forests

MDPI

*Article*

# Phytochemical Study, FTIR and GC-MS Characterization and Evaluation of the Antioxidant Activity of *Letestua durissima* Extracts

**Ley-Fleury Ella Nkogo [1], Marlain Stevy Mikala Mouendou [1], Stéphane Dumarçay [1], Prosper Edou Engonga [2,*] and Philippe Gérardin [1,*]**

[1] UR 4370 USC 1445 LERMAB, National Institute of Agricultural Research (INRAE), Faculty of Sciences and Technologies, Université de Lorraine, 54506 Vandoeuvre-lès-Nancy, France; ley-fleury.ella-nkogo@univ-lorraine.fr (L.-F.E.N.); marlain.mikala-mouendou@univ-lorraine.fr (M.S.M.M.); stephane.dumarcay@univ-lorraine.fr (S.D.)

[2] LAPLUS Multidisciplinary Science Laboratory, Department of Physical Sciences, Normal Superior School, Hight School Avenue, Libreville, Gabon

* Correspondence: edouengong@yahoo.fr (P.E.E.); philippe.gerardin@univ-lorraine.fr (P.G.)

**Abstract:** The current study focused on extract content, phytochemical screening, GC-MS analysis, infrared analysis and antioxidant activity evaluation of bark, sapwood and heartwood of *Letestua durissima*, commonly called Kong Afane in Gabon, using DPPH and ABTS methods. The highest extract contents, reaching 37.3%, were observed in the bark. Phytochemical tests revealed the presence of alkaloids, polyphenols, sterols, terpenes, flavonoids and saponins in the extracts. The quantitative results highlighted high concentrations of polyphenols (95.56 mg GAE $g^{-1}$ of dry extract in the acetone extract), condensed tannins (113.45 mg CE $g^{-1}$ of dry extract in the extract toluene–ethanol) and flavonoids (20.26 mg CE $g^{-1}$ of dry extract in the toluene–ethanol extract) in the bark. Regarding the evaluation of antioxidant activity, the results showed a significant capacity of the extracts to scavenge free radicals. This activity was more pronounced in the toluene–ethanol extracts, with an IC50 of 0.66 for the ABTS method and 2.39 for that of DPPH. The GC-MS identification of phenolic compounds such as hydroxybenzoic acid, protocatechuic acid, gallic acid, catechin and triterpenes could explain the high reactivity of our extracts as antioxidants.

**Keywords:** antioxidant; characterization; extract; *Letestua durissima*; valorization

**Citation:** Ella Nkogo, L.-F.; Mikala Mouendou, M.S.; Dumarçay, S.; Edou Engonga, P.; Gérardin, P. Phytochemical Study, FTIR and GC-MS Characterization and Evaluation of the Antioxidant Activity of *Letestua durissima* Extracts. *Forests* **2024**, *15*, 429. https://doi.org/10.3390/f15030429

Academic Editors: Ana Alves and José Carlos Carvalho Rodrigues

Received: 26 January 2024
Revised: 16 February 2024
Accepted: 20 February 2024
Published: 23 February 2024



## 1. Introduction

*Letestua durissima* is a large tree native to Gabon and the Republic of Congo. It grows in sandy, loamy, clayey and well-drained soils with acidic or neutral pH. It does not grow in shade and prefers moist soil. It is the only recognized species of the *Letestua* genus of the *Sapotaceae* family. *Letestua durissima* tree can reach 50 m high with a straight, cylindrical trunk that can measure 240 cm in diameter. The trunk can have no branches up to 40 m. Its heartwood is dark red-brown, distinguishable from the 5 to 9 cm-wide strip of pale brown sapwood. The wood is very durable. It can be used in a marine environment as it is suitable for hydraulic work, as well as for the manufacture of railway sleepers, bridges and heavy timberwork. In Gabon, it is known by the name of Kong Afane, and it is known as Congotali in Congo [1]. The abandonment of *Letestua durissima* waste in forests in the form of crowns rich in bark and branches of varying diameters, as well as the slabs, chips, shavings and sawdust produced during wood transformation constitutes a considerable source of raw material containing potential biomolecules of interest, which may find applications in high value markets [2]. This situation is not limited to *Letestua durissima* waste but can be extended to all the waste of wood industry. Indeed, it is noticeable that in

Gabon, high amounts of potentially recoverable waste are abandoned during wood processing, justifying research to find potential methods of valorization. Moreover, wood is known to contain secondary metabolites, which have been described to be involved in the tree defenses due to their antioxidant, chelating or antiseptic properties [3]. These same molecules are responsible for wood's natural durability after the harvesting of the tree. The literature reports that 400 million of cubic meters of wood waste are produced in Gabon each year [4]. This waste is often left on the ground or used by the local population as firewood, to produce artisanal charcoal or sometimes for the boilers of wood processing units. Thus, the valorization of wood waste from logging and the wood industry constitutes an important challenge for the sustainable development of the forest and wood industry sectors of the Congo Basin countries. Wood chemistry, among other things, offers new valuable perspectives in this area through the valorization of different kinds of biomolecules to address markets in the area of cosmetic, nutraceutical, human and animal health, agrochemistry (bio-stimulation and bio-control) or animal nutrition [5].

In this context, bioactive molecules extracted from wood have been the subject of numerous studies in the last few decades in view of their valorization [6–10]. Such kinds of valorization represent major economic opportunities through the creation of added value for the sector of forest and wood industry and also creates new jobs.

According to the high natural durability of *Letestua durissima* wood, it seems of interest to investigate the chemical composition of Kong Afane extracts, which have not been studied to date. Indeed, wood resistance to decay has been often associated with the presence of phenolic extractives acting as radical scavengers to prevent wood cell polymers depolymerizing through oxidative systems developing brown and white rot fungi [3]. Such properties could lead to the valorization of extracts as antioxidant agents. The aim of this study is therefore (i) to evaluate the extractives content of the different parts of Kong Afane, namely bark, heartwood and sapwood; (ii) to realize a phytochemical screening and evaluate total polyphenols, total flavonoids and total condensed tannins contents; (iii) to characterize chemicals present in the different extracts by FTIR and GC-MS analysis and, finally, (iv) to evaluate the radical scavenging activities of the different extracts (Figure 1).

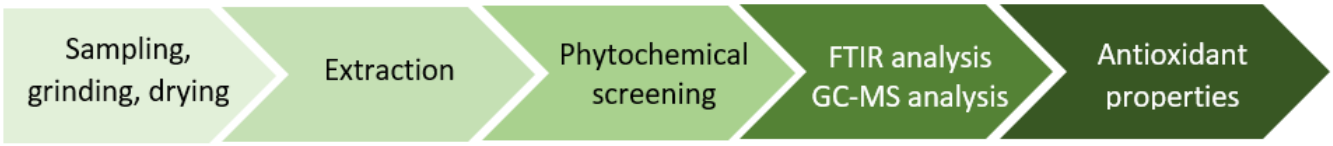


**Figure 1.** Flow diagram of the study process.

## 2. Materials and Methods

### *2.1. Chemical Products*

2,2-Diphenyl 1-pycrilhydrazyl (DPPH), catechin and 3,5-di-tert-4butylhydroxytoluene (BHT) were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). All chemicals necessary for phytochemical screenings like concentrated sulfuric acid (95%–97%), dichloromethane, acetone, absolute ethanol, chloroform, isoamyl alcohol, iron perchloride, ferric chloride, sodium hydroxide, magnesium shavings, chloroform, hydrochloric acid, Fehling's liquor, and Dragendorff's reagent were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France), Carlo Erba (Val de Reuil, France) or Prolabo-Avantor (Rosny-sous-Bois, France) and used without further purification. Deionized water was obtained by an ELGA PURELAB Prima 15 machine (Wycombe, UK).

### 2.2. Plant Material

The sample collection phase was carried out following a previously established procedure. Sampling was carried out in Gabon in the province of Woleu-Ntem. The different parts (bark, sapwood and heartwood) of mature *Letestua durissima* were collected using a machete and a chainsaw on 8 April 2021. The samples were cut into small pieces and then transported to LERMAB in France, where they were air-dried for three weeks. The samples (bark, sapwood and heartwood) were ground to fine sawdust using a FRITSCH PULVERISETTE 9 crusher (Idar-Oberstein, Germany). The sawdust was then sieved to obtain a grain size comprised between 1 and 2 mm and stored away from light in closed glass jars until the time of the chemical tests.

### 2.3. Extraction

Extracted contents were determined using successive Soxhlet extraction with solvents of increasing polarity. Approximately 7 g of sawdust were weighed precisely ($m_0$) and placed in 25 × 80 Sohxlet cartridges with 200 mL of solvent (dichloromethane, acetone, toluene–ethanol (50/50, *v*/*v*) or deionized water) in the extraction flask. Extractions were performed under reflux for 24 h. Solvent was then evaporated or freeze-dried in a case of water and the residual mass of extract was weighed ($m_1$). The extract's contents were calculated using the following equation as a percentage of the final dry mass of extracts from the initial dry mass of sawdust subjected to extraction:

$$Extractive\ Content(\%) = \frac{m_1}{m_0} \times 100$$

### 2.4. Phytochemical Screening

The reagents used to carry out the phytochemical screening of the extracts were prepared and used according to the protocols described in [11–13]. All the different tests were carried out in triplicate. For alkaloids, 2 mL of extract was introduced into a test tube and a few drops of Dragendorff's reagent solution were then added. The appearance of a red-orange precipitate indicated the presence of alkaloids [14,15]. For polyphenols, 2 mL of extract was introduced into a test tube, and then a few drops of the 2% ethanolic ferric chloride solution were added. The appearance of a blue-blackish color indicates the presence of polyphenols [14,15]. The presence of sterols and triterpenes was demonstrated using the Liebermann–Burchard reaction, which consisted of introducing 2 mL of extract into a test tube with 1 mL of acetic anhydride and then 1 mL of concentrated sulfuric acid. The appearance of a purple or purple interphase and the coloring of the green, blue or purple supernatant, respectively, indicates the presence of sterols and triterpenes [16]. The presence of tannins was demonstrated by adding 1 mL of extract, 1 mL of deionized water and 1 to 2 drops of $FeCl_3$ solution (iron perchloride or iron (III) chloride) diluted to 1%. The appearance of a dark green color indicates the presence of tannins [17,18]**.** For reducing the compounds, 2 mL of extract was placed in a test tube, then 2 mL of Fehling's liquor was added. The mixture was then brought to boil in a water bath for 8 min. The appearance of a brick-red precipitates indicated the presence of the reducing compounds [17,18]. For flavonoids, 1 mL of extract was introduced into a test tube then 1 mL of hydrochloric acid was added, followed by 1 mL of isoamyl alcohol and then a few magnesium shavings. The appearance of pink-orange coloring indicates the presence of flavonoids [14,19]. The saponins were identified by introducing 10 mL of each extract into a test tube and then shaking vigorously with a vortex for 15 s. The tube was left to rest for 15 min. The appearance of a persistent foam indicates the presence of saponins [16,17].

### *2.5. Dosage of Total Polyphenols*

The Folin–Ciocalteu colorimetric method described by Wootton–Beard [20] was used for the determination of total phenols content. To carry out the assay, 0.5 mL of extract at 100 ppm diluted in methanol was added to 2.5 mL of the Folin–Ciocalteu reagent (diluted 10 times in deionized water). After adding the Folin–Ciocalteu reagent to the extract, 2 mL of sodium carbonate at 75 $g.L^{-1}$ was added after 8 min. The reaction mixtures were stirred and incubated for 5 min at 50 °C in a thermostatically controlled water bath. After this reaction time, the test tubes was transferred to a cold-water bath for 5 min. The mixture was left to stand for 60 min, and the absorbance was measured at 760 nm using a UV–Visible spectrophotometer (Shimadzu UV-2550, Champs sur Marne, France). A standard range was produced with gallic acid (6 concentration points from 0 to 100 $mg.L^{-1}$). The calculation of the average concentration of polyphenols present in the extracts was determined using a calibration curve in mg of gallic acid equivalent (GAE) $g^{-1}$ of extracts [20].

### *2.6. Dosage of Condensed Tannins*

The acid/butanol technique described by Chamorro [21] was used to evaluate the condensed tannins contained in the extractables. A total of 0.7 g of $FeSO_4.7H_2O$ was introduced into a one-liter flask and dissolved with the butan-1-ol/HCl 950/50 (*v*/*v*) mixture. The whole thing was placed in an ultrasonic bath (DECON Ultrasonics, FS 100 frequency sweep) to completely dissolve the solid. A total of 7 mL of the prepared solution and 50 mg of extractable were introduced into a test tube. The test tubes were shaken carefully to dissolve the extractables, then they were heated to 95 °C for 50 min. After this reaction time, the tubes were cooled in an ice bath and the absorbance of the different solutions was read at 550 nm using a UV–Visible spectrophotometer. The reaction blank was carried out under the same conditions without adding extractives to the test tubes. Condensed tannins (CT) were expressed as equivalents in mg of catechin equivalent (CE) $g^{-1}$ of dry extracts using a calibration curve.

### *2.7. Dosage of Flavonoids*

The quantification of flavonoids was carried out by the $AlCl_3$ colorimetric method described by Amjad M. Shraim with [22] with some modifications. In a test tube, 400 µL of extract, or standard or control deionized water, was added to 120 µL of 5% $NaNO_2$. After 5 min, 120 µL of 10% $AlCl_3$ were added, and the medium was mixed vigorously. After 6 min, 800 µL of 1 M NaOH was added to the medium. A total of 6 concentration points from 0 to 100 $mg.L^{-1}$ were created with catechin to draw the calibration curve, and the different extracts were read at 510 nm using a UV–Visible spectrophotometer.

### *2.8. FTIR Spectroscopy*

The FTIR spectra of the dried extracts were acquired by attenuated total reflectance (ATR) through a diamond cell on a Nicolet 6700. All spectra were recorded at a spectral resolution of 4 $cm^{-1}$, and 16 scans were performed per sample in the 4000–650 $cm^{-1}$ range. Omnic 5.2 (Thermo Scientific, Waltham, MA, USA) software was used for acquisition and data processing.

### *2.9. GC-MS Analysis*

For GC-MS analysis, approximately 1 to 2 mg of dry extracts were placed in vials. Samples obtained with the different solvents underwent derivatization (silylation reaction) before injection to facilitate the solubilization and volatility of all compounds present. For this, 50 µL of BSTFA was added to the pill bottles then left for 24 h in an oven at 70 °C. After this step, the BSTFA was evaporated, and the derivatized extract was then dissolved in 1 mL of ethyl acetate. A total of 1 to 3 µL of this solution was injected into the chromatograph in the gas phase at an inlet temperature of 250 °C in splitless mode. Helium with a flow rate of 1 $mL.min^{-1}$ was used as mobile phase. The temperature program

was as follows: 80 °C was maintained for 2 min, then the temperature was increased to 190 °C at a rate of 10 °C $min^{-1}$, then it was increased at a rate of 15 °C $min^{-1}$ to 280 °C and this temperature was maintained for 10 min, before being increased again at a rate of 10 °C $min^{-1}$ to 300 °C, which was maintained for 14 min. The heating program could be changed slightly to improve the resolution and separation of certain samples. Separation was carried out on a DB-5MS column (stationary phase (95% dimethyl/5% diphenylpolysiloxane; length: 30 m; internal diameter: 0.25 mm; film thickness: 0.25 μm) coupled to a Perkin Elmer Clarus 680 gas chromatograph Perkin Elmer Clarus SQ8 mass spectrometer (Villebon sur Yvette, France). After the separation step, the compounds were transferred to the ionization mass spectrometer via a transfer line heated to 250 °C using the electron impact method (ionization energy 70 eV). The driver software is Turbo Mass v6.1 with the NIST database (American National Standards and Technology, Gaithersburg, MD, USA) (2011 edition). Compound identification was carried out by comparing their mass spectra with the NIST MS Search 2.0 library (2011). Identification is considered relevant for a Match and Reverse Match values greater than 900.

### *2.10. Antioxidant Activity*

Two methods were used to evaluate the antioxidant activity.

#### 2.10.1. DPPH Method

The method described by Anokwuru was slightly modified [23] with the 2,2-diphenyl-1-picrylhydrazyl radical, which allowed us to evaluate the antioxidant activity. A total of 39.4 mg of DPPH was dissolved in 100 mL of methanol. Then, 20 mL of this solution was taken and diluted in a 100 mL flask. Extracts at different concentrations were dissolved in methanol. Amounts of 1 mL of DPPH ($10^{-4}$ M) and 1 mL of extracts dissolved in methanol were introduced into the analysis cell of the spectrophotometer. The mixture was stored in the dark at room temperature for 3 min. After this period, the absorbance was measured at 517 nm using an UV–Visible spectrophotometer. The tests were carried out in duplicate and the antioxidant activity of the extracts ($AA$) was expressed as a percentage based on DPPH alone in methanol without the extract.

$$AA(\%) = \frac{A_0 - A}{A_0} \times 100$$

where $A_0$ is the absorbance of the control and $A$ the absorbance of the DPPH solution in the presence of the extracts. The inhibitory concentration required to consume 50% DPPH (IC50) is determined from the equation of the curve of the percentage of inhibition as a function of the sample concentrations. Low IC50 concentrations correspond to high antioxidant activities.

#### 2.10.2. ABTS+ Method

The antioxidant activity was evaluated by the cationic radical 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic) [24] with a slight modification. ABTS+ (7 mM) was prepared by mixing 384 mg of solid ABTS in 100 mL of potassium persulfate (2.45 mM). The solution was then diluted in a sodium phosphate-buffered solution (5 mM) of pH = 7 in order to achieve an absorbance at 1.4 at 734 nm. Extracts at different concentrations were dissolved in methanol. In test tubes, 1 mL of ABTS+ and 1 mL of extracts dissolved in methanol were introduced. The mixture was stored in the dark at room temperature for 30 s. Then, the absorbance was measured at 734 nm using a UV–Visible spectrophotometer. The tests were duplicated, and the antioxidant activity of the extracts (AA) was expressed as a percentage of inhibition based on DPPH alone in methanol without the extract.

### 2.11. Data Analysis

The analysis of variance of the completely randomized design was analyzed to identify differences between the group samples. The mean differences between the group samples were determined using Duncan's multiple range test at a 5% significance level.

## 3. Results

### 3.1. Extractives Contents

Extractives contents are presented in Table 1.

**Table 1.** Extractives contents of the different wood types (average of three measurements ± standard deviation). Values followed by the same letters do not differ significantly ($\alpha = 0.05$) based on Duncan's test.

| Solvent | Extract Content (%) | | |
|---|---|---|---|
| | Bark | Sapwood | Heartwood |
| Dichloromethane | 1.77 ± 0.66 $^{a}$ | 0.23 ± 0.01 $^{a}$ | 0.10 ± 0.01 $^{a}$ |
| Acetone | 25.33 ± 0.81 $^{f}$ | 3.56 ± 0.29 $^{c}$ | 2.62 ± 0.41 $^{bc}$ |
| Toluene/ethanol | 3.42 ± 1.13 $^{bc}$ | 4.74 ± 0.64 $^{d}$ | 2.68 ± 1.30 $^{bc}$ |
| Water | 6.78 ± 0.27 $^{e}$ | 3.09 ± 0.47 $^{bc}$ | 2.30 ± 0.56 $^{b}$ |
| Total | 37.30 ± 0.72 | 11.61 ± 0.35 | 7.69 ± 0.59 |

### 3.2. Phytochemical Tests

The results of the phytochemical tests of *Letestua durissima* are reported in Table 2.

**Table 2.** Phytochemical screening of *Letestua durissima* extracts obtained by successive extractions.

| Compounds | Reagents | Solvent Extraction | Bark | Sapwood | Heartwood |
|---|---|---|---|---|---|
| Alkaloids | Mayer and Bouchardât | Dichloromethane | + | + | + |
| | | Acetone | + | + | + |
| | | Toluene–ethanol | + | + | + |
| | | Water | + | + | + |
| Flavonoids | Shinoda test | Dichloromethane | − | − | − |
| | | Acetone | + | + | + |
| | | Toluene–ethanol | + | + | + |
| | | Water | + | + | + |
| Polyphenols | Iron (III) chloride | Dichloromethane | − | − | − |
| | | Acetone | + | + | + |
| | | Toluene–ethanol | + | + | + |
| | | Water | + | + | + |
| Saponins | Aphrogenic power | Dichloromethane | − | − | − |
| | | Acetone | + | + | + |
| | | Toluene–ethanol | + | + | + |
| | | Water | + | + | + |
| Sterols and terpenes | Lieberman Bouchard | Dichloromethane | + | + | + |
| | | Acetone | − | − | − |
| | | Toluene–ethanol | − | − | − |
| | | Water | − | − | − |

+: present; −: absent.

### *3.3. Total Polyphenols*

Total polyphenol contents are presented in Table 3.

**Table 3.** Total polyphenol content (average of three tests ± standard deviation). Values followed by the same letter do not differ significantly ($\alpha = 0.05$) based on Duncan's test.

| Solvents | Polyphenol Content (mg GAE $g^{-1}$ of Dry Extract) | | |
|---|---|---|---|
| | Bark | Sapwood | Heartwood |
| Acetone | 95.56 ± 2.47 $^{f}$ | 73.86 ± 1.48 $^{d}$ | 79.91 ± 3.32 $^{e}$ |
| Toluene/ethanol | 92.40 ± 3.19 $^{f}$ | 57.47 ± 2.97 $^{b}$ | 76.89 ± 4.19 $^{de}$ |
| Water | 64.06 ± 3.88 $^{c}$ | 47.64 ± 3.80 $^{a}$ | 55.60 ± 4.04 $^{b}$ |

### *3.4. Total Condensed Tannins*

Total tannin contents are presented in Table 4.

**Table 4.** Condensed tannin content (average of three tests ± standard deviation). Values followed by the same letter do not differ significantly ($\alpha = 0.05$) based on Duncan's test.

| Solvents | Condensed Tannin Content (mg CE $g^{-1}$ of Dry Extracts) | | |
|---|---|---|---|
| | Bark | Sapwood | Heartwood |
| Acetone | 109.12 ± 2.52 $^{f}$ | 61.62 ± 6.53 $^{c}$ | 77.45 ± 2.78 $^{e}$ |
| Toluene/ethanol | 113.45 ± 2.50 $^{f}$ | 43.95 ± 3.91 $^{b}$ | 69.12 ± 4.65 $^{d}$ |
| Water | 62.45 ± 2.18 $^{c}$ | 33.95 ± 3.28 $^{a}$ | 45.45 ± 1.00 $^{b}$ |

### *3.5. Total Flavonoids*

Table 5 presents total flavonoids contents of the different types of *Letestua durissima.*

**Table 5.** Flavonoid content (average of three tests ± standard deviation) Values followed by the same letter do not differ significantly ($\alpha = 0.05$) based on Duncan's test.

| Solvents | Flavonoid Content (mg CE $g^{-1}$ of Dry Extracts) | | |
|---|---|---|---|
| | Bark | Sapwood | Heartwood |
| Acetone | 19.26 ± 0.55 $^{f}$ | 10.93 ± 0.23 $^{d}$ | 14.55 ± 1.25 $^{e}$ |
| Toluene/ethanol | 20.46 ± 0.51 $^{f}$ | 3.97 ± 1.17 $^{bc}$ | 11.98 ± 1.91 $^{d}$ |
| Water | 5.42 ± 0.22 $^{c}$ | 01.11 ± 0.26 $^{a}$ | 3.21 ± 0.51 $^{b}$ |

### *3.6. FTIR Analysis*

Comparison of FTIR spectra of crude extracts is reported in Figure 2.

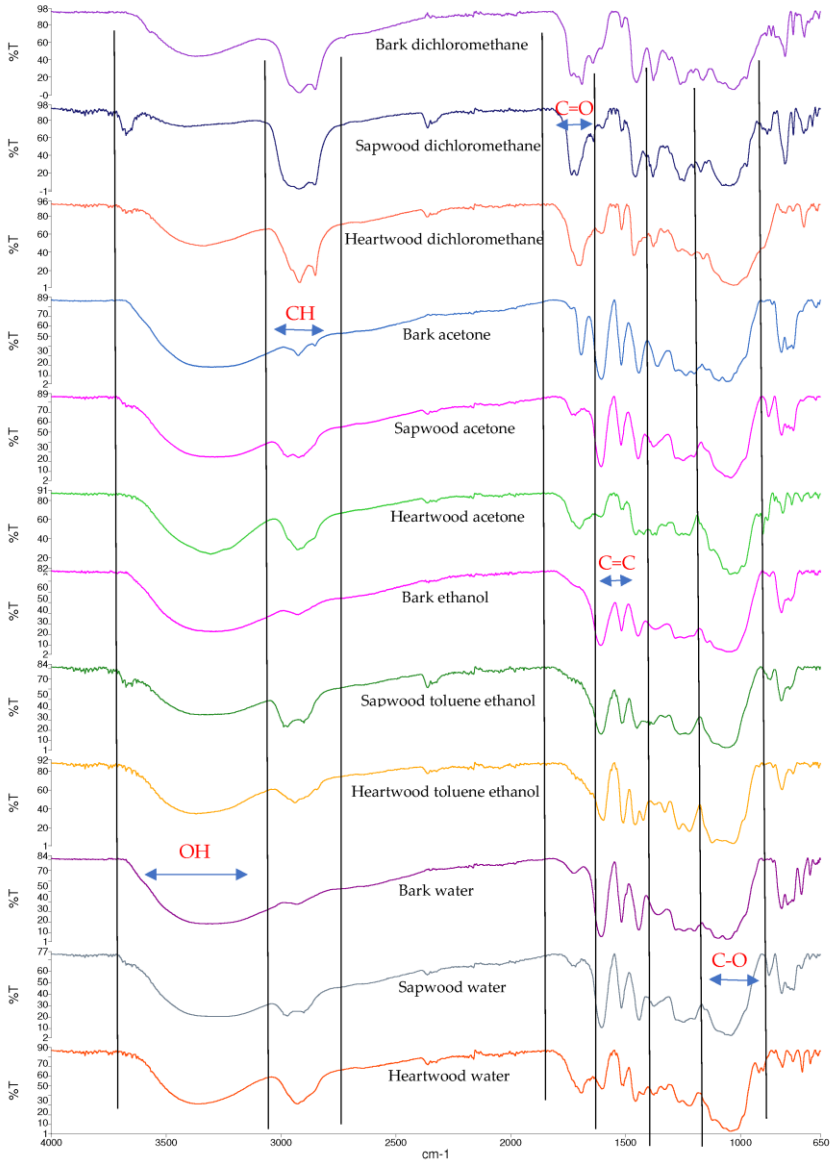


**Figure 2.** FTIR spectra of the different *Letestua durissima* extracts.

### *3.7. GC-MS Analysis*

Annotations of chemicals present in dichloromethane, acetone or toluene/ethanol extracts of the different compartment are presented in Tables 6, 7 and 8, respectively.

**Table 6.** GC-MS analysis of dichloromethane extracts of *Letestua durissima.*

| Identified Compounds | $RT_{(min)}$ | Bark (%) * | Sapwood (%) * | Heartwood (%) * |
|---|---|---|---|---|
| Glycerol | 8.4 | 2 | - | - |
| Hydroquinone | 10.2 | 1 | - | - |
| 3,4-Dihydroxybenzaldehyde | 12.8 | 2 | - | - |
| 4-Hydroxybenzoic acid | 12.9 | 6 | - | - |
| Isovanillic acid | 14.3 | 2 | - | - |
| Protocatechuic acid | 14.8 | 2 | - | 7 |
| Fatty acid | 13.2–24.7 | 14 | 15 | 35 |

| | | | | |
|---|---|---|---|---|
| Taraxerone | 26.9 | 4 | - | - |
| 7,22-Ergostadienone | 27.8 | - | 3 | - |
| Stigmasterol | 27.9 | - | 2 | - |
| β-Amyrone | 28.0 | 7 | 5 | - |
| Stigmasta-4,22-dien-3-one | 28.2 | - | 2 | 2 |
| β-Amyrin | 28.5 | - | 1 | - |
| β-Amyrone isomer | 28.7 | 18 | 16 | 1 |
| α-Amyrin, trimethylsilyl ether | 28.9 | - | - | - |
| β-Amyrin acetate | 29.7 | 7 | 9 | - |
| Lupeol, trimethylsilyl ether | 30.2 | - | 4 | 1 |
| β-Amyrin acetate isomer | 30.7 | 30 | 38 | - |
| Stigmasterol | 31.3 | - | - | 6 |
| Friedelan-3-one | 31.4 | - | - | 4 |
| Betulin | 31.9 | - | - | 2 |
| Epilupeol acetate | 32.2 | 5 | 1 | 3 |

* Percentages of the different compounds are calculated as percentages of the total ion current (TIC).

**Table 7.** GC-MS analysis of acetone extracts of *Letestua durissima.*

| **Identified Compounds** | **RT(min)** | **Bark (%) *** | **Sapwood (%) *** | **Heartwood (%) *** |
|---|---|---|---|---|
| Glycerol | 8.3 | | - | 11 |
| 4-Hydroxybenzoic acid | 12.9 | 2 | - | - |
| Protocatechuic acid | 14.8 | 2 | 7 | 2 |
| Gallic acid | 15.8 | - | - | 13 |
| Sugar | 15.1–24.6 | 18 | 89 | 53 |
| Docosanol, TMS derivative | 19.6 | - | 5 | - |
| Catechin | 21.9 | 6 | - | - |
| Stigmasterol, TMS derivative | 27.2 | - | - | 20 |
| Taraxerone | 27.7 | 6 | - | - |
| β-Amyrin | 28 | 3 | - | - |
| β-Amyrone isomer | 28.7 | 6 | - | - |
| α-Amyrin, trimethylsilyl ether | 28.9 | 12 | - | - |
| β-Amyrin acetate isomer | 30.6 | 35 | - | - |
| Friedelan-3-one | 31.4 | 2 | - | - |

* Percentages of the different compounds are calculated as percentages of the total ion current (TIC).

**Table 8.** GC-MS analysis of toluene/ethanol extracts of *Letestua durissima.*

| **Identified Compounds** | **RT(min)** | **Bark (%) *** | **Sapwood (%) *** | **Heartwood (%) *** |
|---|---|---|---|---|
| Glycerol | 8.3 | 3 | - | - |
| Sugar | 10.0–24.3 | 78 | 95 | 94 |
| Protocatechuic acid | 14.7 | 2 | 4 | - |
| Tricarboxylic acid | 15.2 | 3 | - | 3 |
| Gallic acid | 15.8 | 9 | - | 2 |
| Palmitic acid | 16.6 | 2 | - | - |
| Oleic acid | 17.9 | 2 | - | - |

* Percentages of the different compounds are calculated as percentages of the total ion current (TIC).

*3.8. Antioxidant Activity*

Tables 9 and 10 represent minimum inhibitory concentrations to scavenge half of the DPPH or ABTS radicals, respectively.

**Table 9.** $IC_{50}$ values of the different extracts using DPPH method. Values followed by the same letter do not differ significantly ($\alpha = 0.05$) based on Duncan's test.

| Extraction Solvent | Bark | Sapwood | Heartwood | Catechin | BHT |
|---|---|---|---|---|---|
| Acetone | 3.56 ± 0.07 $^{bc}$ | 6.47 ± 0.06 $^{e}$ | 2.40 ± 0.10 $^{a}$ | | |
| Toluene–ethanol | 2.39 ± 0.79 $^{ab}$ | 5.57 ± 0.01 $^{e}$ | 2.66 ± 0.21 $^{ab}$ | | |
| Water | 4.39 ± 0.30 $^{c}$ | 8.80 ± 0.15 $^{f}$ | 3.31 ± 0.29 $^{b}$ | | |
| | | | | 1.95 ± 0.05 | 3.31 ± 1.54 |

**Table 10.** $IC_{50}$ values of the different extracts using ABTS method. Values followed by the same letter do not differ significantly ($\alpha = 0.05$) based on Duncan's test.

| Extraction Solvent | Bark | Sapwood | Heartwood | Catechin | BHT |
|---|---|---|---|---|---|
| Acetone | 1.50 ± 0.03 $^{a}$ | 3.31 ± 0.15 $^{a}$ | 1.33 ± 0.10 $^{a}$ | | |
| Toluene–ethanol | 1.39 ± 0.18 $^{a}$ | 2.04 ± 0.11 $^{a}$ | 0.66 ± 0.10 $^{b}$ | | |
| Water | 1.90 ± 0.05 $^{a}$ | 3.61 ± 0.10 $^{a}$ | 3.26 ± 0.16 $^{a}$ | | |
| | | | | 0.38 ± 0.01 | 0.46 ± 0.03 |

## 4. Discussion

*4.1. Extractives Contents*

The results obtained in Table 1 indicated that the extractives contents vary from one solvent to another and with the part of the tree used. Extract contents are highest for the bark followed by the sapwood and then by the heartwood. Higher levels of extractives detected in the bark are in good agreement with data from the literature [25–28]. Higher levels of extractives in sapwood compared to heartwood is less common, as heartwood generally contains more extractives than sapwood due to the duramen formation process. This could be consequence of the role of sapwood, which is not only responsible for sap conduction but also for the storage and synthesis of biochemicals. Dichloromethane yields were low in all parts of the tree. The highest dichloromethane content was obtained is in the bark (1.77%) and the lowest in the heartwood (0.10%). For toluene/ethanol extraction, sapwood had the highest extractives content with a yield of 4.74%, and the smallest value was observed in the heartwood (2.68%). For water and acetone, the highest extractives contents were obtained in bark (6.78% and 25.33%, respectively) and the lowest yields were obtained in the heartwood (2.62% and 2.30%, respectively).

Dichloromethane, the first solvent used during the extraction process, presents a low polarity. It mainly solubilizes non-polar substances (oils, fats, terpenes), while polar solvents are able to solubilize more polar compounds, such as polyphenols and sugars. The extraction process which combines apolar and polar solvents makes it possible to partition the extracts into different fractions, facilitating subsequent analyses. The sum of the extracts with each solvent gives an idea of the overall content of extracts in each compartment of the wood. The overall total extractives contents from the different parts studied were 7.69% in the heartwood, 11.61% in the sapwood and 37.3% in the bark.

These extract contents corroborate studies carried out on different tropical species, which have shown different extractives contents with sometimes high levels of 20 to 22% for certain woods [29–31].

*4.2. Phytochemical Tests*

It can be seen from the results reported in Table 2 that alkaloids were present in the dichloromethane, acetone and toluene/ethanol and water extracts of the different parts of

the tree studied (bark, sapwood and heartwood). Flavonoids, saponins and polyphenols were present in the acetone, water and toluene/ethanol extracts and absent in the dichloromethane extracts regardless of the part of the tree studied. Sterols and terpenes were identified in dichloromethane extracts of bark, sapwood and heartwood.

Phytochemical tests revealed the presence of alkaloids, polyphenols, sterols and triterpenes, flavonoids and saponins. The presence of such classes of compounds in tropical woods was already reported in the literature [32]. Fengel and Wegener [33] reported that hot water extraction dissolves alkaloids, proteins, tannins and carbohydrates, while ~~ethanol/~~toluene-ethanol mixture extracted lower molecular mass compounds like stilbenes, lignans, flavonoids and anthocyanins. These compounds have been reported in the literature to possess multiple benefits such as antioxidants, antifungal, antitermite, antibacterial or antimicrobial properties. Flavonoids exhibit antifungal, antibacterial and antitermite activities [34]. Apart from imparting color and natural durability to wood, flavonoids can be considered an important source of antioxidants [35,36]. Alkaloids detected in extracts could have interesting biological activities. Alkaloids have several pharmaceutical applications in humans, and these applications have been clinically proven [37]; they are also reported to act as antibiotics [13]. Polyphenols such as lignans have antifungal and antioxidant properties. They are studied for pharmaceutical and nutraceutical applications [38,39]. Saponins have many applications in the food and cosmetic industry due to their foaming properties. Their potential applications extend to agriculture in soil remediation and bio-control [10]. Sterols and triterpenes are molecules with biological properties such as antibacterial activities [40–44]. The natural durability of wood could be attributed to them along with other extracts such as polyphenols [44].

### *4.3. Total Polyphenols*

The results presented in Table 3 indicate that the average concentration of polyphenols in the different parts of *Letestua* d*urissima* display significant differences from one solvent to another and from one compartment to another. The total polyphenol contents of the different fractions vary between 95.56 and 47.64 mg GAE $g^{-1}$ of dry extract. The highest concentration of phenols was measured in the acetone extract of bark, with a rate of 95.56 mg GAE $g^{-1}$ of dry extract, compared to the toluene–ethanol and aqueous extracts, where values of 92.4 and 64.06 mg GAE $g^{-1}$ of dry extract were recorded, respectively. This result is explained by the fact that acetone is the first polar solvent used followed by the toluene–ethanol mixture after extraction with dichloromethane, which extracts mainly lipophilic compounds or oils acetone, which extracts phenolic compounds. The bark contains the highest concentrations of polyphenols followed by the heartwood and finally the sapwood. Several studies on tropical woods have shown similar results [8,9]. The contents of polyphenols in the acetone and the toluene/ethanol extracts of the heartwood of *Letestua durissima* (respectively, 51.17 mg gallic acid equivalents/g and 44.54 mg gallic acid equivalents/g) are close to those found by Saha Tchinda for moabi heartwood [9], with reported concentrations of 64.52 mg gallic acid equivalents per gram of the dry extract soluble in acetone compared to 30.87 mg gallic acid equivalents per gram of the dry extract soluble in toluene/ethanol. These results corroborate those obtained for extractives contents and phytochemical screening indicating the presence of polyphenols in the acetone, toluene/ethanol and aqueous fractions.

### *4.4. Total Condensed Tannins*

The results presented in Table 4 reveal that the acetone and toluene/ethanol fractions of the bark *of Letestua durissima* contain the highest concentrations of condensed tannins, with a value of 109.12 and 113.45 mg CE $g^{-1}$ of dry extract, followed by heartwood which has a tannin content 77.45 mg CE $g^{-1}$ of dry extract through acetone extraction, 69.12 mg CE $g^{-1}$ of dry extract through toluene-ethanol extraction and 45.45 mg CE $g^{-1}$ of dry extract through water extraction. The smaller fractions were recorded in the sapwood 61.62, 43.95 and 33.95 mg CE $g^{-1}$ of dry extract (respectively, for acetone, toluene/ethanol and water

extractions). This variation can be explained by the fact that the extraction of condensed tannins depends on their chemical nature and molecular weights, on the solvent used and on the operating conditions. The results of Hillis, regarding the main sources of commercial condensed tannins, indicated that the heartwood, sapwood or bark contents of tannins from different trees found in temperate zones did not exceed 55 mg CE $g^{-1}$ of dry extracts [45]. The concentration of condensed tannins of *Letestua durissima* appears much higher than these previous results from the literature. These results are consistent with the extractives contents, the total phenol contents and the phytochemical screening.

### *4.5. Total Flavonoids*

The results show that the total flavonoid contents vary considerably between the different extracts (bark, sapwood and heartwood). The highest concentrations of flavonoids are found in the bark followed by the heartwood and sapwood. The toluene/ethanol extract contains the highest concentration of flavonoids (20.26 mg CE $g^{-1}$ of dry extracts), followed by the acetone extract (19.01 mg CE $g^{-1}$ of dry extracts), while the lowest concentration of flavonoids was measured in the aqueous extract (1.11 mg CE $g^{-1}$ of dry extract). The concentration of flavonoids in the extracts of *Letestua durissima* depends on the polarity of the solvents used for extraction and on the part of the tree studied. Flavonoid contents in acetone extracts and toluene/ethanol mixture of sapwood of *Letestua durissima* are similar to those found for the sapwood of *Coula edulis* wood [27].

### *4.6. FTIR Analysis*

The FTIR spectra of the various extracts of *Letestua durissima* reveals noticeable differences according to the nature of the extraction solvent, especially for non-polar solvents compared to more polar ones. Low polarity solvents highlight compounds such as fatty acids and terpenes, with characteristic peaks at 2913, 2849 or 1462 $cm^{-1}$, which are characteristic of aliphatic absorptions, and at 1720 $cm^{-1}$, which is characteristic of the carbonyl group. On the other hand, the use of more polar solvents indicated the presence of different compounds in comparison to those observed in dichloromethane independently of the wood types analyzed (bark, sapwood and heartwood). In all cases, the acetone, toluene/ethanol (2/1, *v*/*v*) and aqueous extracts exhibit quite similar FTIR spectra, indicating the presence of phenolic and carbohydrate compounds, as demonstrated by the strong absorption at 3218 $cm^{-1}$ corresponding to the elongation vibration of the OH bond of aromatic and aliphatic structures. The peaks at 1601, 1519 and 1442 $cm^{-1}$ are characteristic of the C=C skeletal vibration of the aromatic ring, while the peak at 1698 $cm^{-1}$ is ascribable to conjugated carbonyl groups present in different low molecular weight phenolic extractives. The broad adsorption around 1045 $cm^{-1}$ was attributed to different C-O in alcohol and ether present in different kinds of wood extractives. All these observations corroborate results already obtained during phytochemical screening and total polyphenol, total tannins and total flavonoids.

### *4.7. GC-MS Analysis*

Chromatograms confirmed what was already observed by FTIR concerning the different natures of extractives obtained by extraction with a low polarity solvent like dichloromethane compared to those obtained with higher polarity solvents. The analysis of the bark extract with dichloromethane by GC-MS (Table 7) indicates the presence of numerous terpenes in the dichloromethane (also present in the acetone extract) with β-Amyrin acetate isomer and β-amyrone as main components, 30% and 18% of the total ion current (TIC). Fatty acids and phenolic compounds (3,4-dihydroxybenzaldehyde, 4-hydroxybenzoic acid, isovanillic acid, protocatechuic acid) appearing at earlier retention times (RT) than terpenes were also detected in bark dichloromethane extract. β-Amyrin acetate as well as other terpenoids like lupeol trimethylsilyl ether and β-amyrone were also identified in sapwood dichloromethane extracts mixed with fatty acids. Other

triterpenes such as Stigmasta-4,22-dien-3-one, Friedelan-3-one, Epilupeol acetate or Betulin and fatty acids have also been identified in the dichloromethane extracts of heartwood. The analysis of bark acetone extract indicated the presence of similar compounds to those identified in dichloromethane belonging to the families of phenolic compounds and terpenoids and some new compounds like sugars or catechin. The β-Amyrin acetate isomer and α-amyrin trimethylsilyl ether were present in high quantities, representing 12% and 35% of the total ion current (TIC). Sapwood acetone extract contained mainly sugars (89% of the TIC), while heartwood acetone extracts indicated the presence of glycerol, protocatechuic acid, gallic acid, stigmasterol and sugars. The GC-MS analysis of toluene/ethanol extracts of bark, sapwood and heartwood indicated the presence of high amounts of sugars. The bark contained small amounts of glycerol, fatty acids, protocatechuic and gallic acids.

### *4.8. Antioxidant Tests*

$IC_{50}$ was determined as the concentration of extracts for which 50% of DPPH free radicals are trapped ($IC_{50}$). These values are obtained from the trend lines for which the linear regression coefficient (R2) of the function Y (the percentage of the inhibition of DPPH) as a function of the concentration of extracts is close to 1.

It appears that the antioxidant activity varies according to the extraction solvent used and to the wood compartment considered. Heartwood extracts exhibited the higher antioxidant activities, followed by bark extracts, with sapwood extracts exhibiting the lowest antioxidant activities. The toluene/ethanol mixture appears to be, in general, slightly more efficient to extract antioxidant compounds in comparison to acetone. These results corroborate GC-MS analysis, indicating more phenolic compounds in the heartwood than in the bark. Some higher molecular mass polyphenols like tannins that were not detected by GC-MS were probably present in toluene/ethanol extracts, which would explain their higher reactivity compared to acetone extracts rich in phenolic compounds of low molecular weight. Water was the least efficient solvent to extract antioxidant compounds. Catechin and BHT (butylated hydroxytoluene), used as reference antioxidant compounds, showed higher antioxidant activity than all the extracts studied. However, the $IC_{50}$ values obtained for the more efficient extracts are not very different from values obtained for these two reference compounds. For the DPPH assay, toluene–ethanol bark extract and acetone heartwood extract presented the lowest $IC_{50}$ values, 2.40 and 2.39, respectively, compared to catechin and BHT with values of 1.95 and 3.31, respectively. For the $ABTS^{+}$ assay, toluene/ethanol bark extract and toluene/ethanol heartwood extract presented the lowest $IC_{50}$ values, 1.39 and 0.66, respectively, compared to catechin and BHT with values of 0.38 and 0.46, respectively. Statistical analysis indicated that radical scavenging activities using DPPH method of toluene/ethanol bark and heartwood were not significantly different, while acetone heartwood activities was slightly more efficient. In the case of the $ABTS^{+}$ method, only the toluene/ethanol heartwood extract presented a significantly radical scavenging activity compared to other extracts. The differences observed between the results of the DPPH and ABTS methods may be explained by the different conditions and used reagents. Similar results have already been reported in the literature [46].

Phytochemical screening highlighted the presence of several families of extractives such as flavonoids, tannins and polyphenols, which are reported in the literature to possess strong antioxidant properties [38,39,47,48]. In addition, GC-MS analysis indicated different phenolic compounds such as catechin, 3,4-dihydroxybenzaldehyde, 4-hydroxybenzoic acid, isovanillic acid or protocatechuic acid, which may be also responsible for antioxidant activity. Phytochemical screening revealed that *Letestua durissima* also contains saponins, alkaloids, sterols and terpenes, which are recognized in the literature as potential sources of antioxidant agents [49].

## 5. Conclusions

The determination of extractives contents indicated that *Letestua durissima* bark contained higher amounts of extractives than sapwood and heartwood. The total extractives content of the bark using solvents of different polarities reached up to 37%. Phytochemical analyses showed the presence of flavonoids, polyphenols and saponins in the extracts of the different wood types obtained with polar solvents such as acetone, toluene/ethanol (2/1, *v*/*v*) and water, while terpenes and terpenoids were detected in solvent of lower polarity such as dichloromethane. Alkaloids were detected in all extracts regardless of the wood compartment. The total contents of polyphenols, condensed tannins and flavonoids confirmed high amounts of compounds detected in bark and heartwood extracts from different wood types obtained using polar solvents such as acetone and toluene/ethanol. GC-MS analysis of the different extracts showed that the dichloromethane and acetone bark extracts contained terpenes and terpenoids, with β-Amyrin acetate and *α*-Amyrin, trimethylsilyl ether as the main components. The presence of fatty acids, polyphenols and phenolic acids was detected in bark and heartwood extracts obtained with higher polarity solvents. The measurement of antioxidant activities using ABTS+ or DPPH radical scavenging activity indicated that acetone or toluene/ethanol heartwood and bark extracts had the lowest IC 50 values. Toluene–ethanol bark extract and acetone heartwood extract exhibited quite similar $IC_{50}$ values of 2.40 and 2.39, respectively, for the DPPH assay, while catechin and BHT exhibited values of 1.95 and 3.31, respectively. Toluene/ethanol heartwood extract displayed the lowest $IC_{50}$ values of 0.66 for the ABTS+ assay in comparison to values of 0.38 and 0.46 for catechin and BHT, respectively. A comparison with the antioxidant activities of catechin or BHT indicated that the toluene/ethanol heartwood extract presented antioxidant activities more or less similar to those of these two reference compounds, suggesting a potential valorization as an antioxidant. Structure–activity relationships attribute the antioxidant properties of these extracts to the presence of phenolic compounds contained in the heartwood and bark extracts. Additional studies are necessary to assess the potential for the valorization of tetracyclic and pentacyclic triterpenes present in the bark of *Letestua durissima*.

**Author Contributions:** Conceptualization, P.G. and P.E.E.; methodology, L.-F.E.N.; software, S.D.; validation, P.G., P.E.E. and S.D.; formal analysis, P.G., S.D., L.-F.E.N. and M.S.M.M.; investigation, L.-F.E.N. and M.S.M.M.; writing—original draft preparation, L.-F.E.N.; writing—review and editing, P.G., S.D. and L.-F.E.N.; supervision, P.G., S.D. and P.E.E. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Data Availability Statement:** The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

**Acknowledgments:** LERMaB is supported by a grant overseen by the French National Research Agency (ANR) as part of the "Investissements d'Avenir" program (ANR-11-LABX-0002-01; Laboratory of Excellence ARBRE). The authors thank the National Agency of Grants and Internship of Gabon for the Ph.D. grant of the first author Ley-Fleury Ella Nkogo.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. Gérard, J.; Guibal, D.; Paradis, S.; Cerre, J.C. *Atlas des Bois Tropicaux*, *Caractéristiques Technologiques et Utilisations*; Quae Editions: Versailles, France, 2016; pp. 221–223.
2. Nze Nguema, S. Presentation of the Forestry Sector in Gabon: Report on the Evolution of the Implementation of Government policy in the Forests, Fishing and Aquaculture, Protected Areas and Training Sectors. 2010. Available online: https://pdfslide.tips/documents/prsentation-du-secteur-forestier-au-gabon.html (accessed on 25 January 2024).
3. Schultz, T.P.; Nicholas, D.D. Naturally durable heartwood: Evidence for a proposed dual defensive function of the extractives. *Phytochemistry* **2002**, *54*, 47–52.
4. Eyi Obame, A.P. Niveau de Valorisation des Déchets D'exploitation Forestière: Identification et Estimation des Rebuts de Bois Dans l'AAC 2014 de la *SEEF* à Nzamaligue. Master's Thesis, Ecole Nationale des Eaux et Forêts, Libreville, Gabon, 2017.

5. Isman, M.B.; Machial, C.M. Pesticides based on plant essential oils: From traditional practice to commercialization. In *Naturally Occurring Bioactive Compounds*; Rai, M., Carpinella, M.C., Eds.; Elsevier: Amsterdam, The Netherlands, 2006; pp. 29–44.
6. Verma, M.; Sharma, S.; Prasad, R. Biological alternative for termite control: A review. *Int. Biodeterior. Biodegrad*. **2009**, *63*, 959–972.
7. Vek, V.; Keržič, E.; Poljanšek, I.; Eklund, P.; Humar, M.; Oven, P. Wood Extractives of Silver Fir and Their Antioxidant and Antifungal Properties. *Molecules* **2021**, *26*, 6412.
8. Mounguengui, W.S. Caractérisation HPLC de Marqueurs Permettant de Prévoir L'évolution de Certaines Propriétés Macroscopiques du Bois Lors de Différents Processus de Dégradation. Ph.D. Thesis, Université Henri Poincaré, Nancy, France, 2008.
9. Saha Tchinda, J.B. Characterization and Valorization of Extractable Substances from Five Major Cameroonian Species in the Wood Industry: Ayous, Moabi, Movingui, Padouk and Tali. Ph.D. Thesis, Université de Lorraine, Nancy, France, 2015.
10. Pizzi, A. Tannins: Prospectives and Actual Industrial Applications. *Biomolecules* **2019**, *9*, 344.
11. Houghton, P.; Raman, A. Laboratory Handbook for the Fractionation of Natural Extracts; Springer: New York, NY, USA, 1998.
12. Akinjogunla, O.J.; Yah, C.S.; Eghafona, N.O.; Ogbemudia, F.O. Antibacterial activity of leave extracts of *Nymphaea lotus* (Nymphaeaceae) on Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Staphylococcus aureus* (VRSA) isolated from clinical samples. *Ann. Biol. Res*. **2010**, *1*, 174–184.
13. Badiaga, M. Etude Ethnobotanique, Phytochimique et Activités Biologiques de *Nauclealatifolia Smith*, une Plante Médicinale Africaine Récoltée au Mali. Ph.D. Thesis, Université Blaise-Pascal, Clermont Ferrand, France, 2011.
14. Satyan, R.S.; Aveek, N.; Eganathan, P.; Parida, A. Comparative histochemical localization of secondary metabolites in seed-raised and in vitro propagated plants of *Excoecaria agallocha* Linn. (Euphorbiaceae), the milky mangrove tree of historical significance. *Biotech. Histochem.* **2010**, *85*, 285–293.
15. Medza M'Ella, D.L.; Ép Mezui-Mbeng, M.A.; N'Negue; Edou Engonga, P.; Mengome, L.E.; Obiang Engone, N.; Aboughe-Angone, S. Phytochemical study and anti-radical activity of extracts from the oil seeds of *Desbordesia glaucescens* (Engl.) Tiegh. from Gabon. *J. Med. Plants Res.* **2022**, *16*, 306–314.
16. Jamaludin Malik, J.; Santoso, A.; Mulyana, Y.; Ozarska, B. Characterization of Merbau Extractives as a Potential Wood-Impregnating Material. *BioResources* **2016**, *11*, 7737–7753.
17. Bentabet Lasgaa, N. Etude Phytochimique et Evaluation des Activités Biologiques de Deux Plantes *Fredoleiareteoides* et *Echmimu valgare* de L'ouest Algérien. Ph.D. Thesis, University of Abou Bakr Belkaid, Tlemcen, Algeria, 2015.
18. Hamid, E.; Moncef, B.; Assia, B.; Hind, T.; Rachid, B. Screening phytochimique d'une plante médicinale: *Mentha spicata* L. *Am. J. Innov. Res. Appl. Sci.* **2018**, *7*, 226–233.
19. Doloking, H.; Mukhriani; Ningsi, S.; Tahar, N. Flavonoids: A review on extraction, identification, quantification, and antioxidant activity. *Ad-Dawaa J. Pharm. Sci.* **2022**, *5*, 26–51.
20. Wootton-Beard, P.C.; Aisling, M.; Ryan, L. Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after in vitro digestion measured by FRAP, DPPH, ABTS and Folin–Ciocalteu methods. *Food Res. Int.* **2011**, *44*, 217–224.
21. Chamorro, S.; Viveros, A.; Alvarez, I.; Vega, E.; Brenes, A. Changes in polyphenol and polysaccharide content of grape seed extract and grape pomace after enzymatic treatment. *Food Chem.* **2012**, *133*, 308–314.
22. Shraim, A.M.; Ahmed, T.A.; Rahman, M.M.; Hijji, Y.M. Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *LWT Food Sci. Technol.* **2021**, *150*, 111932.
23. Anokwuru, C.; Sigidi, M.; Zininga, T.; Tshisikhawe, M.; Shonhai, A.; Ramaite, I.; Traoré, A.; Potgieter, N. Phenolic contents, antioxidant activity and spectroscopic characteristics of *Pterocarpus angolensis* DC. stem bark fractions. *Indian J. Tradit. Knowl.* **2017**, *16*, 400–406.
24. Piccolella, S.; Fiorentino, A.; Pacifico, S.; D'Abrosca, B.; Uzzo, P.; Monaco, P. Antioxidant properties of sour cherries (*Prunus cerasus* L.): Role of colorless phytochemicals from the methanolic extract of ripe fruits. *J. Agric. Food Chem*. **2008**, *56*, 1928–1935.
25. Brennan, M.; Fritsch, C.; Cosgun, S.; Dumarcay, S.; Colin, F.; Gérardin, P. Yield and compositions of bark phenolic extractives from three commercially significant softwoods show intra- and inter-specific variation. *Plant Physiol. Biochem*. **2020**, *155*, 346–356.
26. Pohjamo, S.P.; Hemming, J.E.; Willför, S.M.; Reunanen, M.HT.; Holmbom, B.R. Phenolic Extractives in *Salix Caprea* Wood and Knots. *Phytochemistry* **2003**, *63*, 165–169.
27. Vek, V.; Šmidovnik, T.; Humar, M.; Poljanšek, I.; Oven, P. Comparison of the Content of Extractives in the Bark of the Trunk and the Bark of the Branches of Silver Fir ( *Abies alba* Mill.). *Molecules* **2023**, *28*, 225.
28. Dedrie, M.; Jacquet, N.; Bombeck, P.L.; Hébert, J.; Richel, A. Oak barks as raw materials for the extraction of polyphenols for the chemical and pharmaceutical sectors: A regional case study. *Ind. Crops Prod*. **2015**, *70*, 316–321.
29. Bopenga Bopenga, C.S.A.; Meyo Degboevi, H.; Candelier, K.; Edou Engonga, P.; Dumarcay, S.; Thévenon, M.F.; Gérardin Charbonnier, C.; Gérardin, P. Characterization of Extracts from the Bark of the Gabon Hazel Tree (*Coula edulis Baill*) for Antioxidant, Antifungal and Anti-Termite Products. *J. Renew. Mat*. **2021**, *1*, 17–33.
30. Brocco, V.F.; Paes, J.B.; Gonçalves da Costa, L.; Brazolin, S.; Chaves Arantes, M.D. Potential of teak heartwood extracts as a natural wood preservative. *J. Clean. Prod*. **2017**, 142, 2093e2099.
31. Sirmah, P.; Dumarçay, S.; Gérardin, P. Effect of Unusual amount of (-)-mesquitol of from the heartwood of *Prosopis juliflora*. *Nat. Prod. Res.* **2009**, *23*, 183–189.
32. Mounguengui, W.S.; Saha Tchinda, J.B.; Kor Ndikontar, M.; Dumarçay, S.; Attéké, C.; Perrin, D.; Gelhaye, E.; Gérardin, P. Total phenolic and lignin contents, phytochemical screening, antioxidant and fungal inhibition properties of the heartwood extractives of ten Congo Basin tree species. *Ann. For. Sci.* **2016**, *73*, 287–296.

33. Fengel, D.; Wegener, G. *Wood Chemistry, Ultrastructure, Reactions*; Walter de Gruyter: Berlin, Germany; New York, NY, USA, 1984; pp. 27–65.
34. Arima, H.; Ashida, H.; Danno, G. Rutin-enhanced Antibacterial Activities of Flavonoids against *Bacillus cereus* and *Salmonella enteritidis*. *Biosci. Biotechnol. Biochem*. **2002**, *66*, 1009–1014.
35. Harborne, J.B.; Williams, C.A. Advances in Flavonoid Research. *Phytochemistry* **2000**, *55*, 481–504.
36. Pietta, P.G. Flavonoïdes comme antioxydants. *J. Prod. Nat.* **2000**, *63*, 1035–1042.
37. Silvestrini, A.; Pasqua, G.; Botta, B.; Monacelli, B.; Van der Heijden, R.; Verpoorte, R. Effects of alkaloid precursor feeding on a *Camptotheca acuminate* cell line. *Plant Physiol. Biochem*. **2002**, *40*, 749–753.
38. Eklund, P.C.; Långvik, O.K.; Wärnå, J.P.; Salmi, T.O.; Willför, S.M.; Sjöholm, R.E. Chemical studies on antioxidant mechanisms and free radical scavenging properties of lignans. *Org. Biomol. Chem*. **2005**, *3*, 3336–3347.
39. Gérardin, P.; Hentges, D.; Gérardin, P.; Vinchelin, P.; Dumarçay, S.; Audoin, C.; Gérardin-Charbonnier, C. Knotwood and branchwood polyphenolic extractives of silver fir, spruce and douglas fir and their antioxidant, antifungal and antibacterial properties. *Molecules* **2023**, *28*, 6391.
40. Lee, Y.S.; Shin, K.H.; Kim, B.K.; Lee, S. Anti-diabetic activities of fucosterol from *Pelvetia siliquosa*. *Arch. Pharm. Res*. **2004**, *27*, 1120–1122.
41. Murkies, A.L.; Wilcox, G.; Davis, S. Phytoestrogens. *J. Clin. Endocrinol. Metab*. **1998**, *83*, 297–303.
42. Hooper, L.; Aedin, C. A Review of the Health Care Potential of Bioactive Compounds. *J. Sci. Food Agric.* **2006**, *86*, 1805–1813.
43. Dzubak, P.; Hajduch, M.; Vydra, D.; Hustova, A.; Kvasnica, M.; Biedermann, D.; Markova, L.; Urba, M.; Sarek, J. Pharmacological activities of natural triterpenoids and their therapeutic implications. *Nat. Prod. Rep.* **2006**, *23*, 394–411.
44. Huang, Z.; Hashadi, K.; Makino, R.; Kawamura, F.; Kuniyoshi, S.; Ryuichiro, K.; Ohara, S. Evaluation of biological activities of extracts from 22 African tropical wood. *J. Wood Sci.* **2009**, *55*, 225–229.
45. Hillis, W.E. Wood properties and uses. In *Black Wattle and Its Utilisation*; Brown, A.G., Ho, C.K., Eds.; RIRDC Publication: Canberra, Australia, 1997; pp. 89–93.
46. Sillero, L.; Prado, R.; Labibi, J. Simultaneous microwave-ultrasound assisted extraction of bioactive compounds from bark. *Chem. Eng. Process.* **2020**, *156*, 108100.
47. Treml, J.; Šmejkal, K. Flavonoids as potent scavengers of hydroxyl radicals, comprehensive reviews. *Food Sci. Food Saf*. **2016**, *15*, 720–738.
48. Kawamura, F.; Ramle, S.; Sulaiman, O.; Hashim, R.; Ohara, S. Antioxidant and antifungal activities of extracts from 15 selected hardwood species of Malaysian timber. *Eur. J. Wood Prod.* **2011**, *69*, 207–221.
49. Burlacu, E.; Nisca, A.; Tanase, C. A comprehensive review of phytochemistry and biological activities of Quercus species. *Forests* **2020**, *11*, 0904.