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Extraction and Characterization of *Aucoumea klaineana* Pierre (Okoume) Extractives

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Abstract: In order to promote convenient strategies for the utilization of wood wastes from aucoumea klaineana pierre (okoume) timber industry, various chemical analysis were carried out on samples from different origins. Total extractives content of the bark, sapwood and heartwood of okoume were evaluated. thermogravimetric analyses were performed and the stiasny number was calculated. it was found that the bark was richer in fatty acid of high molecular weight while the sapwood was rich in fatty acid of low molecular weight. the condensed tannins content varied according to the origin and the part of the tree. these new findings should be useful for green okoume based tannin adhesives.

Keywords: Extractibles; fatty acids; tannins; polyphenols; stiasny number; TGA

1 Introduction

Aucoumea klaineana Pierre (Okoume) is one the best wood species mainly used for its good quality in panels or plywoods industry. This wood presents remarkable capacities for unwinding. Characteristics such as straightness; log dimensions, low density fairly uniform quality and abundance in forest render that wood species popular for peeling indeed.

Since the Gabonese government prohibited exporting logs in 2010, the trade of local transformed wood increased significantly. Therefore, the utilization of local wood species such as Okoume or African mahogany increased in the Gabonese industry. However, the timber industry in this central Africa country is mainly focused on wood cutting, peeling and sawing; that generates a high content of non-valorized wood wastes accounting about for 50% of wood transformed.

Among the 400,000,000 m^3 of exploited woods in Gabon per year, Okoume represented 130,000,000 m^3 (about 31%) in 2016 [1].

Analyzing Okoume wood revealed the presence of resin which pointed out some bactericide properties. These properties are the fact of phenolic compounds located in its essential oil [2]. Additionally, the analysis of Okoume's essential oil revealed antiradical and antioxidant activities [3].

Apart the studies listed above, the chemical characterization of Okoume oleoresin was extensively investigated, and patented by Renimel Isabelle in 1999 [4].

However, the chemical composition of the non-resin parts of Okoume has received little attention.

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Our research focused on extracting and characterizing extractible molecules from Okoume wood by Soxhlet apparatus, thermogravimetric analysis (TGA), Stiasny number and condensed tannin quantification.

2Materials and Methods

2.1 Materials

The bark, sapwood and heartwood of Okoume were obtained from SED (Société Equatoriale de Déroulage) in different areas of Gabon natural forest. Thus, trees from the West were collected in Nzamaligue (Nzama) forest, and those from the South were obtained in Milolé forest. Trees from the North were sampled in Mitzic forest by the SNBG (Société Nationale des Bois du Gabon). The wood was collected in February 2016 and sampled as follows: One piece of each tree (83 cm × 10 cm diameter x thickness) was taken at 1.3 m from the soil. Samples were first air dried for one week and then dried in an oven for 24 hours at 105°C. Further, samples were cut and grounded with a grinder (Retsh SK1 rotating knife) at 60 meshes. All the chemicals used in this study were purchased from Fisher Scientific and Sigma-Aldrich. Solvents and reactants were used without further purification

2.2 Methods

2.2.1 Soxhlet Extraction with Different Solvents

A weighed dried sample (10 g) of the powdered material was extracted at 40-70°C for 4 hours under reflux with 400 mL of solvent (Petroleum ether, hexane and acetone (70%, v/v)) in a round bottomed flask heated. The experiment was repeated three (3) times. After extraction, the content was concentrated with a rotating vapor vacuum pump coupled and the yield of the crude was calculated according to the equation below:

$$\text{Yield of crude} = \frac{\text{mass of the solid residue}}{\text{dry mass of sawdust}} x\ 100$$

2.2.2 Tannins Extraction

The tannins were extracted from Okoume wood according to a previously published procedure [5,6] as follows: a sample-water ratio of 1/9 was put in water containing 5% of sodium hydroxide, 0.25 % sodium bisulfite and 0.25% of sodium sulfate. The mixture was maintained under continuous magnetic stirring for 2 hours at 80°C. The obtained tannin extracts were filtered, dried in an oven at 50 °C and yielded as expressed in the equation below:

$$\text{Extraction yield} = \frac{\text{mass of extract recovered}}{\text{mass of dry sample}} x\ 100$$

2.2.3 Thermogravimetric Analysis

The thermogravimetric analysis was carried out on a computerized thermobalance (TGA Q50 instrument) using a furnace which allows a heating rate of 10 °C/min. The thermobalance configuration gives a sensitivity of ± 0.4 lg. It allows to use a small sample mass (10-50 mg) which is needed to ensure isothermal conditions in the samples. In order to establish an inert atmosphere (Nitrogen) during all experiments, a controlled air flow (fixed at 60 mL. min^{-1}, 1 atm) sweeps the measurement cell that is purged for 20 min before starting the heating program. The temperature program was 25 to 600°C.

2.2.4 Stiasny Number

The Stiasny number reaction was used to determine the reactivity of tannins toward formaldehyde. According to Hoong et al., (2010), 50 mL of sample (0.4%, w/w) tannin solution was pipetted into a 150 mL flask. Aqueous formaldehyde (37%, 5 mL) and hydrochloric acid solution (10 M, 5 mL) were then added and the mixture was heated under reflux for 30 minutes. At the end of this reaction, the mixture was filtered through a sintered glass filter (filter nº 3) while it was still hot. The precipitate was dried to constant weight in an oven at 105°C. The Stiasny number was performed in triplicate and determined as follows:

$$\text{Stiasny Number} = \frac{\text{oven−dried weight of precipitate}}{\text{extracted sample dry weight}}\ x\ 100$$

3 Results and Discussion

3.1 Soxhlet Extraction with Different Solvents

In this study, different solvents were used to extract various compounds such as fatty acids with high molecular weight (petroleum ether solvent), fatty acids with low molecular weight (hexane solvent) and tannins (acetone solvent) in order to study the variability of Okoume extracts in the first collection of Nzama site (Nzama 1). The results presented in Tab. 1 pointed out significant differences between the solvents ($p < 0.05$). With the exception of the bark, the highest extracts content was obtained with petroleum ether. Extracts obtained with petroleum ether displayed significant differences between the bark, sapwood and heartwood ($p < 0.0001$). It was noteworthy that fatty acids were more abundant in the bark (12.24 ± 6.5%) than the sapwood (8.72 ± 3.3%) and the heartwood (6.60 ± 2.0%). But, hexane exhibited also significant differences regarding Okoume extracts ($p < 0.0001$), and the highest content on fatty acids of low molecular weight was found in the sapwood (17.66 ± 6.90%) than the heartwood (15.87 ± 8.70%) and the bark (7.57 ± 5.70%). However, acetone extracts confirmed the presence of tannins in that hardwood species. Although the limited number of trees, Okoume bark and sapwood tannins content didn't exhibit significant ($p > 0.05$) as depicted in Tab. 1, while the heartwood was the least abundant in tannins (1.33 ± 0.60%). The presence of tannins in Okoume was previously described by Mounguengui et al. [8] who found a polyphenol content of 0.64 ± 0.05% for Okoume heartwood. However other authors using acetone (70%, v/v) as solvent found that Okoume extracts were more abundant in the heartwood than sapwood [9], for samples from Mitzic natural forest. That result underlined the variability and the complexity of wood extracts study depending at least on wood origin and sunshine of heliophilia plant like Okoume. Nevertheless, a deep investigation of Okoume wood extracts variability and other related properties shall concern future studies.

Extractive molecules are assumed to be present in the porous structure of plants [10]. In wood species, polyphenols compounds such as tannins, flavonoids, or other molecules like fatty acids, terpens, fats or oil [10-13] accounted for extracts in the wooden bark.

Table 1: Extracts yield in % of dry matter, obtained by the Soxhlet method of the first collect of Nzamaligue (Nzama 1)

	Petroleum ether	Hexane	Acetone
Sample	Mean scores (± SD)	Mean scores (± SD)	Mean scores (± SD)
Bark	12.24 ± 6.50^{a}	7.57 ± 5.70^{d}	$5.51 \pm 1.90^{d,g}$
Sapwood	8.72 ± 3.30^{b}	17.6 ± 6.90^{e}	4.55 ± 0.50^{g}
Heartwood	6.60 ± 2.00^{c}	15.87 ± 8.70^{f}	1.33 ± 0.60^{h}

N = 3. Means with the same letters are not statistically different at the 0.05 level of significance. Mean ± S.D

3.2 Extracting Yield of Tannins

Water-soluble extractive yields at 80°C are presented in Tab. 2. These extractives should be mainly composed of condensed tannins and polysaccharide residues. The results showed certain homogeneity in the tannins content of Nzamaligue forest which did not display significant difference on their tannins content ($p > 0.05$), excepted between the sapwood and the heartwood of Nzama 2 which showed a $p <$

0.05. However, no significant difference was found for Milole forest tannins content which showed a $p > 0.05$ between the three part of Okoume (Tab. 2). Nevertheless, the samples collected at Mitzic natural forest suggested significant difference between the bark, sapwood and heartwood condensed tannins ($p < 0.05$). When Nzama 1 and 2 are considered as one group labelled Nzamaligue, a fine analysis based on three groups (Nzamaligue, Milolé and Mitzic) exhibited a clear significant difference between the tannins content of Nzamaligue and Mitzic ($p < 0.05$). However, no difference was found between Milolé and Mitzic bark ($p > 0.05$); but some differences on tannins content were found between the bark of Milolé and the heartwood of Mitzic, and the heartwood of Milolé and the sapwood of Mitzic, both displayed a $p < 0.05$. In addition, no difference was observed between the tannins content of Milolé and Nzamaligue bark ($p > 0.05$), while Milolé and Nzamaligue heartwood as well as Nzamaligue heartwood and Milolé sapwood exhibited pointed out significant difference on their tannins content ($p < 0.05$). These results suggested the existence of three distinct forest blocs which should contain differences regarding the tannins content.

Taking into account that chemical compounds do not have the same reaction according to their concentration in a solution, the trees origin should have provoked difference regarding their reactivity toward solvent; thus explaining in some extent the variability observed. Similar results pointed out high standard deviation were already observed in wood pine by Chupin [6].

Table 2: Extract yield obtained with water salt solvent at 80°C, expressed in % of dry matter

	Nzama1	Nzama2	Milolé	Mitzic
Sample	Mean scores (± SD)	Mean scores (± SD)	Mean scores (± SD)	Mean scores (± SD)
Bark	29.33 ± 5.00^{a}	28.33 ± 13.80^{b}	50.33 ± 11.84^{e}	35.17 ± 1.44^{f}
Sapwood	31.67 ± 14.15^{a}	$19.33 \pm 3.62^{b,c}$	27.17 ± 14.68^{e}	48.17 ± 2.02^{g}
Heartwood	32.67 ± 4.31^{a}	$31.17 \pm 4.49^{b,d}$	28.83 ± 12.11^{e}	20.00 ± 1.32^{h}

N = 3. Means with the same letters are not statistically different at the 0.05 level of significance. Mean ± S.D

3.3 Thermogravimetric Analysis

Fig. 1 showed the TGA curves conducted under nitrogen. According to Galletti et al. [14], tannins pyrolysis could lead to the formation of catechin and catechol moiety. A study conducted by Garro Galvez et al. [15] on the thermal decomposition of gallic acid showed that the degradation occurred mainly in three steps. The first one is at 260°C (26-27%) corresponds to the release of carbon dioxide during heating (decarboxylation). The second one is at 360°C (29%) may be due to an additional loss of hydroxyl groups. The last one occurred at 503°C (45%) corresponded to important residues of carbon oxidation (CO_2, H_2O, CO). First derivative peak observed from 150 to 250°C should correspond to sugars degradation as it is commonly observed during various heat treatments of wood. Phenolic groups may be probably degraded in secondary process after 300°C. The different stages of Okoume phenolic thermal decomposition depends strongly on zhtheir structure composition, degree of polymerization and interflavonoids bonds nature as well.

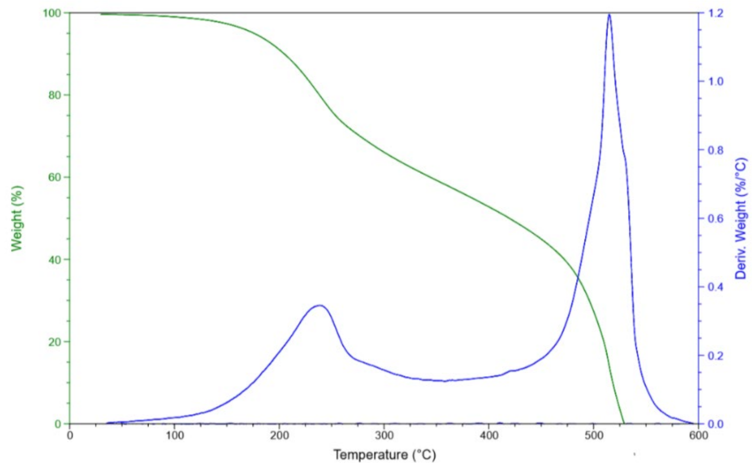

Figure 1: The TGA of Okoume water extractives at room temperature heated at 10 °C/min under nitrogen

3.3 Stiasny Index

The results are presented in Tab. 3. The Stiasny number gave us informations about our extracts regarding formaldehyde reactivity. This test permit us to appreciate the adhesive capacity of tannins [16,17]. Yazaki et al. [18] assessed that the minimum Stiasny value to produce high quality adhesives is 65%. However, Ping et al. [15] produced good adhesives quality with a Stiasny number of 45%. In this study, we obtained Stiasny numbers in the range 50% to 93%. So, whatever the origin of Okoume wood wastes, a strong capability for adhesives was obtained through this Stiasny index analysis.

Table 3: Stiasny number

	Nzama1	Nzama2	Milolé	Mitzic
Sample	Mean scores (± SD)	Mean scores (± SD)	Mean scores (± SD)	Mean scores (± SD)
bark	83.33 ± 11.55	73.33 ± 37.86	50.00 ± 26.46	76.67 ± 25.17
Sapwood	73.33 ± 15.28	93.33 ± 15.28	66.67 ± 15.28	70.00 ± 10.00
Heartwood	53.33 ± 11.55	66.67 ± 49.33	66.67 ± 5.77	60.00 ± 40

N=3

4 Conclusions

The results obtained showed that Okoume is rich in various polar and no polar compounds. The variability of results is high and the maximum amount of extractives through soxhlet extraction method is around 40%. Further work has to be done to analyse the molecular content of each extracts. In addition to its traditional use as panel or plywood, that hardwood wastes revealed good reactivity with formaldehyde with Stiasny test. This property could be used in future, to valorize okoume extractives as raw material for green adhesives production.

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