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Chemical composition of African mahogany (*K. ivorensis A. Chev*) extractive and tannin structures of the bark by MALDI-TOF

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ABSTRACT

The total phenolic and the condensed tannins content of the bark, the sapwood and the heartwood of *Khaya ivorensis* (*K. ivorensis*) from the natural forest of Gabon was investigated by Folin-Ciocalteu, vanillic assay and acid/butanol methods All these methods showed that the bark, the sapwood and the heartwood of *K. ivorensis* did not display a significant difference ($p > 0.05$) regarding their phenolic content and their condensed tannins content as well. The acetone/water extracted tannins from the bark were characterized by Matrix Assisted Laser Desorption/Ionization time of flight (MALDI-TOF) mass spectrometry and by Fourier Transform InfraRed spectroscopy (FTIR). The presence of fisetinidin, gallocatechin, and trihydroxyflavan monomers was observed for the first time. Flavonoid oligomers linked to sugars were found within *K. ivorensis* condensed tannins. An heptamer formed by mixed fisetinidin, trihydroxyflavan, three dihydroxyflavans, and a carbohydrate dimer including mannose was also identified for the first time in the bark of *K. ivorensis*. That mahogany hardwood species did not exhibit any antifungal activity against the white rot fungus *Coriolus versicolor*. However, bark powder decreased the radial fungus growth.

1. Introduction

In African traditional medicine, the bark, the leaves, the seeds, and the roots of *K. ivorensis* trees have been extensively used in pharmacology for decades as an anti-inflammatory remedy (Lompo et al., 2007). Such a mahogany wood species was used as an ingredient in cosmetics (Iwu and Kiayman, 1992; Wu et al., 2014), and displayed antimalarial, antifungal, and cytotoxic activities. Furthermore, limonoids extracted from fruit, flowers, stem bark or leaf of *K. ivorensis* and *Khaya senegalensis* (*K. senegalensis*) displayed antifungal activities against *Botryos phaeriarhodona*, *Botrytis cinerea Pers* (brown rot) rots (Haluk and Roussel., 2000; Abdelgaleil et al., 2004, 2005; Takin et al., 2013). In addition, methyl angolensate located in the wood, bark, and fruits of these mahogany species were assumed to exhibit the highest biocide activity. However, all the studies regarding the chemical composition of mahogany wood species mainly concerned extractives from the stems, barks, branches, leaves, roots, and seeds (Lompo et al., 1998) whereas the chemical composition of mahogany xylem including the sapwood and the heartwood remained to be investigated.

The forest inventory of 2006 showed that the total volume of logs exported from Gabon averaged 1,500,000 m^3 per year (Nze Nguema, 2009). Ten wood species, including *K. ivorensis,* represented 70% of that total annual volume of exported sawns and veneers (Bayol et al., 2014; Ndiade Bourobou et al., 2010; Koumba Zaou et al., 1998). Considering the national and international pressure demand on transformed wood based products, the governments of the Congo basin decided to increase their local timber transformation. Consequently, Gabon adopted a new forestry code in 2001 (Etat Gabonais, 2001). These new forest policies were reinforced in 2009 by the total ban on exporting logs. This has led to the logs being used for new market sectors based on second and third transformation products instead of the traditional plywood industry of *Aucoumea klaineana Pierre (A. klaineana)*. In 2014, Gabon forest

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industry was the second economic sector and forest products exports were presenting 5.8% in value of all export activities. The wood transforming industry increased the production of a considerable amount of underutilized wood wastes (bark, first and last board of trunks, rotary cutting heartwoods, abandoned blocks from cut down woods, spurs, forks…). With the exception of *Aucoumea* for which its chemical structure and composition as well as its sapwood and heartwood potential for cellulose and ethanol production have been investigated (Safou-Tchiama et al., 2007, 2016, 2017). The chemical composition of the most of woods from the Congo basin, including *K. ivorensis* remains little known.

A *K. ivorensis* tree can reach 50 m in height and a diameter up to 1.2 m. It has an average density of 0.6 which renders that mahogany buoyant in water. This tropical hardwood displays little interspecies variability (Détienne, 1979; França et al., 2016) as found for another mahogany, *Khaya anthotheca*. However, *K. ivorensis* displays a low natural resistance to fungal decay (França et al., 2016). According to the Gabonese forest policies, the minimum diameter for exploitation of *K. ivorensis* is 80 cm (Etat Gabonais, 2003). In Gabon, this african mahogany is very adapt for afforestation such as *A. klaineana* (Koumba Zaou et al., 1998) and as easily everywhere else in the world (Hung and Trueman, 2011; Tchoundjeu and Leakey, 1996). The interest in african mahogany is growing on the world timber market due to its good quality (Heryati et al., 2011). However, wood processing produces a high content of waste (wood waste volumes of Mahogany may be estimated at around 50,000 m^3/year). Actually, suitable wood waste recovering strategies are lacking, in particular about mahogany wood. This limits a proper enhancement of its potential by products which could help to develop the timber industry in Central Africa in general and in Gabon in particular.

The aim of the present study is to enhance chemical knowledge of Mahogany wood wastes for future valorizations. For that, we want to determine the polyphenols content of the bark, sapwood, and heartwood of *K. ivorensis*. An attempt is made to determine the molecular structure of condensed tannins extracted from the bark by MALDI-TOF and FTIR.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

All the chemicals used in this study were purchased from Fisher and Sigma Aldrich.

2.1.2. Wood sampling

The bark, sapwood, and heartwood were collected from a *K. ivorensis* of 80 years hold sampled from a section disk of 10 cm of thickness and 85 cm of diameter. The wood was harvested at Mitzic in the North of Gabon by the SNBG (Société Nationale des Bois du Gabon) in February 2016. The fresh samples were put in sterilized bags, air-dried for one week in the laboratory and oven-dried (105 °C) for 48 h. The dried samples were grinded to pass through 60 mesh (≈1 mm diameter) with a rotative knife grinder (Retsch SK1).

2.2. Methods

2.2.1. Biocidal activity control of K. ivorensis wood powders

The biocide activity of the bark, sapwood, and heartwood powders from *K. ivorensis* against the radial growth of the white rot-fungus *Coriolus versicolor* (*C. versicolor*) was controled by adaption of the international standard XP CENT/TS 15083-1. 2006 version.

Preparation of the culture medium – The culture medium used contained malt (4 g)-agar (2 g) in 100 ml of demineralized water. Each culture received different powder concentrations (0% to 6% m/v), and was then placed in a VOTSCH climate control chamber at 70% relative humidity and 25 °C. The radial growth was controlled every two days for 15 days.

2.2.2. Extraction of polyphenols at room temperature

350 mg of dried wood powder (M_{dried}) from each sample was mixed separately at room temperature with 30 ml of acetone/water solution (7:3, v/v). The mixtures were stired for 3 h. The supernatant was recovered, then acetone was evaporated. Four repetitions of each extraction were performed.

2.2.3. Total phenolic content measurement

The total phenol content was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965) as follows: 1 ml of extracts were diluted with 9 ml of demineralized water, and 0.5 ml of the diluted extracts was poured into 2 ml of Folin-Ciocalteu reagent (1/10, v/v in demineralized water). Then, 2.5 ml of sodium carbonate solution (0.7 M) was added to the Folin-Ciocalteu reagent containing the extracts. The final solution obtained was placed into test tubes and left for 5 min in a water bath maintained at 50 °C. The absorbance was registered at 760 nm. The results were expressed as gallic acid equivalent, based on the extracted dry powder amount. The total phenol content was determined according to the following equation below (1):

$$Total \quad phenols(\%) = \frac{C \times D \times V}{1000 \times M_{dried}} \times 100 \tag{1}$$

C: total phenol concentration (ppm). D: degree of dilution (10). V: volume of starting solution (30 ml). M_{dried}: mass of dry powder.

2.2.4. Determination of proanthocyanidin content

2.2.4.1. Anthocyanes measurement by the acid hydrolysis in butanol method. 0.5 ml of dried aqueous extracts were added to 5 ml of ferrous sulfate solution obtained by dissolving 77 mg of $FeSO_4{\cdot}7H_2O$ previously poured into 500 ml of HCl (37%) in nBuOH (2/3, v/v). The mixtures obtained were placed for 15 min in a water bath maintained at 95 °C. After cooling, the absorbances were recorder at 530 nm and the results were expressed as cyanidin equivalent based on the dried wood extracts content. Proanthocyanidins (PA) was determined (Saad et al., 2012) according to the following Eq. (2):

$$[PA] = \frac{A \times V \times D \times V' \times M}{\varepsilon \times v \times m} \tag{2}$$

PA: Proanthocyanidins content (mg cyaniding E/g dry weight expressed as mg cyaE/g DM); V: volume of reaction (ml). D: dilution factors (10). V': volume of the aqueous extract recovered after extraction with diethyl ether (ml). M: cyaniding molar mass (287 g/mol). v: volume of the sample (ml). A: absorbance of the sample. m: mass of dry powder samples (g). ξ: molar extinction coefficient (34700 $M^{-1}\,cm^{-1}$) according to Scalbert et al. (1989).

2.2.4.2. Condensed tannins measurement by the acid condensation of vanillin method. Condensed tannins in an acid medium were measured according to the vanillin condensation method described by Broadhurst and Jones (1978) as following: 0.5 ml of aqueous extracts contained in a tube were mixed with 3 ml of vanillin reagent dissolved in methanol (4%, w/v). Then, 1.5 ml of concentrated HCl (37%) was added, and the mixture was kept in the dark at 20 °C for 15 min. Absorbances were registered at 500 nm. The results obtained were expressed as catechin equivalent based on the amount of dry extracted samples. The calibration was carried out using an aqueous solution of catechin (30 mg/l).

2.2.5. MALDI-TOF analysis

Tannins extracted from *K. ivorensis* bark were obtained using an acetone/water solvent (7/3,v/v). The samples were oven-dried at 105 °C for 24 h and dissolved in a solution of water/acetone (1:1, v/v) up to 7.5 mg/ml. To increase ion formation, NaCl solution (1.5 µl of a