The role of extractives in the natural durability of the heartwood of Dicorynia guianensis Amsh: new insights in antioxydant and antifungal properties

Jean-Baptiste Say Anouhe, Florence Bobelé Niamké, Milcard Faustin, David Null Virieux, Jean-Luc Pirat, Augustin Amissa Adima, Seraphin Kati-Coulibaly, Nadine Amusant

Annals of Forest Science (2018) 75: 15
https://doi.org/10.1007/s13595-018-0691-0

ORIGINAL PAPER

The role of extractives in the natural durability of the heartwood of *Dicorynia guianensis* Amsh: new insights in antioxydant and antifungal properties

Jean-Baptiste Say Anouhe[1] · Florence Bobelé Niamké[2] · Milcard Faustin[3] · David Virieux[4] · Jean-Luc Pirat[4] · Augustin Amissa Adima[2] · Seraphin Kati-Coulibaly[1] · Nadine Amusant[3]

Received: 13 July 2017 / Accepted: 9 January 2018 / Published online: 6 February 2018

Abstract

• ***Key message*** **The natural durability of *Dicorynia guianensis Amsh*'s Heartwood is conferred by the high content of antioxidant phenolic compounds, especially tannins and flavonoids combined with the presence of fungistatic alkaloids. The content of phenolic compounds increases according to the natural durability classes, from durable wood (class 2) to moderately durable wood (class 3) and correlated to the antioxidant capacity.**

• ***Context*** The heartwood of *Dicorynia guianensis* Amsh is resistant to white rot fungi decay, but the mechanism of this natural durability is not fully elucidated.

• ***Aims*** Biochemical studies were carried out in order to better understand the role of extractives in natural durability of *D. guianensis*.

• ***Methods*** The powders from durable and moderately durable heartwood were extracted with methanol, ethanol, and hot water. The quantity of total phenols, tannins, and flavonoids as well as antioxidant activity, evaluated by 2,2′-azino-bis-(3-ethylbenzo-thiazoline-6-sulfonic acid) were determined using colorimetric methods. Antifungal activity was assessed by using two white rot fungi. The bioactive fractions and compounds were obtained using bio-guided fractionation, HPLC isolation, MS and RMN spectroscopic analyses.

• ***Results*** Durable woods contain higher amounts of heartwood extract and antioxidant activity. Antioxidant activity was highly correlated with the content of phenolics. The purification of the most antioxidant fraction FII affords the characterization of (+)-

Handling Editor: Jean-Michel Leban

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13595-018-0691-0) contains supplementary material, which is available to authorized users.

✉ Florence Bobelé Niamké
bobele.niamke@inphb.ci; bobeleflo@yahoo.fr

Jean-Baptiste Say Anouhe
janouhes@gmail.com; janouhes@yahoo.fr

Milcard Faustin
glicha152@yahoo.fr

David Virieux
david.virieux@enscm.fr

Jean-Luc Pirat
jean-luc.pirat@enscm.fr

Augustin Amissa Adima
amissaaug@gmail.com

Seraphin Kati-Coulibaly
katicoulibalys@yahoo.fr

Nadine Amusant
nadine.amusant@cirad.fr

[1] Laboratoire de Nutrition et Pharmacologie, UFR Biosciences, Université Felix Houphouët Boigny de Cocody-Abidjan, 22, BP 582, Abidjan 22, Côte d'Ivoire

[2] LAPISEN, Groupe de recherche en Chimie des Eaux et des Substances Naturelles, Institut National Polytechnique Houphouët Boigny, BP 1313, Yamoussoukro, Côte d'Ivoire

[3] CIRAD, UMR EcoFoG, AgroParisTech, CNRS, INRA, CIRAD, Université des Antilles, Université de Guyane, 97300 Cayenne, France

[4] AM2N, Institut Charles Gerhardt, UMR 5253, ENSCM 8 rue de l'Ecole Normale, 34296 Montpellier, France

catechin (−)-epicatechin, neoastilbin, astilbin, and isoastilbin. Alkaloid fraction FIII exhibits dose-dependent fungistatic activity against *Pycnoporus sanguineus* Linnaeus and *Trametes versicolor* Quelet.
• ***Conclusion*** Phenolic antioxidants and fungistatic alkaloids positively impact the natural durability of *D. guianensis*.

Keywords *Dicorynia guianensis* · Heartwood · Natural durability · Antifungal · Antioxidant · Phenols · Alkaloid

1 Introduction

Trees are exposed to various biotic constraints (fungi, virus, bacteria, and vertebrae) and other harsh environmental conditions, which induce oxidative stress. Plants build a defense against stress or disease through physical and chemical barriers. They protect themselves from oxidative effects by enhanced synthesis of some secondary metabolites. Secondary metabolites played a key role in plant growth, development, and defense against infections or injuries (Benhamou and Rey 2012). In heartwood, various bioactive compounds have been discovered, and they are mainly composed of terpenoids (Dickson et al. 2007), alkaloids (Lee et al. 2009), and phenolic compounds such as stilbenes and flavonoids (Nascimento et al. 2013). Extractives act as either antifungal compounds or antioxidants (Salido et al. 2015**)**. However, the biological activities of the extracts of many tropical woody species have not been fully investigated.

The Guiana shield is one of the largest continuous areas of lowland tropical rainforest in the world. *Dicorynia guianensis* Amshoff (Fabaceae) is abundant in eastern Surinam and western French Guiana where it may make up 10% of the forest stands (Paradis et al. 2011). *D. guianensis* is well known for its high and inherent natural durability. Its heartwood is classified from very durable to moderately durable when challenged by white rot fungi (Amusant et al. 2004). This species is the most exploited in French Guiana's timber market and represents more than the half of the 73,000 annual cubic meters of local wood production (IEDOM 2014). Wood production's activities generate large amounts of biomass residues (sawdust, wood chips, and barks) which are left in the field or burned for energy production (Berlioz 2012). Recent studies have reported the antiradical capability of alkaloids fractions from its heartwood (Anouhe et al. 2015).

Several studies showed that pure or aqueous mixtures of methanol and ethanol were used for the extraction of bioactive compounds such as phenolic compounds, from the plant matrix (Lolita et al. 2012).

We tested that durable heartwood would contain more extractives (phenolics, flavonoids, tannins) and would exhibit high radical scavenging activity than less durable heartwood. Therefore, the objective of this study was to investigate the extractives obtained from the heartwood of four trees of *D. guianensis* with varying durability classes by using different solvents and measuring the extractive yields, phenolic compound content, and ABTS radical scavenging activities of durable and moderate-durable heartwood. Subsequently, the antifungal activity of heartwood extracts was performed against white rot fungi, and some antioxidant compounds were characterized.

2 Materials and methods

2.1 Materials

A total of four trees of *Dicorynia guianensis* Amsh were harvested in February 2012 from Paracou experimental forest station (5° 15′ N, 52° 55′ W) in French Guyana. Two chips of 50 cm of heartwood from each tree were obtained at 1.3 m from the tree base and stored in freezer until use. One was used for natural durability tests and the second for chemical analyses (Amusant et al. 2013). Heartwood natural durability classes are previously assessed using a soil block test by measuring the absolute mass losses (x %) for individual test. They are regrouped in two classes as recommended by NF EN 350-2 (Afnor 2016); durable (class 2) and moderately durable (class 3) against soil microflora. For durable wood, $5 < x < 10$ and for moderately durable wood $10 < x < 15$. After 72 h, chips are grounded to obtain a fine and fresh powder (less than 0.25 mm) using a crusher type Reich SM 100 (Eragny-sur-Oise, France) and stored at − 10 °C for further analyses.

For the antifungal assay, two white rot fungi, *Pycnoporus sanguineus* (Linnaeus ex Fries) and *Trametes versicolor*, Quelet (CYB 863-A), provided by the Laboratory of preservation of UR BioWoEb of CIRAD Montpellier (France), are tested in malt extract agar media (MEA).

2.2 Extraction procedure

The extractions are carried out using methanol and ethanol from Sigma-Aldrich (Steinrhein, Germany) and hot distilled water (100 °C). The wood powders (250 g) are extracted with 1 L of solvent according to the methods described by Royer et al. (2013) with some modifications. Aqueous extracts were obtained under reflux for 1 h. After the filtration on a Buchner funnel, the aqueous filtrate was freeze-dried on Bioblock Scientific lyophilizer (Kirch, France). The two different organic extracts were obtained at a room temperature of 32 °C within 24 h with an orbital shaker (Lab-Line model 3520) of

250 rpm. The solvent was removed by using a rotavapor (Flawil, Switzerland) for the evaporation at 40 °C, and the dry extracts are weighed. The experiment is carried out three times for each type of heartwood powder. The extraction yield is expressed as a percentage of the wood dry weight.

2.3 Determination of total phenols, tannins, and flavonoids

2.3.1 Total phenols

The total phenol contents are determined in *D. guianensis*'s heartwood using Folin-Ciocalteu's reagent by the method of Singleton et al. (1999) following the procedure described by Luís et al. (2014). This non-specific method for the quantification of phenolics was used as the content of non-phenolic compounds was negligible (data not shown). Gallic acid (Sigma-Aldrich, Steinrhein, Germany) is used as the standard. Fifty microliters of samples at 5 mg/mL concentration are mixed up with 450 μL of distilled water, 2.5 mL of a 0.2 N Folin-Ciocalteu's reagent from Fluka (Buchs, Swiss), and 2 mL saturated sodium carbonate solution (75 g L^{-1}). The final mixture is heated at 50 °C for 10 min, and the absorbance was later read at 765 nm compared to a blank (solution with only the solvent used for the dissolution of other extracts) using a UV-visible spectrophotometer Jasco V-530 pro (Tokyo, Japan). The standard curve was prepared using 750, 500, 250, 125, and 62.5 mg/L solutions of gallic acid in methanol ($y = 0.0010x + 0.0216$; $R^2 = 0.9947$). The calibration curve of absorbance vs. concentration of standard was used to quantify total phenol content. For each 103 heartwood extracts, the assay was carried out in triplicate. Results were expressed as milligram gallic acid equivalent per gram of dry extract (mg GAE g^{-1} of dry extract).

2.3.2 Flavonoids

Flavonoid content in various extracts of heartwood was assessed using the aluminum chloride method (Luís et al. 2014). Five hundred microliters of each solution of the extract was mixed separately with 1.5 mL of methanol, 100 μL of 10% aluminium chloride (Merck, Darmstadt, Germany) solutions, 100 μL of 1 M potassium acetate (Merck, Darmstadt, Germany), and 2.8 mL of distilled water (Milli-Q TGI water system, USA). After incubation at room temperature for 30 min, the absorbance was measured at 415 nm with a spectrophotometer UV-Vis – Jasco V-550 pro (Tokyo, Japan). The standard curve was prepared using 37.5, 75, 125, and 200 mg L^{-1} solutions of quercetin from Sigma (St. Louis, MO, USA). The flavonoid content values are determined using an equation obtained from the calibration curve of quercetin graph ($y = 0.0035x + 0.0014$; $R^2 = 0.9997$). The flavonoid content of each extract was expressed in mg quercetin equivalent per gram of dry extract (mg QE g^{-1} of dry extract). Each sample was carried out in triplicate.

2.4 Determination of biological activities

2.4.1 Determination of antioxidant activity

The antioxidant activity is determined using the radical $ABTS^{+\cdot}$ scavenging activity method of Re et al. (1999) as described by Gülçin et al. (2010) with some modifications. The $ABTS^{+}$ was produced by reacting 2 mM ABTS in water with 2.45 mM potassium persulfate from Merck (Darmstadt, Germany), stored in the dark at room temperature for 16 h. The $ABTS^{+}$ solution is diluted to give an absorbance of 0.70 ± 0.02 at 734 nm in 0.1 M sodium phosphate buffer (pH 7.4). Extracts at different concentrations are dissolved in methanol (100 μL) and mixed up with 3900 μL of $ABTS^{+\cdot}$ methanol solution. The absorbance is recorded 30 min after the mixing, and the percentage of radical scavenging is calculated for each concentration compared to a blank, lacking scavenger. The pure methanol (100 μL) is used as the control for this experiment. Ascorbic acid from Scharlau Chemie (Barcelona, Spain) is used as positive control. The assay is carried out in triplicate, and the percentage of inhibition is calculated using the following equation:

$$(\%I) = [(A_0 - A)/A_0] \times 100 \quad (1)$$

where A_0 is the absorbance of the blank and A is the absorbance of samples.

The concentration providing 50% of inhibition (IC_{50}) is determined from the plot of inhibition percentage against the concentration of samples. Extracts activities are compared to positive references such as the activity of ascorbic acid from Scharlau Chemie SA (Barcelona, Spain).

2.4.2 Determination of antifungal activity

The antifungal efficiency of the methanolic extracts and fractions was tested with two white rot fungi: *Pycnoporus sanguineus* (Linnaeus ex Fries) and *Trametes versicolor*, Quelet (CYB 863-A) according to the method developed by Niamké et al. (2012). The strains are maintained by periodic subculture on malt-agar culture media and preserved at 4 °C until their use. Liquid (40 g of malt and 1000 mL of pure water) and solid (40 g malt, 20 g of agar and 1000 mL of pure water) media are used after their sterilization in an autoclave at 121 °C for 25 min and a pressure of 15 psi. Antifungal activity is determined by using the broth microdilution technique in 96 U-bottomed wells (250 μL) sterile microplates Elisa type from Corning Inc., (New York, USA). Well working volume of 100 μL is used.

Stock solutions of extractives are prepared by dissolving 5 mg of extractives in 1 mL of methanol–water (1:1) (*v*/*v*). The test extracts and fractions are added in serial double dilution in medium from stock solution. A volume of 20 μL of each solution is mixed up with 80 μL of sterilized malt extract in wells. A final concentration of extract on malt mixture of 62.5, 250, and 1000 μg mL^{-1} are obtained. Then, 1 mm of diameter of the mycelium of each fungal colony was aseptically transferred into the wells. The commercial biocide tebuconazole [(3-R/S)-1-(4-chlorophenyl)-4, 4-dimethyl-3-(1H-1,2,4-triazolylmethyl)-pentane-3-ol] from Sigma-Aldrich (Steinrhein, Germany) at concentration of 0.43 μg mL^{-1} is used as a positive control and water as a negative control. Each test is repeated three times for each extract and for each fungus. The microplates are incubated at 21 °C and 70% humidity for *Trametes versicolor* and 30 °C for *Pycnoporus sanguineus* in an enclosure at 70% humidity in the dark.

After 72 h, inoculums are subsequently removed from microplates and transferred on petri dishes (55 cm, Greiner, France) that were previously filled with a malt-agar culture medium. When the fungi radial growth reaches the border of the negative control's plate, the antifungal index (AI%) was calculated over negative control on which water was used. The fungal toxicity, expressed as the inhibition activity on the mycelial growth, is calculated versus the negative control. The fungal growth inhibition expressed by antifungal index (AI) was determined as follows:

$$Antifungal\ index\ (AI\%) = (1 - Da\ Db) \times 100 \quad (2)$$

where *Da* is the diameter of the growth zone in the experimental dish (cm) and *Db* is the diameter of the growth zone in the control dish. The processing of radial growth measurement data was done with free software ImageJ version 1.43 m Java 1.6.0_14 of Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, MD, USA, https://imagej.nih.gov/ij/, 1997–2016.

2.5 Fractionation of methanolic extract and characterization of compounds

2.5.1 Fractionation

One hundred grams of methanolic extract was successively fractioned using the n-hexane, chloroform (pH 5, pH 9, and pH 12), and ethyl acetate (Sigma-Aldrich, Steinrhein, Germany) at pH 7. Solvents were obtained from Sigma-Aldrich (Steinrhein, Germany). The different filtrates were evaporated on Rotavapor® (Flawil, Switzerland) under vacuum at 40 °C and resulted respectively to fraction FI, fraction FII, fraction FIII, fraction FIV, and fraction FV. The residual water fraction is lyophilized to give fraction FVI (Anouhe et al. 2015). An amount of 3100 mg of fraction FII is chromatographed over a column of silica gel 60 (Merck, 230–400 mesh). Elution is performed by chloroform (Sigma-Aldrich, Steinrhein, Germany) containing increasing amounts of methanol until 30% (*v*/*v*). Analytical thin-layer chromatography (TLC) on silica gel F254 plates (Merck, Germany) of the fractions were used to identify similar fractions and yield to two subfractions; FII-A1 (1225 mg) and FII-A2 (1152 mg).

2.5.2 Experimental procedure for NMR and MS characterization

The main isolated compounds are characterized by mass spectrometry and nuclear magnetic resonance spectroscopy. Liquid chromatography-quadrupole-time of flight mass spectrometry (LC-Q-TOF-MS) has been applied by using micromass Q-TOF III spectrometer of Waters (Manchester, UK) to determine each isolated compound mass. NMR spectroscopy analyses are performed in methanol-d4 from Sigma-Aldrich (Steinrhein, Germany) in Advance 600 and 400 MHz spectrometer of Bruker (Rheinstteten, Germany). Chemical shifts (δ) are expressed in ppm, and the multiplicities are given as singlet (s), doublet (d), triplets (t), multiplets (m), and coupling constants are reported as ^{n}J value in Hertz (Hz).

2.6 Statistical analysis

The XLSTAT 7.1 software package (Paris, France) is used, and results are presented as main values ± standard deviation. The non-parametric Kruskal-Wallis and Mann-Whitney tests are used to compare the radical scavenging activities and phytochemical between durable and moderately durable wood. The Spearman correlation test is performed between the IC_{50}, total phenolic, tannins, and flavonoid content. Values are statistically significant at $\rho < 0.05$.

3 Results

3.1 Extraction yield, total phenols, tannins, and flavonoid content of extracts

The extraction yield of heartwood of *D. guianensis* using methanol, ethanol, and hot water, total phenol, tannins, and flavonoid content as well as antioxidant activity are presented in Table 1. The durable heartwood had more extractives, larger total phenol, tannins, and flavonoid content than the moderately durable heartwood. The average of extractive contents varied from 3.00 to 5.91%, and there is a significant difference between the two classes of durability ($\rho < 0.05$) except in ethanol extracts ($\rho > 0.05$). The total phenol contents varied between 333.9 and 537.25 eq. gallic acid per gram of extract.

Table 1 Extraction yields (%), total phenols, tannins, and flavonoid content and concentrations that inhibited 50% of ABTS free radical (IC_{50}) of *Dicorynia guianensis* according to solvents of extraction. Different letters within the column of a line indicate significant differences between extraction solvents ($\rho < 0.05$). Values are means ± standard deviation, $N = 3$

	Solvents of extraction			
	Decay resistance	Methanol	Ethanol	Hot water
Extraction yields (%)	Moderate	3.40 ± 0.09a	3.00 ± 0.04a	4.44 ± 0.02a
	Durable	4.99 ± 0.05b	3.93 ± 0.05a	5.91 ± 0.05b
Total phenols (mg EGA g^{-1})	Moderate	474.98 ± 16.04a	396.07 ± 13.88a	333,39 ± 16,40a
	Durable	537.25 ± 20.67b	440.62 ± 10.97b	389.31 ± 12.87b
Tannins (mg EGA g^{-1})	Moderate	120.70 ± 6.48a	84.30 ± 6.28a	114.96 ± 5.78a
	Durable	159.48 ± 23.60b	116,55 ± 15,85b	136.23 ± 17.38b
Flavonoids (mg EQ g^{-1})	Moderate	39.16 ± 3.91a	32,16 ± 4,16a	22.66 ± 2.86a
	Durable	55.42 ± 5.53b	57,39 ± 5,40b	32.70 ± 1.71b
IC_{50} (mg L^{-1})	Moderate	4.73 ± 0.21a	5.52 ± 0.11a	8.64 ± 0.59a
	Durable	3.43 ± 0.13b	4.66 ± 0.20b	5.45 ± 0.12b

Methanolic and ethanolic extracts content gave higher total phenol than hot water extracts. The tannin content ranges from 84.30 to 159.48 mg eq. gallic acid per gram of extract. Hot water and methanol provide a higher content of tannins and extractives, compared to ethanol. Flavonoid contents of extracts are between 22.66 and 57.39 mg QE g^{-1}. Methanol and ethanol provide extract in average higher amounts of flavonoids.

3.2 Antioxidant activity

The IC_{50} of heartwood extracts varied from 3.43 to 8.64 μg mL^{-1}, and the IC_{50} depend on the class of durability and the solvent of extraction (Table 1). The IC_{50} of extractives from durable heartwood was lower than those of moderate-durable wood. Methanol provides extracts with lowest IC_{50}. The Spearman's rank correlation coefficients between radical scavenging activity expressed by IC_{50} and phenolic compound content from the different extraction solvents are presented in Table 2. Regardless of the solvent, the total phenolic content in extractives is highly correlated with radical scavenging ($-0.96 < R < -0.92$, $\rho < 0.05$). We also found a high coefficient of correlation for tannins ($-0.95 < R < -0.86$, $\rho < 0.05$) and flavonoids ($-0.93 < R < -0.90$, $\rho < 0.05$).

3.3 Structural identification of flavonoid-type compounds from antioxidant fractions

We investigated the radical scavenging inhibitory activity of six fractions obtained from initial methanol extract of heartwood (Fig. 1). Fractions FI and fraction FVI above mentioned had IC_{50} in 20 μg mL^{-1} whereas fractions FV and fraction FIV had IC_{50} between 11 and 12 μg mL^{-1}. Fraction FII and FIII had the lowest IC_{50}, respectively, at 1.93 and 2.47 μg mL^{-1}. The most active, fraction FII, was purified in two subfraction FII-A and FII-B and led to the isolation of four compounds. The compounds **1** and **2** were obtained from FII-A whereas compounds **3** and **4** were obtained from FII-B. The mass spectrum analysis of compound **1** and compound **2** showed the presence of a positive molecular ion $[M+H]^+$ at $m/z = 291.0870$ that suggests the empirical formula of $C_{15}H_{13}O_6$. Compound **1** and compound **2** have the maxima of absorption at 280 nm with a respective [α]D 20 °C in methanol of + 18.01 (c = 0.06) and − 19.1 (c = 0.05). The ^{13}C and ^{1}H-NMR characteristics of compounds **1** and compounds **2** are described in Table 3.

The compound **3** and compound **4** have UV maximum of absorption at 300 nm. Their molecular formula is established as $C_{21}H_{23}O_{11}$ by mass analysis at $m/z = 451.1234$ $[M+H]^+$. The ^{1}H-NMR chemical shift of compound **4** shows a pure stereoisomer whereas compound **3** consists in a mixture of two stereoisomers: **4a** astilbin and **4b** isoastilbin (Table 4). The chemical structures of isolated compounds are presented in Fig. 2.

3.4 Antifungal activity of methanolic extracts and fractions

The antifungal activity of methanolic extract and its fractions are presented in Table 5. Fraction FIII is found to display an inhibitory effect on the growth rate of *Pycnoporus sanguineus* and *Trametes versicolor*. The antifungal efficiency of this fraction against the two white rot fungi is the one on which the concentration dependent. It relies on concentrations varying

Table 2 Spearman rank correlation coefficients between ABTS free radical scavenging activity and the content of phenolic compounds (total phenols, tannins, and flavonoids) of extracts from *Dicorynia guianensis*. All significant correlations are in italics

	Methanol	Ethanol	Hot water
Total phenol	*− 0.923*	*− 0.965*	*− 0.965*
Tannins	*− 0.867*	*− 0.909*	*− 0.951*
Flavonoids	*− 0.909*	*− 0.951*	*− 0.937*

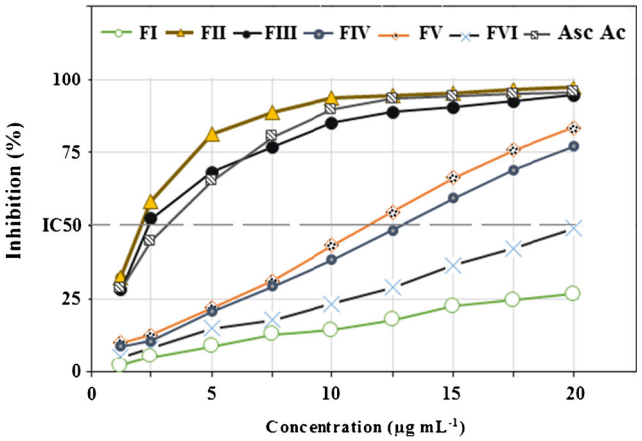

Fig. 1 Percentage of inhibition of ABTS free radical by methanol extracts' fractions using concentrations between 1 to 20 µg/mL in comparison to ascorbic acid (Asc Ac). FI, FII, FIII, FIV, and FV are organic fractions and FVI is the residual aqueous fraction

from 1000 to 62.5 µg mL^{-1} based on the AI. Among the wood-degrading fungi tested, *P. sanguineus* showed the lowest growth inhibition to fraction FIII. However, the growth rate of *T. versicolor* is also less affected by the same concentration of FIII. For example, the antifungal index of fraction FIII at the concentration of 1000 µg mL^{-1} is 43.6 and 18.7%, respectively, against *P. sanguineus* and *T. versicolor*. Tebuconazole inhibited the growth of the two white rot fungi at the concentration 0.43 µg mL^{-1} (Table 5).

Table 3 ^{1}H NMR and ^{13}C NMR chemical shifts of (+)-catechin (**1**) and (-)-epicatechin (**2**) isolated from the heartwood of *Dicorynia guianensis*

No.	δ_c	Compound 1 δ_H, mult, J(Hz)	Compound 2 δ_H, mult, J(Hz)
2	81.5	4.55; 1H; *d* (7.4)	4.81; 1H; s
3	67.45	4.03; 1H; *dq* (7.83; 5.43)	4,15; 1H; *m*
4	27.2	α-2.82; 1H; *dd* (5.5; 16.1)	α-2.83; 1H; *dd* (5.4; 16.0)
		β-2.41; 1H; *dd* (5.5; 16.1)	β-2.75; 1H; *dd* (5.4; 16.0)
5	156.46	–	–
6	94.92	5.85; 1H; *d* (2.3)	5.81; 1H; *d* (1.96)
7	157.1	–	–
8	97.2	5.92; 1H; *d* (2.3)	5.92; 1H; *d* (1,96)
9	155.47	–	–
10	99.92	–	–
1'	130.80	–	–
2'	113.90	6.83; 1H; *d* (1.9)	6.95; 1H; (*s*)
3'	144.90	–	–
4'	147.2	–	–
5'	120.4	6.77; 1H; *d* (1.9)	6.72; 1H; *d* (1.8)
6'	118.8	6.75; 1H; *dd* (1.9; 8.1)	6.81; 1H; *d* (1.8; 8.1)

Table 4 ^{1}H NMR and ^{13}C NMR spectroscopic data for compound **3** and compound **4**

N°	δC	Compound **3** δH, mult, J(Hz)	Compound **4a** δH, mult, J(Hz)	Compound **4b** δH, mult, J(Hz)
2	83.54	5.04; 1H; *d* (10.4)	5.12; 1H; *d* (9.8)	4.94; 1H; *d* (2.0)
3	78.37	4.53; 1H; *d* (10.4)	4.52; 1H; *d* (9.5)	4.58 ; 1H; *d* (2.0)
4	195.5	-	-	-
5	164.1	-	-	-
6	97.25	5,78 ; 1H; *d* (1.9)	5.84; 1H; *d* (1.5)	5.80; 1H; *d* (1.9)
7	172.1	-	-	-
8	97.25	5,76; 1H; *d* 1.9)	5.86; 1H; *d* (1.2)	5.80 ; 1H; *d* (1.9)
9	164.2	-	-	-
10	101.8	-	-	-
1'	130.2	-	-	-
2'	115.8	6,87; 1H; *d* (1.9)	6.83; 1H; *d* (1.7)	6.86; 1H; *d* (1.9)
3'	146.5	-	-	-
4'	147.2	-	-	-
5'	115.8	6.80; 1H; *m*	6.69; 1H; *d* (8.2)	6.80; 1H; *m*
6'	120.4	6.82; 1H; *m*	6.73; 1H; *d* 8.2	6.81; 1H; *m*
1"	101.7	4.07; 1H; *s*	4.2; 1H; *brs*	5.08; 1H; *s*
2"	71.7	3.57; 1H; *m*	3.38; 1H; *brs*	4.01; 1H; *m*
3"	71.6	3.40; 1H; *m*	3.42; 1H; *dd* (9.4, 2.9)	3.67 ; 1H; *m*
4"	73.3	3.22; 1H; *t* (9,6)	3.13; 1H; *t* (9.4)	3.31; 1H; *t* (9.6)
5"	70.1	2.34; 1H;*m*	3.81; 1H; *m*	4.16; 1H; *m*
6"	18.3	0.81; 3H; *d* (6,3)	1.01; 3H; *d* (6.3)	1.09; 3H; *d* (6.0)

^{1}H NMR and ^{13}C NMR chemical shifts of neoastilbin (**3**), astilbin (**4a**) and isoastilbin (**4b**) isolated from the heartwood of *Dicorynia guianensis*

4 Discussion

4.1 Extraction yield, total polyphenols, tannins, and flavonoid content

4.1.1 Extraction yield

Extractive content obtained using polar solvents have shown intraspecific variation in the amount of extractives related to wood durability of *D. guianensis*. Durable heartwood had higher extractive content than moderate-durable woods. The higher percentage of extractives in the durable heartwood was expected, as extracts are active to preserve the internal tissue of the tree against interior attack (Amusant et al. 2014). These results are supported by previous studies, which showed that very durable and durable tropical hardwood timbers have higher extractives content in the heartwood than moderate and non-durable woods (Lukumandaru 2013). In addition, the extraction yield depends on the solvents used, even though some compounds are not extractable, as they are bonded to the wood matrix. For instance, hot water provided large amounts of extractives. Indeed, high temperatures increase the diffusivity of water and improve the mass transfer of dissolved

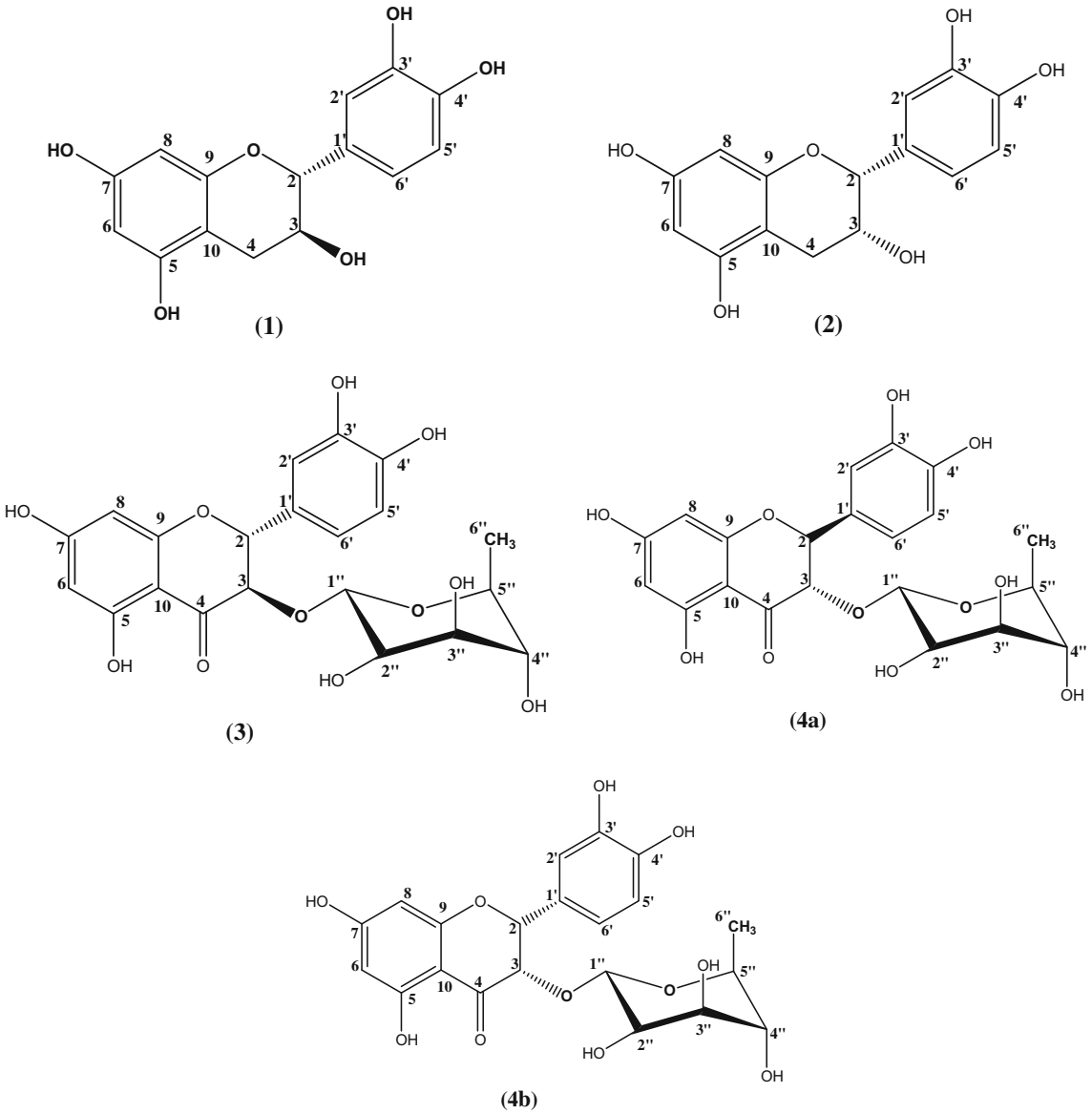

Fig. 2 Flavonoids characterized from the subfraction FIIA: (+)-catechin (**1**), (−)-epicatechin (**2**) and from the subfraction FIIB: neoastilbin (**3**), astilbin (**4a**), and isoastilbin (**4b**)

Table 5 Antifungal index expressed in percentage of the methanolic extract of *Dicorynia guianensis* and its fractions on growth inhibition of *Trametes* versicolor and *Pycnoporus sanguineus*

	Antifungal index (%)					
	Trametes versicolor			*Pycnoporus sanguineus*		
Concentration (μg mL^{-1})	62.5	250	1000	62.5	250	1000
Methanol extract	0	0	2.1	0	0	4.5
Fraction FI	0	0	0	0	0	0
Fraction FII	0	0	1.3	0	0	1.95
Fraction FIII	0	7.4	18.6	5.8	15.8	43.6
Fraction FIV	0	0	1.8	0	0	3.7
Fraction FV	0	0	0	0	0	0
Fraction FVI	0	0	1.3	0	0	4
Tebuconazole (0.43 μg mL^{-1})	100			100		

compounds, thus improving the extraction efficiency (Mustafa and Turner 2011). Regardless of the nature of the wood, these findings are consistent with the data reported by Royer et al. (2011) from *Acer rubrum* L. and Ket and Kang (2012) from *Acacia* sp. (Pietarinen et al. 2006). Extraction yields by methanol and ethanol were significantly different ($\rho < 0.05$). The difference of the polarity of solvents (Shabbir et al. 2011) could explain this observation.

4.2 Total phenols, tannins, and flavonoid content

The highest level of total phenols, tannins, and flavonoid content was obtained from durable wood when extracted with methanol and ethanol. The extracts contained high amounts of phenolics, likewise that reported in heartwood extracts of other Leguminosae. Ethanol extract of *Acacia confusa* Merr. contained 529.7 mg GAE g^{-1} of dry extract (Chang et al. 2001). Sergent et al. (2014) reported values of total phenols from 597 to 603 $mg.g^{-1}$ of dry extract for *Robinia pseudoacacia* L. Some other studies had also investigated the phenolic composition of wood extracts and concluded that phenolic compounds of most resistant woods were positively correlated with natural durability (Kadir and Hale 2016; Niamké et al. 2011). Contrastingly, in some other woody species, complex relationship was found between natural durability and the composition of extractives. For instance, the natural durability of *Thuja plicata* Donn ex D. and *Chamaecyparis nootkatensis* D.Don is weakly correlated with individual extractives content (Taylor et al. 2006).

4.3 Antioxidant activities

Free radical scavenging activity of non-durable wood extracts was generally lower than those of durable wood probably due to the content of phenolic antioxidant compounds. The radical scavenging activity expressed by IC_{50} was strongly correlated to total phenol content. Some other studies involving in tropical wood have shown the major contribution or a crucial role of phenolic compounds in oxidative scavenging activity (Kawamura et al. 2011). The Spearman's correlation coefficient pointed out tannins and flavonoids as compounds responsible for the radical scavenging activity of the samples. Flavonoid compounds are known as high-level antioxidants because of their ability to scavenge free radicals such as singlet oxygen, superoxide free radicals, and hydroxyl radicals (Treml and Šmejkal 2016). They are also known to chelate trace elements involved in free radical production and upregulate antioxidant defenses (Agati et al. 2012). Tannins also contributed to radical scavenging activity. It is well known that their radical scavenger property depends on the flavanols' building blocks and the degree of oligomerization (Rhouma-Martin 2013). The ability to scavenge free radicals was the important characteristic of polyphenols. This suggests their potential to slow down wood degradation process by quenching free radicals produced by the extracellular fungal enzyme.

4.4 Major flavonoids isolated

The final structure of isolated compounds was confirmed by comparing the spectrometric NMR and the mass values with those of the literature. (+)-Catechin (**1**) and (−)-epicatechin (**2**) are respectively characterized by Royer et al. (2013) and Shu-Hua et al. (2003). The molecular formula of compound **3** and compound **4** was determined to be $C_{21}H_{23}O_{11}$. The ^{1}H and ^{13}C chemical shift values assigned these two compounds were thus in complete conformity *i*th those described by Xiang et al. (2009) for neoastilbin (**3**), astilbin (**4a**), and its isomer isoastilbin (**4b**). These isolated flavonoids are already being isolated from others heartwood tissues of Leguminosae such *Eperua falcata* (Rodrigues et al. 2010; Royer et al. 2010) and *Acacia* sp. Extractives protect heartwood against fungal colonization by fungicidal activity and/or being excellent free radical scavengers (Schultz and Nicholas 2000). Radical scavenging activity is particularly important because both white rot and brown rot fungi are believed to use radicals to disrupt cell walls (Pietarinen et al. 2006). Although the radical scavenging activity of (+)-catechin, (−)-epicatechin (Muzolf-Panek et al. 2012) and astilbin (Petacci et al. 2010) is well documented, these compounds do not exhibit any antifungal activity (Royer et al. 2010). However, others flavonols such as dihydromorin, aromadedrin, and pinobanksin are active against wood destroying basidiomycete such as *Coriolus versicolor* (Nascimento et al. 2013). Beyond their role in woody tissue, the isolated flavonoids are known for multiple pharmacological activities, such as neuroprotective (Cheng et al. 2016), anti-inflammatory (Huang et al. 2011), as well as an anti-diabetic nephropathy (Li et al. 2009) properties.

4.5 Antifungal activity of methanolic extract and its fraction

Fraction FIII has shown moderate and weak fungistatic activities respectively against *Pycnoporus sanguineus* and *Trametes versicolor*. *P. sanguineus* was more sensitive to the action of fraction FIII compared to *T. versicolor*. *T. versicolor* was more destructive on *D. guianensis* heartwood than *P. sanguineus* (Amusant et al. 2004). The fungistatic activity of heartwood extractives may be imputed to alkaloids present in fraction FIII. Previous study of this fraction resulted in the isolation of indols and β-carboline alkaloids from this fraction (Anouhe et al. 2015). Due to the good radical scavenging activity of fraction FIII, its alkaloids (Anouhe et al. 2015) would retard the initial step of fungal degradation process (Tanaka et al. 2007). The fraction FII, which contains mainly flavonoids, did not exhibit antifungal activity against the two wood degradation fungi. White rot fungi produce peroxidases

and laccases, which degrade cellulose, hemicelluloses, lignin, and some extractives (Lekounougou et al. 2008). These enzymes are able to metabolize polyphenolic compounds limiting their toxicity and therefore the effectiveness of the extract to inhibit mycelium growth (Gochev and Krastanov 2007). Other plant defense mechanism in wood such as anatomical barriers might provide additional protection against fungal colonization (Kirker et al. 2013).

5 Conclusion

The antioxidant activity associated to the phenolic compound content allows a preliminary insight about the differences between durable and moderately durable wood of *D. guianensis*. Trees with high content of extractives, mainly tannins and flavonoids, are more resistant against fungi than others. Phenolic compounds are able to quench free radicals and slow the fungal biodegrading process. Nevertheless, the alkaloid fraction has a direct fungistatic property. Heartwood of *D. guianensis* represents a source of extractives with biological properties. Antioxidant and antifungal activities of some extractives may be of interest in formulations of high-value-added products, where such properties are required. The isolated chemicals would be an additional revenue source for the forestry industry that could be utilized for wood preservation, cosmeceutical and nutraceutical applications

Acknowledgements This work was financially supported by the Cooperation and Cultural Action (SCAC) of the Embassy of France in Côte d'Ivoire, CIRAD and French National Research Agency (CEBA, ref. ANR-10-LABX-0025) as part of the "Investissement d'Avenir" program.

Co-author contributions J.B Anouhe assured the conception and realization of all chemical and antioxidant experiments. He wrote the first draft of the paper. Nadine Amusant supervised the experimental work and coordinated the research project. Florence Niamké coordinated the antifungal activity tests and helped in writing and editing the manuscript. David Virieux and Jean-Luc Pirat assured the training of J-B Anouhe on spectroscopic measurements. Milcard Faustin, Amissa Adima, and Seraphin Kati-coulibaly were involved in the critical reading of the results. The final correction of the paper was read and approved by all authors.

References

Afnor NF EN 350 (2016) Durabilité du bois et des matériaux dérivés du bois - Méthodes d'éssai et de classification de la durabilité vis-à-vis des agents biologiques du bois et des matériaux dérivés du bois - Durabilité du bois et des matériaux dérivés du bois - Méthodes d'éssai et de classification de la durabilité à l'eau du bois et des matériaux à base de bois

Agati G, Azzarello E, Pollastri S, Tattini M (2012) Flavonoids as antioxidants in plants: location and functional significance. Plant Sci 196: 67–76. https://doi.org/10.1016/j.plantsci.2012.07.014

Amusant N, Beauchene J, Fournier M, Janin G, Thevenon MF (2004) Decay resistance in *Dicorynia guianensis* Amsh: analysis of inter-tree and intra-tree variability and relations with wood colour. Ann For Sci 61(4):373–380. https://doi.org/10.1051/forest:2004030

Amusant N, Anouhé J-B, Amissa A, Beauchène J, Niamké F, Nicolinni E (2013) Tree crown architecture: a tool for decay resistance evaluation. IRG/WP 16-108, annual meeting, Stockholm, pp 1–12

Amusant N, Nigg N, Thibaut B, Beauchene J (2014) Diversity of decay resistance strategies of durable tropical woods species: *Bocoa prouacencsis* Aublet, *Vouacapoua americana* Aublet, Inga alba (Sw.) wild. Int Biodeterior Biodegrad 94:103–108. https://doi.org/10.1016/j.ibiod.2014.06.012

Anouhe J-B, Adima A, Niamke B, Stien D, Amian B, Blandinières P-A, Virieux D, Kati-Coulibaly S, Pirat JL, Amusant N (2015) Two new β carboline type alkaloids from *Dicorynia guianensis* heartwood. Phytochem Lett 12:158–166. https://doi.org/10.1016/j.phytol.2015.03.012

Benhamou N, Rey P (2012) Stimulateurs des défenses naturelles des plantes : une nouvelle stratégie phytosanitaire dans un contexte d'écoproduction durable. I. Principes de la résistance induite. Phytoprotection 92(1):1–23. https://doi.org/10.7202/1012399ar

Berlioz E (2012) Economy of the forest-wood sector in Guyana. French Academy of Agriculture, pp 1–3

Chang S, Wu J, Wang S, Kang P, Yang N, Shyur F (2001) Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. J Agric Food Chem 49(7):3420–3424. https://doi.org/10.1021/jf0100907

Cheng T, Wang W, Li Q, Han X, Xing J, Qi C, Lan X, Wan J, Potts A, Guan F, Wang J (2016) Cerebroprotection of flavanol (−)-epicatechin after traumatic brain injury via Nrf2-dependent and – independent pathways. Free Radic Biol Med 92:15–28. https://doi.org/10.1016/j.freeradbiomed.2015.12.027

Dickson A, Houghton J, Hylands J (2007) Antibacterial and antioxidant cassane diterpenoids from *Caesalpinia benthamiana*. Phytochemistry 68(10):1436–1441. https://doi.org/10.1016/j.phytochem.2007.03.008

Gochev V, Krastanov A (2007) Fungal laccases. Bulg J Agric Sci 13:75–83

Gülçin I, Bursal E, Hilal S, Bilsel M, Ahmet C, Gören A (2010) Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food Chem Toxicol 48(8-9):2227–2238. https://doi.org/10.1016/j.fct.2010.05.053

Huang Q, Cheng Z, Shi H, Xin W, Wang T, Yu L (2011) Isolation and characterization of two flavonoids, engeletin and astilbin, from the leaves of *Engelhardia roxburghiana* and their potential anti-inflammatory properties. J Agric Food Chem 59(9):4562–4569. https://doi.org/10.1021/jf2002969

IEDOM: Institut d'Emission des Départements d'Outre-Mer (2014) Guyane annual report, 186 p

Kadir R, Hale M (2016) Antioxidant potential and content of phenolic compounds in extracts of twelve selected Malaysian commercial wood species. Eur J Wood Prod 1–8

Kawamura F, Ramle S, Sulaiman O, Hashim R, Ohara S (2011) Antioxidant and antifungal activities of extracts from 15 selected hardwood species of Malaysian timber. Eur J Wood Prod 69(2): 207–221. https://doi.org/10.1007/s00107-010-0413-2

Ket L, Kang C (2012) Influence of different extraction solvents on lipophilic extractives of Acacia Hybrid in different wood portions. Asian J Appl Sci 5:107–116

Kirker G, Blodgett A, Arango R, Lebow K, Clausen C (2013) The role of extractives in naturally durable wood species. Int Biodeterior Biodegrad 82:53–55. https://doi.org/10.1016/j.ibiod.2013.03.007

Lee J, Oh H, Yang H, Baek N, Kim S-H, Cho H, Ki D (2009) Cytotoxic alkaloids from the wood of Picrasma Quassioides. J Korean Soc Appl Biol Chem 52(6):663–667. https://doi.org/10.3839/jksabc.2009.110

Lekounougou S, Mounguengui S, Dumarcay S, Rose C, Courty P, Garbaye J, Gerardin P, Jacquot P, Gelhaye E (2008) Initial stages of *Fagus sylvatica* wood colonization by white – rot basidiomycetes *Trametes versicolor*. Enzymatic characterization. Int Biodeterior Biodegrad 61(4):287–293. https://doi.org/10.1016/j.ibiod.2007.06.013

Li G, Jiang W, Yue X, Qu G, Tian J, Wu J, Fu F (2009) Effect of astilbin on experimental diabetic nephropathy in vivo and in vitro. Planta Med 75(14):1470–1475. https://doi.org/10.1055/s-0029-1185802

Lolita T, Zanda K, Ruta G (2012) Comparison of different solvents and extraction methods for isolation of phenolic compounds from horseradish roots (Armoracia rusticana). World Acad Sci Eng Technol 64: 903–908

Luís Â, Neiva D, Pereira H, Gominho J, Domingue F, Paula D (2014) Stumps of Eucalyptus globulus as a source of antioxidants and antimicrobial polyphenols. Molecules 19(10):16428–16446. https://doi.org/10.3390/molecules191016428

Lukumandaru G (2013) The natural termite resistance of teak wood grown in community 424 forest. J Ilmu Teknol Kayu Tropis 11: 131–139

Mustafa A, Turner C (2011) Pressurized liquid extraction as a green approach in food and herbal plants extraction: a review. Anal Chim Acta 703(1):8–18. https://doi.org/10.1016/j.aca.2011.07.018

Muzolf-Panek M, Gliszczynska-Swigło A, Szymusiak A, Szymusiak H, Tyrakowska B (2012) The influence of stereochemistry on the antioxidant properties of catechin epimers. Eur Food Res Technol 235(6):1001–1009. https://doi.org/10.1007/s00217-012-1826-4

Nascimento M, Santana D, Maranhão C, Oliveira L, Bieber L (2013) Phenolic extractives and natural resistance of wood. In: Chamy R, Rosenkranz F (eds) Biodegradation – Life of Science, 349–370 p

Niamké B, Amusant N, Charpentier J-P, Chaix G, Baissac Y, Boutahar N, Adima A, Kati-Coulibaly S, Jay-Allemand C (2011) Relationships between biochemical attributes (non-structural carbohydrates and phenolics) and natural durability against fungi in dry teak wood (*Tectona grandis* L. f.) Ann For Sci 68(1):201–211. https://doi.org/10.1007/s13595-011-0021-2

Niamké B, Amusant N, Stien D, Chaix G, Lozano Y, Kadio A, Lemenager N, Goh D, Adima A, Kati-coulibaly S, Jay-allemand C (2012) Dihydroxy-epiiso- catalponol, a new naphthoquinone from *Tectona grandis* L.f. heartwood, and fungicidal activity. Int Biodeterior Biodegrad 74:93–98. https://doi.org/10.1016/j.ibiod.2012.03.010

Paradis S, Guibal D, Vernay M, Beauchêne J, Brancheriau L, Chalon I, Daigremont C, Détienne P, Fouquet D, Langbour P, Lotte S, Mejean C, Thévenon M.F, Thibaut A, Gérard J (2011) Caractéristiques technologiques de 245 essences tropicales et tempérées. Tropix 7.0. CIRAD – (CD-Rom)

Petacci F, Freitas S, Brunetti I, Khalil N (2010) Inhibition of peroxidase activity and scavenging of reactive oxygen species by astilbin isolated from *Dimorphandra mollis* (Fabaceae, Caesalpinioideae). Biol Res 43(1):63–74

Pietarinen S, Willfor M, Virkstrom F, Holmbom B (2006) Aspen knots, a rich source of flavonoids. Wood Sci Technol 26:245–258

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26(9-10):1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rhouma-Martin G (2013) Enzymatic oligomerization of flavonoids and evaluation of the biological activities of synthesized oligomers. Doctoral Thesis in Biological Sciences and Biotechnologies. University of Lorraine, 196 p

Rodrigues A, Theodoro T, Eparvier V, Basset C, Silva R, Beauchene J, Espindola S, Stien D (2010) Search for antifungal compounds from the wood of durable tropical trees. J Nat Prod 73(10):1706–1707. https://doi.org/10.1021/np1001412

Royer M, Stien D, Beauchêne J, Herbette G, McLean J-P, Thibaut A, Thibaut B (2010) Chemical extractives of the tropical wood Wallaba are natural anti-swelling agents. Holzforschung 64:211–215

Royer M, Diouf N, Stevanovic T (2011) Polyphenol contents and radical scavenging capacities of red maple (*Acer rubrum* L.) extracts. Food Chem Toxicol 49(9):2180–2188. https://doi.org/10.1016/j.fct.2011.06.003

Royer M, Maria P, Martha E, Niokhor D, Tatjana T (2013) Study of nutraceuticals, nutricosmetics and cosmeceutical potentials of polyphenols bark extracts from Canadian forest species. PharmaNutrition 6:1–10

Salido S, Pérez-Bonilla M, Adams P, Altarejos J (2015) Phenolic components and antioxidant activity of wood extracts from 10 main Spanish olive cultivars. J Agric Food Chem 63(29):6493–64500. https://doi.org/10.1021/acs.jafc.5b02979

Schultz T, Nicholas D (2000) Naturally durable heartwood: evidence for a proposed dual defensive function of the extractives. Phytochemistry 54(1):47–52. https://doi.org/10.1016/S0031-9422(99)00622-6

Sergent T, Kohnen S, Jourez B, Beauve C, Schneider J, Vincke C (2014) Characterization of black locust (*Robinia pseudoacacia* L.) heartwood extractives: identification of resveratrol and picéatannol. Wood Sci Technol 48(5):1005–1017. https://doi.org/10.1007/s00226-014-0656-x

Shabbir G, Anwar F, Sultana B, Khalid M, Afzal M, Khan Q, Ashrafuzzaman M (2011) Antioxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of Gold Mohar [*Delonix regia* (Bojer ex Hook.) Raf.] Molecule 16(12):7302–7319. https://doi.org/10.3390/molecules16097302

Shu-Hua Q, Da-Gang W, Yun-Bao M, Xiao-Dong L (2003) A novel flavane from *Carapa Guianensis*. Acta Bot Sin 45:1129–1133

Singleton V, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. Methods Enzymol 299:152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Tanaka H, Yoshida G, Baba Y, Matsumura K, Wasada H, Murata J, Agawa M, Itakura S, Enoki A (2007) Characterization of a hydroxyl radical producing glycoprotein and its presumptive genes from the white rot Basidiomycetes *Phanerochaete chrysosporium*. J Biotechnol 128(3):500–511. https://doi.org/10.1016/j.jbiotec.2006.12.010

Taylor M, Gartner B, Morrell J, Tsunoda K (2006) Effects of heartwood extractive fractions of Thuja Plicata and Chamaecyparis nootkatensis on wood degradation by termites or fungi. J Wood Sci 52(2):147–153. https://doi.org/10.1007/s10086-005-0743-6

Treml J, Šmejkal K (2016) Flavonoids as potent scavengers of hydroxyl radicals, comprehensive reviews. Food Sci Food Saf 15(4):720–738. https://doi.org/10.1111/1541-4337.12204

Xiang Z, Qiang X, Jian-Xin L, Ting C (2009) Structural revision of two flavanonols glycosides from *Smilax glabra*. Planta Med 75:654–655