who demonstrated that autohydrolysis of pine wood led to both extraction and degradation of hemicelluloses due to high temperature and reaction time, ultimately transforming them into oligosaccharides and simple sugars. Thus, the hemicellulose depletion in the final stage of extraction emphasizes its vulnerability and confirms the chemical intensity of the applied treatments on the bark biomass.

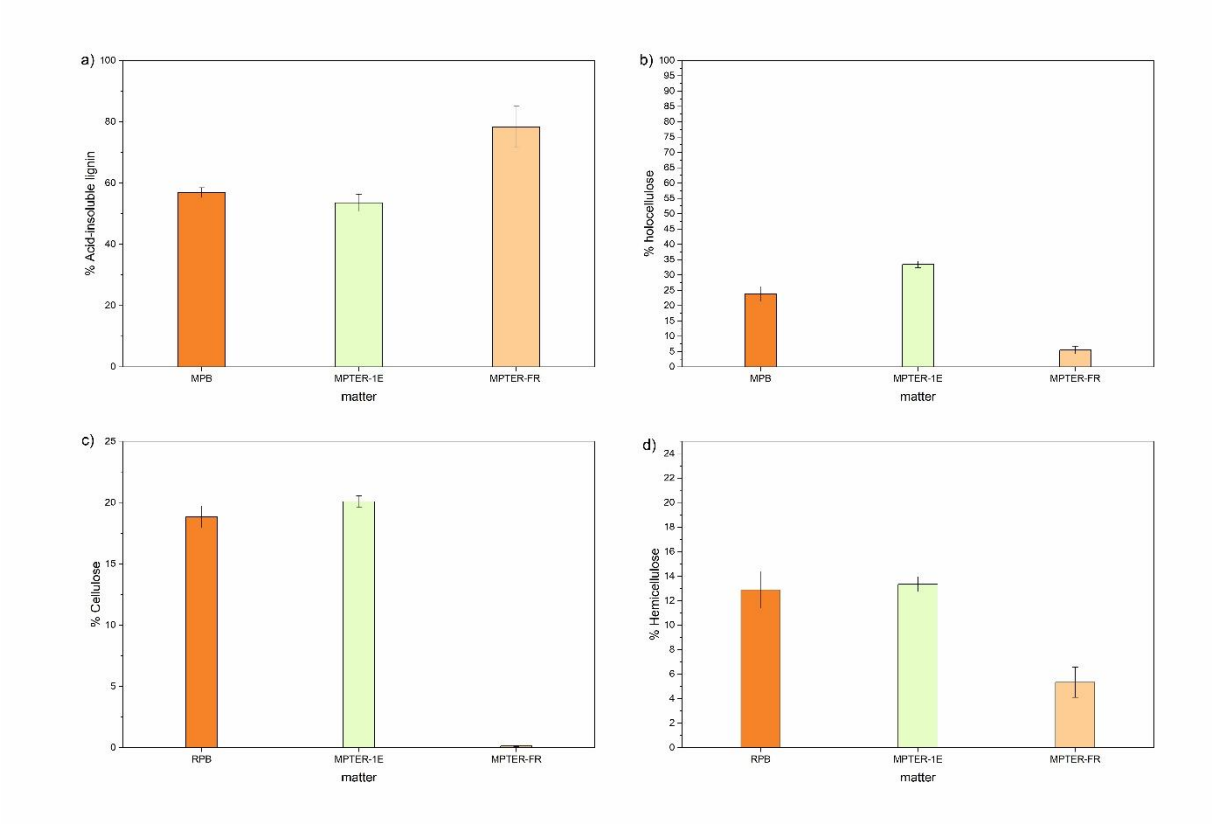
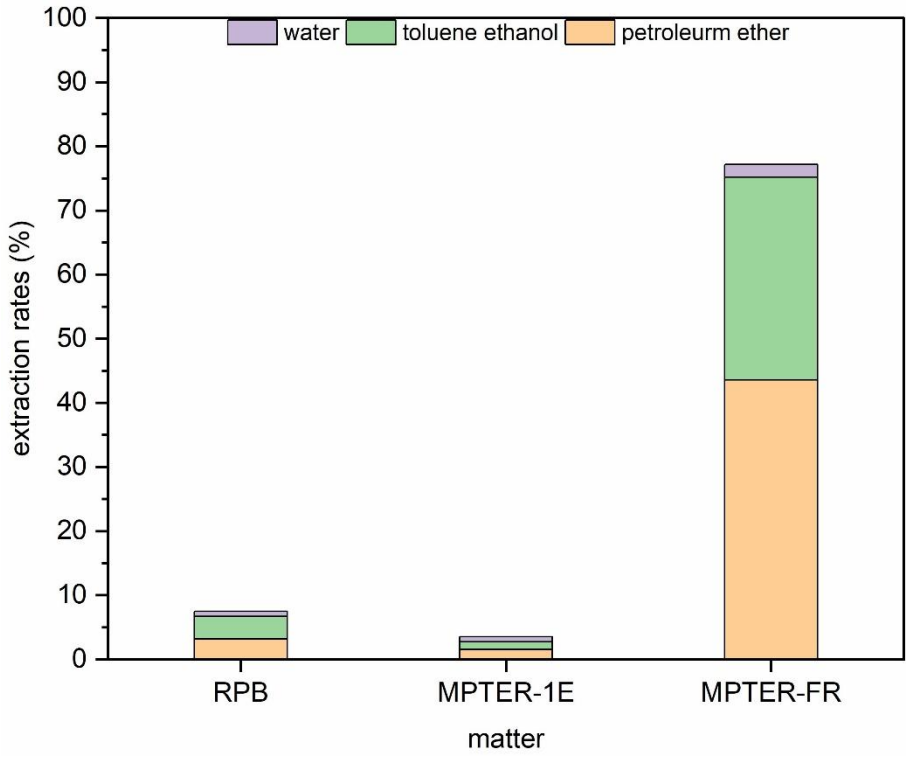


Figure 4. (a) Percentage of acid-insoluble lignin in MPB, MPTER-1E, and MPTER-FR; (b) Holocellulose content (%) in RPB, MPTER-1E, and MPTER-FR; (c) Cellulose content (%) in RPB, MPTER-1E, and MPTER-FR; (d) Hemicellulose content (%) in RPB, MPTER-1E, and MPTER-FR.

3.4 sequential solvent extraction (TAPPI T 264 standard)

Figure 5 presents the extractives content of three different samples, MPB, MPTER-1E, and MPTER-FR that were determined by sequential extraction with solvents (petroleum ether, toluene/ethanol (2:1), and water) of increasing polarity following the TAPPI T264 standard. This technique indicates the distribution of the solubility of lipophilic and polar compounds

over various stages of extraction and their changes during the process. In MPB, the total extractives content was 8.24%. The material extracted with petroleum ether constituted 3.21%, while 3.50% and 1.53% were extracted with toluene/ethanol and water, respectively. Such a profile fits the expected one for unprocessed bark, natively composed of resins, waxes, phenolics, and simple sugars in moderate quantities. While after the first tannin extraction from maritime pine bark (MPTER-1E) material presented a total extractable fraction of 3.51% that was divided into 1.51% petroleum ether-soluble, 1.23% toluene/ethanol-soluble, and 0.77% water-soluble compounds. Essentially, this decline manifests that the initial step of the extraction process has depleted the stage with the most accessible compounds, tannins and low-molecular-weight polyphenols, substantially. Finally, the MPTER-FR displayed a significant rise in extractable content, reaching a total of 77.18%. The portions of extractable compounds were reconverted to petroleum ether, toluene/ethanol, and water, as shown in the figure, with 43.54%, 31.64%, and 2.00%, respectively, revealing the great accumulation of extractable materials that remain in the last residue. This dramatic rise was predominantly associated with petroleum ether and toluene/ethanol fractions, suggesting the release or formation of low-polarity and mid-polarity compounds due to structural degradation. The elevated petroleum ether-soluble content is indicative of enriched non-polar components—likely resins, fatty acids, or depolymerized lignin fragments—while the toluene/ethanol-soluble fraction points to aromatic and polyphenolic residues, possibly modified during oxidative or alkaline treatments. These results are consistent with the hypothesis that advanced processing disrupts the polymeric matrix, generating smaller, more soluble degradation products. Such solvent-extractable increases in heavily processed biomass residues have also been reported by Vieito et al. [60], who revealed that solvent polarity and order of extraction influence considerably the yield and pattern of extractables of pine bark with water/ethanol mixtures extracting more polyphenolics and lipophilic compounds than each of the solvents alone.



607

608 Figure 5: Extraction Rates (%) of RPB, MPTER-1E, and MPTER-FR Using Petroleum Ether,
609 Toluene-Ethanol and Water, According to TAPPI T 264 Standard

610 **Conclusion**

611 This study evaluated the potential for valorizing residues obtained after industrial tannin
612 extraction from maritime pine bark through a combination of complementary analyses.
613 Together, these methods allowed a clear understanding of how the material evolves chemically
614 and structurally during extraction. The raw bark has low measurable amounts of phenolics, not
615 due to a lack of bioactive compounds but to their limited solubility, which hinders extraction
616 and quantification. The FTIR spectra show a decrease of hydroxyl-rich signals and an increase
617 in aliphatic features as the extraction progressed, confirming the gradual removal of soluble
618 compounds. The evolution of the material's composition reflects these chemical changes:
619 polysaccharides such as hemicellulose and cellulose were progressively degraded or dissolved,
620 while lignin became predominant in the final residue reaching about 80%. The observed
621 increase in fixed carbon content and heating value with lignin enrichment indicates a
622 transformation from a lignocellulosic matrix into a carbon-rich residue suitable for
623 thermochemical conversion. The decrease in molecular and the abrupt rise in extractability
624 suggest that the structure gets more fractured and soluble, hence accounting for higher phenolic
625 content and antioxidant activity in the final residue compared with the unextracted bark. In

other words, the breakdown of macromolecules during extraction enhances phenolic solubility and concentration while making them better at holding energy. These findings show that, rather than inert wastes, tannin extraction residues are chemically modified materials with high extractable phenolic content and strong energy potential, making them promising candidates for bioenergy applications and phenolic compound recovery within a circular bioeconomy framework.

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