Relationships between chemical composition and decay resistance of *Coula edulis* Baill as an alternative wood species in Gabon

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Context and objectives

The natural durability of wood is defined as its ability to resist to biological degradation caused by fungus and insects and weathering without any chemical treatment (Rowell et al. 2005). In reality, only the heartwood of some species is durable; sapwood being in general easily degraded. Although several factors like hydrophobicity, density, lignin and extractives contents have been reported to be involved in wood natural durability, most of the authors agree with the import role played by extractives (Antwi-Boasiako and Pitman 2009). Extractives content varies widely in wood (Antwi-Boasiako et al. 2010, Kilic and Niemz 2012). Extractives are low molecular weight substances found in the porous structure of wood. These are generally lipophilic or hydrophilic secondary metabolites that are not essential for tree growth. The content and composition of the extractive substances vary according to the wood samples, the part of the tree, its age, the location and the season of harvesting as well as the period and conditions of storage. Extractive substances are responsible for wood characteristics such as color, odor, natural durability and acoustic properties (Aloui et al. 2004). They protect wood from ultraviolet rays, pathogens and herbivores (Harborne and Williams 2000). They consist of several families of compounds such as waxes, fats, terpenoids, quinones and a large variety of phenolic compounds (simple phenols, flavonoids, lignans, stilbenes, tannins…). Phenolic compounds are the most important class of extractives presenting antifungal, antioxidant and other properties that are potentially valuable (Hillis 1972). In addition, naturally durable wood species contained toxic compounds that are generally polyphenols such as tannins, flavonoids and stilbenes. Some species also have non-phenolic extractives such as quinones and terpenoids. For example, the molecules responsible for the natural durability of *Tectona grandis* consist of toxic quinones, which present strong antitermitic and fungicidal properties. The toxicity of extractive substances correlates with their antioxidant activity (Amusant et al. 2007). The most important agents of wood biodegradation are fungi and termites (Antwi-Boasiako et al. 2010). However, while natural durability of most of temperate wood species has been well documented (Aloui et al. 2004, Guilley et al. 2004), less information is available on the natural durability of many Congo Basin wood species (CIRAD 2008, Bopenga bopenga et al. 2020a,b,c). The Congo Basin hosts an exceptional biodiversity for trees as well as for flora and fauna. Only a few wood species are exploited because of their high market value (AGEOS 2015). The direct consequence of this selective exploitation is the over-utilization of certain wood species. Several lesser-known species are available in Gabon including the Gabonese hazel (*Coula edulis* Baill), whose wood is used by the local populations for its longevity because of its resistance to fungi and insects, including termites. The wood is used in the construction of huts as poles or lintels (Moupela C et al. 2010, 2013). The goal of this study was to better

understand the reasons for the natural durability of this species including: (i) the effect of extractives on decay resistance, (ii) the effect of extractives on the fungal growth inhibition, and (iii) the role of wood chemistry in relation to durability.

Material and method

Material. Heartwood of Gabon hazel (C. edulis) was obtained from a 50-cm-diameter log taken from between 1.50 and 2.50 m above the ground. The tree was harvested at the Ayémé primary forest in Gabon located in the Estuary province. Tree age was estimated between 50 and 75 years. 30×10×5 mm3 heartwood blocks were cut in the longitudinal, radial and tangential directions, respectively. The blocks were randomly used for the different tests performed. Sawdust of *C. edulis* Baill was prepared using a ball mill (Retsch SM 100), sieved (Ø=0.160 mm) and stored in glass fasks after drying in an oven at 70 °C to avoid extractives degradation.

Method. For the extraction blocks, blocks of heartwood were extracted to evaluate the effect of extractives on wood decay durability. The blocks were Soxhlet extracted for three weeks using acetone-water (1/1, v/v), the apparatus being refuelled when needed (Fig. 1). A duration of three weeks was chosen to improve extraction, which is known to be more difficult on blocks due to a lower exchange surface compared to sawdust. The blocks were then dried at 103 °C and weighed (m1). Extraction yield was determined using:

$$\text{Yield of extraction (\%)} = [(m_0 - m_1) / m_0] \times 100 \quad (1)$$

where m_0 was the anhydrous mass of the blocks before extraction dried at 103°C and m1 the anhydrous mass of the blocks dried at 103°C after extraction.

Fig 1: (a) Sanded specimens 30×10×5 mm3; (b) Extraction of the test pieces using Soxhlet.

For the extraction sawdust, 10 g of finely ground dried heartwood was successively Soxhlet-extracted for 24 hours with solvents of increasing polarity: dichloromethane, acetone, toluene-ethanol (2/1, v/v) and water. each extraction was performed in triplicate. The extracts were concentrated using a rotary evaporator under vacuum, except for water extracts which were lyophilized. The extracts were then dried in a desiccator under vacuum in the presence of P_2O_5 and weighed regularly until constant mass (m_e**)**. The yield of extract is determined using:

$$\text{Yield of extract (\%)} = (m_e / m_s) \times 100 \quad (2)$$

where m_e was the mass of extract and m_s the mass of sawdust used for extraction. The extracts were then recovered and stored in closed bottles in the dark.

Decay resistance tests. The mass loss (ML) caused by fungal attack in Fig. 2 and the moisture content (MC) is determined as follows:

$$\text{ML (\%)} = [(m_0 \text{ or } m_1) - m_3)] / (m_0 \text{ or } m_1) \times 100 \quad (3)$$

$$\text{MC (\%)} = (m_2 - m_3)/m_3 \times 100 \quad (4)$$

where m_0 was the initial anhydrous mass of unextracted blocks, m_1 the anhydrous mass of extracted blocks before fungal exposure, m_2 the mass of wet blocks after fungal exposure and m_3 the dried mass of blocks after fungal exposure.

Corrected mass loss was calculated as follows:

$$ML_{corrected}\ (\%) = ML\ (\%) - ML_{diffusion}\ (\%) \quad (5)$$

where $ML_{diffusion}$ was the mean of mass loss values obtained for diffusion tests. The inhibition of fungal growth by extracts is shown in Fig. 3 and the analysis of extracts in Fig. 4.

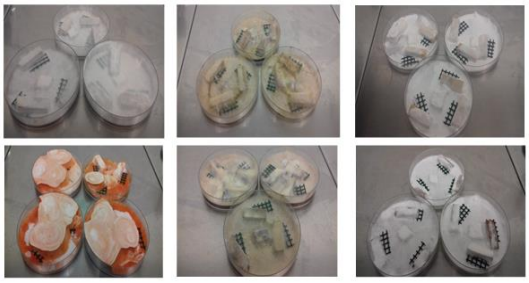

Fig. 2: Comparison of wooden test pieces with different fungi.

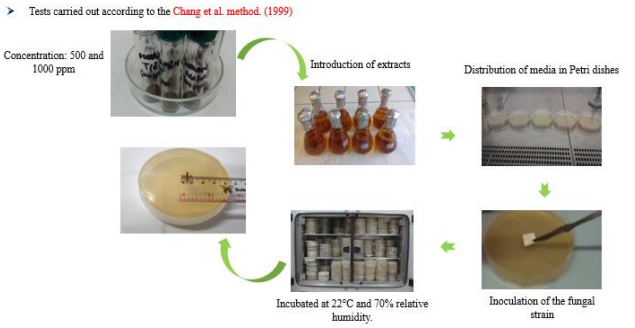

Fig. 3: Inhibition of fungal growth by extracts test

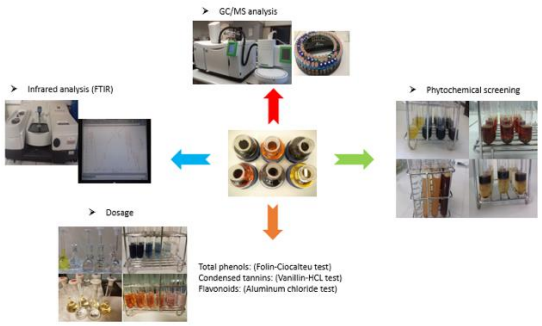

Fig. 4 : Analysis of extracts tests

Results and discussion

Extractive contents. Extractive yield varied with solvent to another (Tab. 1). The yield was lowest with dichloromethane (1.1%), probably due to the limited amounts of non-polar compounds in *C. edulis* heartwood. The highest extract content was obtained with acetone (8.5%). None of the solvents used removed all extractives because of their different solubilities. Previous studies suggest that the non-polar solvents mainly extracted oils, grease or terpenes, while polar solvents removed polyphenols such as lignans, stilbenes, flavonoids, tannins….The total yield of extractives reached 16.1%, which is similar to the extractives content of many tropical species. Mass losses of the virulence controls ranged from 48.9 % for *T. versicolor* to 50.2% for *P. sanguineus* for beech and 40.6% for *R. placenta* and 48.5% for *C. puteana* for pine sapwood (Tab. 2). To be valid, the European standard EN 113 requires minimum WL of 30%. All fungi tested met this requirement. Mass losses of all non-extracted heartwood blocks of *C. edulis* were less than 2%, regardless of the fungus used indicated that this species is resistant to attack by white or brown rot (Tab. 3).

Natural resistance of wood blocks against fungal attack. The moisture content of the blocks ranged from 20.8 to 53.7%, indicating that conditions were generally suitable for wood degradation. The results also indicated that extracted wood absorbed more moisture showing that the extractives influenced wood moisture content. Steric hindrance and hydrophobic character of extractives may explain the higher water content of extracted samples.

Tab. 1. Extractives recovered from *C. edulis* heartwood sawdust with solvents with increased polarity

Solvent	Yields (%)[a]
Dichlorométhane	1.1 ± 0,2
Acetone	8.5 ± 0,2
Toluène/éthanol	2.9 ± 0,4
Water	3.6 ± 0,2
Total	**16.1±1**

[a] Values represent means of 3 replicates ± standard deviation.

Tab. 2: Corrected mass loss of *C. edulis* wood blocks after 16 weeks exposure to different fungi

	$ML_{corrected}$ (%)[a]			
Sample	*Trametes versicolor*	*Pycnoporus sanguineus*	*Rhodonia placenta*	*Coniophora puteana*
Beech control	48.9 ± 3	50.2 ± 9.5	-	-
Pine control	-	-	40.6 ± 7.5	48.5 ± 8.3
C. edulis	1.3 ± 0.4	1.28 ± 0.9	0.4 ± 0.1	0.5 ± 0.1
Extracted *C. edulis*	19.8 ± 0.9	17.5 ± 0.9	10.8 ± 0.9	12.8 ± 0.3

a Values represent means of 3 replicates ± standard deviation.

Tab. 3: Classification of durability based on EN 350 (2016).

Sample	*T. versicolor*	*P. sanguineus*	*R. placenta*	*C. puteana*
C. edulis	1 (x=0.02)	1 (x=0.02)	1 (x=0.01)	1 (x=0.01)
Extracted *C. edulis*	3 (x=0.40)	3 (x=0.35)	2 (x=0.27)	2 (x=0.26)

[a] 1= Very Durable; 2= Durable; 3= Moderately Durable; 4= Weakly Durable; 5= Non Durable

Tab. 4: Average moisture content of wood blocks after 16 weeks of exposure to a fungal attack

	Moisture content (%)[a]			
Sample	*T. versicolor*	*P. sanguineus*	*R. placenta*	*C. puteana*
Beech control	50.3 ± 10.7	51.3 ± 4.9	-	-
Pine control		-	49.0 ± 5.3	53.7 ± 6.7
C. edulis	27.4 ± 0.9	27.1 ± 1.1	20.8 ± 1.0	25.7 ± 1.8
Extracted *C. edulis*	45.0 ± 4.4	40.9 ± 3.6	32.7 ± 8.0	43.5 ± 7.7

[a] Values represent means of 3 replicates ± standard deviation

The *GC-MS analysis* is shown in Tab. 5

Tab. 5: Compounds identified by GC-MS and their relative abundance relative to Total Ion Current (TIC)

Solvent	Retention time (min)	Compound	Abundance(%)
Dichloromethane	22.64	Tetracosanoic acid	20
	25.31	Hexacosanoic acid	54
	27.68	Docosanoic acid	5
	29.68	Unknown	11
	33.74	Unknown	11
Acetone	16.09	Gallic acid	53
	20.31	Unknown	3
	21.38	Unknown	4
	22.73	Unknown	6
	24.46	Unknown	3
	28.22	Ellagic acid	30
Toluene/ethanol	16.09	Gallic acid	20
	28.29	Ellagic acid	80

Inhibition of fungal growth by extracts. Fractions extracted with dichloromethane had lower antifungal properties as demonstrated by the weak inhibition of fungal development (Fig 5). Acetone, toluene/ethanol and water extracts presented significant antifungal properties. Growth of brown rot fungi began only on the sixth day with R. placenta and on the eighth day for *C. puteana* at 500 ppm concentrations. The higher concentrations (1000 ppm) completely inhibited mycelial growth. *P. sanguineu*s did not start growing until the fifth day at 500 ppm and the sixth day at 1000 ppm. *T. versicolor* growth at 500 ppm and 1000 ppm started on the third day. Acetone, toluene/ethanol and aqueous fractions inhibited growth of the brown rot fungi more than the white rot fungi. Oxidizing enzymes such as laccases and peroxidases excreted by white rot fungi may be capable of degrading phenolic compounds such as extractive substances.

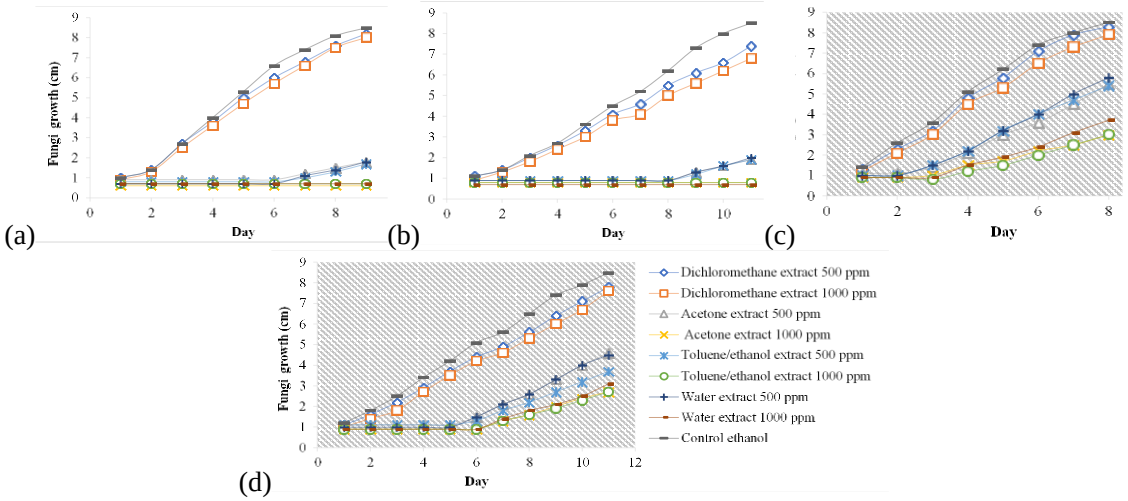

Fig 5. Activity of extracts on the growth of the different fungi:
(a) *Rhodonia placenta*; (b) *Coniophora puteana*; (c) *Trametes versicolor*; (d) *Pycnoporus sanguineus.*

Conclusions

The effect of extractives on the natural durability of *C. edulis* baill was studied in Petri dishes on extracts free or not heartwood blocks exposed to different brown rot and white rot fungi. Mass losses recorded on the extracted blocks were much greater than those measured on the non-extracted blocks indicating clearly the effect of extractives on wood natural durability. With mass losses lower than 2% after 16 weeks exposure to the fungi, this species can be classify as “very durable”, allowing to design utilisation as construction wood for indoors or outdoors applications by local populations as an alternative to wood shortage in Gabon. Growth inhibition tests have shown that at concentrations above 500 ppm, most of the extracts presented an important fungistatic effect on the growth of white rot and brown rot fungi and completely inhibited the last ones growth inhibition at 1000 ppm. GC/MS and FTIR analysis indicated the presence of different polyphenolic compounds like gallic acid, ellagic acid and tannins, which may explain in great part natural durability of this species to fungi.

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