



Inhibition of fungi with wood extractives and natural durability of five Cameroonian wood species

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ABSTRACT

The natural durability of five Cameroonian wood samples was evaluated using the European standard EN 350-1 with a slight modification. The capacity of extracts to inhibit the fungal growth was evaluated at different concentrations with propiconazole (a commercial fungicide for wood) as standard. Scanning Electron Microscopy (SEM) of wood blocks was carried out before and after extraction in different solvents. Unextracted and extracted wood blocks were exposed to fungal attack, with beech as a reference wood. Results showed that mass losses of non-extracted samples ranged from 0.1 to 59% and those of extracted samples from 3% to 40%. Before extraction, all wood samples under study were classified as very resistant to fungi attack (class 1) with respect to the standard. After extraction, some of the wood samples became vulnerable to fungal attack. Movingui, padouk, and tali became less resistant to fungi attack (class 3). The SEM of wood revealed that the extractives were removed from the fibres. The effect of extractives on fungal growth showed that extracts weakly inhibited fungal growth up to concentrations of 250 µg/mL but extracts of tali and movingui totally inhibited fungal growth at a concentration of 500 µg/mL.

1. Introduction

Wood plays an important role in human activity. It is used as material for products, building construction, etc. Wood can be used inside or outside, as packaging, for plywood and as panels. The wood of *Triplochiton scleroxylon* (Ayous) was used in the construction of parts of the ceiling of Roissy Airport (Vernay, 2005). For these applications, wood must possess a good natural durability against insect and fungal attacks, as well as a good dimensional stability and weather resistance (Ali et al., 2011). Some tropical woods have very good natural durability against wood destructive agents. These species are most often exported to Europe, Asia and the United States of America. Recently, Cameroon passed a law that banned the exportation of certain wood samples, thereby favoring the local processing of logs. This local transformation leads to the production of a huge amount of wood waste

(sawdust, bark and board residue) that is usually burnt or abandoned but which could be valorized (Saha Tchinda, 2015). In 2006, this transformation generated 245 million m³ of waste, about 1.20 million tonnes. In many industrialized countries, such waste is valorized in the production of particle board, composite materials and energy, thus this waste represents a real economic potential. To optimize the use of waste from timber exploitation, the valorization of wood extractives can be envisaged without stifling other uses; extraction could be carried out prior to other transformation processes. Extractives are low molecular weight substances located in the porous structure of wood. They are generally lipophilic or hydrophilic secondary metabolites i.e. compounds that are not essential for the growth of the tree. The content and composition of extractives vary with wood samples, the part of the tree, its age, geographical site of harvest, season of the harvest and storage time and conditions. Extractives are responsible for wood

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characteristics such as color, odor, natural durability and acoustic properties (Aloui et al., 2004). They protect wood against ultraviolet radiation, pathogens and herbivores (Harborne and Williams, 2000). They consist of several families of compounds such as terpenoids, waxes and fats, and phenolics. Phenolic compounds are the most significant class of compounds shown in previous studies to possess interesting properties. Phenolic compounds have been used as antioxidants, anti-fungals and natural dyes for fibres (Xie et al., 2017; Saha Tchinda et al., 2013, 2014; Taylor et al., 2006). Reyes-Chilpa et al. (1998) isolated compounds such as flavonoids and isoflavonoids (4,2',5'-trihydroxy-chalcone, isoliquiritigenin (4,2',4'-trihydroxychalcone), (+) - liquiritigenin, (+) - medicarpin, and homopterocarpin) that inhibited the growth of *Lenzites trabea* and *Coriolus versicolor* isolated from the heartwood of *Platymiscium yucatanum*.

It should be noted that the natural durability of Cameroonian wood species has not been amply studied. The earliest study dates back to 2005 (Nzokou et al., 2005). Given the growing demand for Cameroonian timber in the Central African sub-region, it would be interesting to study the natural durability of these wood species. In this perspective, the current study was carried out under tropical conditions with tropical fungi, to investigate the natural durability of tropical heartwood and the effect of leaching of wood species under those conditions. The wood species chosen for this study are highly exploited in Cameroon and legally banned from exportation. According to the Cameroon Ministry of Environment and Forests, the most exploited wood species are ayous (37.2%), sapelli (18.9%), azobé (6.6%), tali (5.7%), movingui (2.0%), moabi (1.8%), padouk (1.3%) and others (26.5%). Descriptions of these wood species were recently published (Saha Tchinda et al., 2013; Mounguengui et al., 2016). The scientific names of the wood species studied are given in the Table 1 below.

The demand of these woods on the local construction market and the Central Africa sub-region has increased tremendously.

Extractives of durable tropical wood may play a key role in conferring durability to vulnerable wood. Extractives can thus be valorized as a source of antifungal to protect wood against insect and fungal attacks. To better understand the reasons for the natural durability of these species and the effect of extractives on natural durability, *in vitro* natural durability of wood species and the effect of leaching on fungal attack was carried out.

The objectives of the present study were: (i) to evaluate the natural resistance or natural durability of five Cameroonian wood species: ayous (*Triplochiton scleroxylon*); moabi (*Baillonella toxisperma*), movingui (*Distemonanthus benthamianus*), padouk (*Pterocarpus soyauxii*) and tali (*Erythrophleum suaveolens*). Specifically, no such studies have been carried out on movingui and moabi; (ii) to study the effect of leaching of these species on durability for their use under other climatic conditions, and (iii) to evaluate the effect of extractives on the growth inhibition of white rot and brown rot fungi

Table 1
Wood species tested for decay under laboratory conditions.

Wood species	Commercial name	Family
<i>Triplochiton scleroxylon</i>	Ayous (Cameroon, Equatorial Guinea, Gabon, France, United Kingdom)	Malvaceae
<i>Baillonella toxisperma</i>	Moabi (Angola, Congo, Cameroon) Adjap (Cameroon, Equatorial Guinea)	Sapotaceae
<i>Distemonanthus benthamianus</i>	Movingui (Cameroon, Gabon)	Fabaceae- caesalpinioideae
<i>Pterocarpus soyauxii</i>	Ayan (Benin, Nigeria, Ghana) Padouk (Central African Republic, Cameroon)	Fabaceae
<i>Erythrophleum suaveolens</i>	Tali or Elone (Cameroon, Ivory Coast, Senegal)	Erythroxyloaceae

2. Materials and methods

2.1. Reagents

Reagent-grade dichloromethane (Carlo Erba, 99.9%), acetone (Prolabo, 99.9%), ethanol (Sigma-Aldrich, 99.8%), toluene (Prolabo Chemicals, 100%), propiconazole, malt extract for microbiology (Fluka), and agar (Fischer Scientific) were used without further purification.

2.2. Raw materials

For this study, heartwood of all species (ayous, moabi, movingui, padouk and tali) was obtained from the Centre region, Mefou and Afamba division, Mfou district, Metet village (Cameroon) and identified by a wood specialist of the "Herbier National du Cameroun". Three trees of each species were sampled. The average characteristics of the trees were: 70–80 years of age, height of 50 m, heartwood diameter of 2.00 m, and sapwood thickness of 2 cm. Samples were taken from between 1.50 and 2.50 m above the ground. Heartwood sample blocks of dimensions 25 × 5 × 5 mm³ were made in the longitudinal, radial and tangential directions respectively. Sawdust of different wood samples, obtained as left over from the fabrication of test blocks, ground into fine particles using a cutter mill (Retsch SM 100), sieved and the particle size between 0.2 and 0.4 mm retained for Soxhlet extractions. Beech wood, used in this study as a virulence control, was graciously offered by the "Laboratoire d'Etudes et de Recherche sur le Matériau Bois" (LERMaB) in Nancy, France.

2.3. Extraction

Extraction was carried out on 5–7 g of heartwood sawdust in 200 mL of solvent (dichloromethane, acetone, toluene-ethanol mixture (2:1, v/v) or distilled water) in a Soxhlet extractor in the increasing order of polarity of these solvents. Sawdust was extracted for 12 h at the rate of 7 siphons per hour. This time of extraction was chosen to ensure that a maximum of extractives was removed. It should be noted that, even after twelve hours of extraction of padouk, the solvent remained slightly colored in the Soxhlet. For the other wood species, a clear extract was obtained after seven hours indicating that a maximum of colored extractives had been removed. After extraction, the solvent was evaporated under reduced pressure (or lyophilized in the case of water) and the crude extract was dried under vacuum in a desiccator over phosphorus pentachloride, P₂O₅, weighed and stored in the dark for further use (Neya et al., 2004; Mburu et al., 2007). The yields of extracts in polar and non-polar solvents were combined to obtain the total yield. The yield extraction of sawdust extracted with one solvent was calculated using Eq. (1).

$$\text{Yield of extractives (\%)} = \frac{m_{b+e} - m_b}{m_s} \times 100 \quad (1)$$

Where m_{b+e} is the mass of flask plus extractives; m_b the mass of empty flask and m_s the oven dry mass of sawdust.

Heartwood blocks (dimensions 25 × 5 × 5 mm³ in the longitudinal, radial and tangential directions respectively) were extracted in the same solvents as above. After extraction, the blocks were dried at 103 °C, weighed and retained for a study of the effect of leaching on durability. Each extraction was performed in quadruplet and an average of the results reported. The total yield of extraction of heartwood blocks was calculated by Eq. (2).

$$\text{Total yield of extractives of heartwood blocks (\%)} = \frac{m_d - m_{de}}{m_d} \times 100 \quad (2)$$

where m_d is the mass of oven dry blocks and m_{de} the mass of oven dry blocks after extraction.

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