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Within-tree variability of chemical constituents of *Tessmaniaafricana* from Gabon

[1] Mbagou Jean Bosco, [2] Edou Engonga Prosper, [3] Yves Fortin, [4] Tatjana Stevanovic Janezic

[1, 3, 4] Centre de Recherche des Matériaux Renouvelables (CRMR), Université Laval, Québec, Canada

[2] Département des Sciences Physiques, Ecole Normale Supérieure de Libreville, Laboratoire Pluridisciplinaire des Sciences (LAPLUS), Avenue des Grandes Ecoles, Libreville, Gabon

Corresponding Author: **Edou Engonga Prosper**

Abstract

This work studies the intra-tree variability of the chemical constituents of *Tessmania africana* from Gabon from two trees, at three height levels (DBH, mid-height and under the crown) and two radial positions (sapwood and heartwood). The contents of ethanol-toluene and hot water extracts in tree 1 gave 10.1% (heartwood) and 7.5% (sapwood), and in tree 2; 9.8% (heartwood) and 5.7% (sapwood). The cellulose content is 46.8% (heartwood) against 46.3% (sapwood) for tree 1 and 47.6% (heartwood) and 47.2% (sapwood) for tree 2; with very little variation from tree to tree. The hemicelluloses (pentosanes) gave an average content of 17.1% (heartwood) against 19.4% (sapwood) for tree 1 and 17.4% (heartwood) and 19.0% (sapwood) for the tree 2. The total lignin content in tree 1 is 28.3% (heartwood) and 28.2% (sapwood) and for tree 2; 28.1% (heartwood) and 27.8% (sapwood). Total phenols, flavonoids, hydroxycinnamic acids, proanthocyanidins or condensed tannins are determined by UV-visible spectroscopy. In tree 1, total phenols vary from 248 to 434 mg gallic acid equivalents/g of dry extract and are more abundant in the heartwood, particularly at mid-height. For tree 2, the total phenols are also concentrated in the heartwood, 397 mg gallic acid equivalents/g of dry extract. The content of hydroxycinnamic acids varies between 112.6 and 193.1 mg of chlorogenic acid equivalent/g of dry extract in the heartwood against 82.0 and 153.5 mg of chlorogenic acid equivalent/g of dry extract in the sapwood for tree 1, and values varying from 103.5 and 166.8 mg of chlorogenic acid equivalent/g of dry extracts for the heartwood and 81.4 and 179.2 mg of chlorogenic acid equivalent/g of dry extracts in the sapwood for tree 2. The hydroxycinnamic acids indicate a higher concentration in the heartwood than in the sapwood and maximum values at mid-height in the sapwood for the two trees. Proanthocyanidins vary between 6.3 and 74.8 mg cyanidin chloride equivalent/g dry extract in sapwood and 43.6 and 93.6 mg cyanidin chloride equivalent/g extract dryness in the heartwood for tree 1 and values varying between 17.6 and 44.8 in mg of cyanidin chloride equivalent/g of dry extract in the sapwood; 17.6 and 68.0 in mg of cyanidin chloride equivalent/g of dry extract in the heartwood for tree 2. *Tessmania africana* is a tropical species rich in phenolic compounds which are more concentrated in the heartwood and in the mid- height.

Keywords: Tessmania Africana, Cellulose, Pentosans, Lignin, Extract Content, Polyphenol Assay

1. Introduction

Tessmaniaafricana or Wamba is a tropical tree species from the Congo Basin forests (Maisonneuve and Manfredini 1988) [16]. This designation is accepted in all the countries of the Central African sub-region. Wamba has three varieties and is part of the botanical family *Caesalpiniaceae: Tessmanialescrauwaetii*, *Tessmaniaabnormala* and *Tessamaniaafricana.* Three clues make it easy to differentiate them in the forest. These are their base, bark and foliage. *Tessmaniaafricana*has a thickened base. Its bark is dark gray, vertically fissured, hard and brittle. Its greyish-pink sapwood is quite different from its reddish-brown heartwood. There is little literature on this species. The *Tessmaniaafricana* type Wamba bears fruit with circular, flat pods five centimeters in diameter covered with thorn-like warts. These fruits secrete a colorless sticky resin. The seeds generated by this tree range from 1 to 4 per fruit and are black in color. They are hard, flattened and smooth. Botanical details on the other two varieties are available in the volume of Faure and Vivien (2011) "Trees and tropical forests". In general, the Wamba is a large tree with a diameter of between 0.80 m and 1.30 m, for a height that varies between 25 and 48 m. Its trunk is without buttresses or considerable thickenings. Depending on the country of origin, this species may have a specific name. For example, in Gabon it is known as Nkaga, in Cameroon by Esingang, in Congo by Pamiel and in the Democratic Republic of Congo by Wamba. this species is "Wamba". Some authors estimate that this wood would be a hardwood and that its density would be between

800 and 950 kg/m3 (Maisonneuve and Manfredini 1988) [16] while (Faure and Vivien 2011) estimate that it would be between 1000 and 1100 kg/m^3. No chemical literature review currently exists on these species (*Tessmania*spp.). The Wamba is a tropical species little known from the Gabonese evergreen forest by industrialists and researchers in Wood Sciences for its potential uses in the wood industries. However, tropical woods are considered noble for several reasons: their high density, their hardness, their natural durability, their richness in color, their varied and sought-after appearance as well as their ease of implementation. In addition, a large number of tropical species produce wood with superior properties to wood from temperate climate species (Fouquet 2009) [11], which allows them to be used in construction for jobs subject to high stresses. Our article is based on a stratified sampling reduced to two trees. The diameters of the two trees cut at breast height were 90 cm for the first and 60 cm for the second. The study of the variability of these different chemical constituents will be based on two radial positions (sapwood and heartwood) and three levels of height (breast height, mid-height and close to the crown). This publication therefore highlights the variability intra-tree chemical constituents of Wamba wood by determining the chemical indices (cellulose, pentosans or hemicelluloses, lignin), the dosage of phenolic compounds (total phenols, flavonoids, hydroxycinnamic acids, proanthocyanidins or condensed tannins), the contents of extracts totals. Indeed, several authors have highlighted the role of extractables on the durability of wood and mainly polyphenols. These chemical substances influence the physico-mechanical properties of wood (Déon *et al*. 1980).

2. Materials and methods

Purchase of chemicals

All these products were required for analysis. Concentrated H_2SO_4(sulfuric acid) (95-97%), Anhydrous ethanol, toluene, Folin-Ciocalteu's reagent, gallic acid, $AlCl_3$(aluminum trichloride), quercetin, butanol, cyanidin chloride, HCl (hydrochloric acid), Arnow's reagent, NaOH (sodium hydroxide), chlorogenic acid; HNO_3(nitric acid), dinitro-2-4-phenylhydrazine DPNH were also purchased from Sigma-Aldrich, Carlo Erba or Prolabo and used without further purification.

Plant material

The samples of *Tessmaniaafricana* wood come mainly from two trees most representative of the marketable dimensions taken in May 2012 in the forest concession under sustainable management of the company John-Bitar Co. Ltd located in Otoumbi in the department of AbangaBigné (Ndjolé), Gabon.

The diameters of the trees sampled as part of our study are as follows: Tree 1: DBH1: 0.90 m, total trunk length: 24.3 m; Tree 2: DBH2: 0.60 m, total trunk length: 18 m. Log cutting and sample preparation The ridge sampling phase at three height levels was carried out using a commercial chainsaw. Thus, a ridge 1 m long was taken at breast height (HP), mid-height (MT) and under the crown (SC). From each ridge, a central plateau 12 cm thick was cut. These trays, previously identified by a number, were coated with a CH_3BR antiseptic solution at a concentration of 50 g/m3 for 20 h at 30° C.

Upon their arrival at University of Laval, the wooden trays were kept in the freezers of the Department of Wood and Forest Sciences of University of Laval until the start of sample preparation in the fall of 2013. Throughout the text, the following acronyms will be used. For tree number 1 we have: HP1: wood located between 1.3 and 2.3 m from the ground; MT1: wood between 13.3 m and 14.3 m from the ground; SC1: wood located between 23 and 24 m from the ground. For tree number 2: HP2: wood located between 1.3 and 2.3 m from the ground; MT2: wood between 8 m and 9 m from the ground; SC2: wood located between 17 and 18 m from the ground.

Preparation of sawdust

Obtaining the sawdust for the various tests began with the fragmentation and grinding of the wood, a crucial step in determining the chemical composition of the wood. The wooden sticks were first shredded with a chisel to facilitate the grinding on the one hand and to avoid any possible modification of the various chemical elements by excessive heating of the machine during this operation. The fragments obtained were then introduced in small quantities into the two hammer mills. This approach is necessary to obtain a suitable grain size whose fraction is between 40 and 60 mesh, thus corresponding to the requirements of the ASTM D1105 (1996) standard in terms of grain size to quantify the rate of wood extract. Finally, the powder collected was sieved and kept in the identified plastic bags until the time of the chemical tests. Contents of total extracts Soxhlet extraction.

The extraction of the wood powder was first carried out using a Soxhlet in an anhydrous ethanol-toluene mixture (Stevanovic and Perrin 2009) [25]. The extraction is carried out continuously for 6 h. This consists of weighing 20 g of sawdust in an anhydrous state and depositing it at the bottom of the cellulose cartridge in the central tube of the extraction Soxhlet. A cotton pad then closes the cartridge at its upper level. The ethanol-toluene mixture is obtained in a ratio of 1000 ml of ethanol for 427 ml of toluene thus corresponding to an azeotropic mixture. A sample of this mixture is then poured into a 250 ml flask weighed beforehand before placing it in a boiling flask heater at 60° C. Thus, under the effect of the heat, the solvent evaporates from the flask then condenses at the level of the reflux condenser and falls drop by drop on the sawdust immediately engaging a liquid solvent level equilibrium with the siphon. The last drop, which will break this balance, automatically draws all the solvent with the dissolved extracts into the flask. The solvent condenses again in the condenser and the cycle begins again. At the end of the operation, the flask containing the extracts dissolved in the solvent is placed in a rotary evaporator under vacuum. The solvent is thus evaporated and the extracts remain stuck to the inner wall of the flask. After placing the flask in an oven at 102 ºC for 24 h followed by 60 min of cooling in the desiccator, the mass of the flask with extracts is determined. Thus, knowing the mass of the empty balloon and that with the extracts, it is possible to calculate the percentage of extractables with the ethanol-toluene mixture compared to the mass of the initial wood using the equation (1):

$$R(\%) = \frac{Mext}{Méch} \times 100 \quad (1)$$

Where

R: Soxhlet extraction yield in %;

Mext: mass of the extract after evaporation in mg;

Mech: anhydrous mass of the wood powder sample in mg before Soxhlet extraction.

Each extraction was made with three repetitions for each type of extraction in order to reduce the risk of experimental errors.

Hot water extraction Hot water extraction consists of putting about 2 g of wood powder in a 250 ml flask and pouring 100 ml of distilled water into a heating mantle under reflux condenser. After boiling for 3 h, the contents of the flask were filtered with a filtering crucible of porosity C, weighed beforehand and then proceeded to wash this same powder with 1 liter of hot water. The crucible is dried in an oven at 102°C for 24 hours. The extracted powder was weighed to determine the percentage of hot water extractables compared to the initial anhydrous mass. The extraction of the remaining wood powder was air dried to determine the moisture using equation (2):

$$R(\%) = \frac{(M1-M2)}{M1} \times 100 \quad (2)$$

Where

EC: hot water extraction yield;

M1: mass of anhydrous wood powder after Soxhlet extraction;

M2: mass of anhydrous wood powder after extraction with hot water.

The powder is then air-dried and stored in polyethylene bags for determination, after stabilization, of the starting humidity of the sawdust in order to assess the content of cellulose, hemicelluloses (pentosans) and total lignin. In order to determine the different classes of polyphenols of ethanolic extracts in Wamba wood, the so-called maceration method was used. Thus, each wood powder from the two radial positions (sapwood-heartwood) and from the three height levels was placed in a 1:10 ratio (10 g of dry fibers in 100 ml of 95% ethanol) in an Erlenmeyer flask of 250 ml glass closed with a rubber stopper covered with aluminum foil. The aluminum prevents the ethanol from coming into contact with the rubber, which prevents possible contamination from the rubber. The Erlenmeyer flasks were also entirely covered with aluminum foil to prevent the photosensitive molecules from degrading by light. The mixtures were then placed under stirring on a shaker (11 orbital Barnstead 4633) for 24 h at 200 rotations per minute. After 24 h, each sample was separated by vacuum filtration through a Whatman No. 4 filter in a Büchner type funnel and the fibers were rinsed with an additional 100 ml of 95% ethanol to ensure that all extracts are found in the filtrate rather than adsorbed on the fiber surface. The extracts were then dried in a pre-weighed flask on a rotary evaporator in a bath at 40°C until complete evaporation and were then placed in a vacuum oven at 40°C for approximately 36 h or until a constant mass is measured. The dry extracts were re-dissolved in 100% ethanol (Greenfield Ethanol Inc.) at 10 mg/ml, filtered using syringes on 0.45 µm cellulose acetate filters (VWR Inc.) and stored at -20°C in amber glass bottles. It should be noted that a color difference is observable between the sapwood and the heartwood overall. This method has already been followed by several authors, including St. Pierre (2012) [26].

Determination of macromolecular chemical constituents

Determination of cellulose

For the dosage of cellulose, it was first necessary to submit the wood powder to the attack of a mixture of nitric acid and ethanol. Thus, lignin is transformed into alcohol-soluble and water-insoluble nitrophenol products. Hemicelluloses, for their part, are largely hydrolyzed, degraded and go into solution. The method therefore consists of weighing approximately 1 g of wood powder in a 250 ml Erlenmeyer flask. Then pour these powders into a beaker with 15 ml of HNO_3, then quickly 60 ml of alcohol, previously measured in the graduated cylinder. This mixture is poured into the Erlenmeyer flask with a ground neck containing the sawdust. The mixture is brought to a water bath with reflux condenser while gently swirling the flask frequently as boiling begins. After boiling for 1 hour, the contents of the flask are filtered through a crucible of porosity C previously weighed and placed on the vacuum flask. A fresh mixture of alcohol (50 ml) and nitric acid (10 ml HNO_3 - 40 ml ETOH) is poured three times into a flask. Finally, vacuum filtering followed by careful washing of the residue, first with ethanol then with cold water and finally with hot water (about 1 liter) is carried out. Afterwards, this process is followed by drying of this sawdust in an oven at 102°C for 24 hours. A new weighing of the dried powder carried out, the cellulose content is determined according to the average of the triplicate obtained. The cellulose index was determined using the Kurschner and Hoffner method (Stevanovic and Perrin 2009) [25]. The determination of this index was carried out using wood powder extracted with ethanol-toluene beforehand. A total of 36 replicates (3 replicates per height level in sapwood and heartwood from six samples per tree) were needed to determine the cellulose index and ultimately assess its level of intra-tree variation in the *Tessmaniaafricana.*

Determination of pentosans

Hemicelluloses were determined as pentosan levels, based on the CPPA-G-12 standard (Stevanovic 2014b) [24]. The measurement consists in subjecting the wood powder to attack by concentrated hydrochloric acid (azeotrope at 131 ± 0.5 g/l) and in dosing the furfural thus obtained, after the precipitation of the hydrazone obtained by the action of dinitro-2-4-phenylhydrazine (DPNH) on the aldehyde group of furfural. By weighing the hydrazone formed, it was possible to determine the weight of the furfural and, taking into account the yield of furfural, the level of pentosans, also called the furfural index. 300 to 500 g of ground material are weighed (to the nearest 0.1 g) in a 250 ml flask in which a few grains of pumice stone and 120 ml of concentrated hydrochloric acid at 131 g/l have been placed. The flask is placed on the pentosan device while introducing 30 ml of concentrated hydrochloric acid at 131 g/l into the flask to be separated and the valve of the latter being closed. The mixture was boiled for 10 min to collect about 30 ml of distillate. A few drops were decanted from the contents of the flask to return another 30 ml of acid to the flask to be decanted and continue the experiment until reaching 270 ml of distillate. After having transferred the contents of the test tube into a 600 ml beaker and rinsed the test tube with 2N hydrochloric acid, 150 ml of DNPH, filtered on an F

crucible, are added while rubbing the walls, leaving to stand for 18 h. The crucible of porosity F, previously weighed, is placed on the vacuum flask in order to let the contents of the beaker pass with the minimum possible vacuum. The precipitate is washed with 50 ml of a 2N hydrochloric acid solution (dissolution of excess DNPH), then in water until the Cl^- ions disappear (negative test for $AGNO_3$). Finally, drying in an oven at 102° C. for 24 hours is carried out before weighing and determining the rate of pentosans relative to the dry wood (average of 3 tests). The mass of furfural is given by formula (3):

Mass of furfural = Mass of precipitate x 0.348 (3)

Equivalence to the level of pentosans is obtained by using an average yield of 80.5% for furfural (88.4% for xylans and 74.3% for arabans).

where:

Mass of pentosans = Mass of furfural x 1.71 (4)

Determination of total lignin Klason

The lignin determination was carried out according to the modified Klason lignin method (Stevanovic 2014b) [24]. It consists of accurately weighing in a 16 x 100 mm test tube (test tube), 225 to 250 mg of extracted wood powder which is mixed with 3 ml of 72% H_2SO_4. After impregnation using a glass rod, the specimen is placed in a temperature-controlled bath (30°C) for one hour. The contents of the test tube are then transferred to a 500 ml Erlenmeyer flask using 84 ml of deionized water. Before being transferred to the autoclave (for 1 hour at 125°C), the 500 ml Erlenmeyer flask was covered with another, but smaller one (50 ml). The filtering of the lignin on a crucible takes place after the samples have been cooled. The lignin is then washed with demineralised water until a neutral pH is obtained. Finally, the lignin is dried in an oven at 103°C until constant weight. The lignin percentage is calculated using equation (5) below:

Lignin (%) = (Mass of lignin) / (Anhydrous mass of sawdust) × 100 (5)

Determination of acid-soluble lignin Acid-soluble lignin (a particularly important measurement for hardwood lignins, which are susceptible to degradation and solubilization in acidic conditions) is measured by filtrate spectrophotometry by introducing, first by pipette, 1 ml of concentrated filtrate into a 16 x 150 mm test tube to which 15 ml of 4% H_2SO_4 has been added. The absorbance of the solution was finally measured at 205 nm using a quartz cuvette and 4% H_2SO_4 as reference solution. The following formula allows the determination of the acid-soluble lignin concentration (6):

Undiluted filtrate: Lignin (g/*l*) *c*= A110 (6)

Where

A: absorbance. Diluted filtrate: Lignin (*g*/*l*) *c*= (A/110) × (7)

Where

D =Vfinal/(Vinitial) (9)

With: Vend: End volume Vinitial: Initial volume

All this analysis is carried out taking into account the volume of the substrate and the dry mass of the sawdust analyzed:

L a.s=(C.V.100)/(1000 ((Mo)(100⁄100-e))×100 (9)

Where

L a.s: concentration of acid-soluble lignin in the non-extracted dry ground material (%);
C: concentration of lignin in the filtrate (g/l);
V: total volume of the filtrate (87 ml)
Mo: anhydrous mass of the crushed material extracted in (g);
E: percentage of extracts contained in the wood powder (%).

Essays of phenolic compounds

Dosage of total phenols

The determination of total phenols was carried out using the Folin-Ciocalteu method adapted by Diouf *et al*. (2009) [7]. It is a colorimetric method based on the fact that Folin's reagent is reduced, during the oxidation of phenols, to a mixture of blue tungsten oxide and molybdenum. The blue color produced, whose maximum absorption is at 755 nm, is proportional to the amount of polyphenols present in the aqueous extract. The determination of the content of total phenols in the extract requires drawing a calibration curve of a standard phenol standard. The calibration curve used during our experimentation with gallic acid as a standard concerns total phenols. The results are expressed in mg gallic acid equivalent/g of dry plant matter by referring to the gallic acid calibration curve. Dosage of flavonoids Flavonoids are another category of phenolic compounds. Their contents were measured by a method adapted by Diouf *et al*. (2009) [7] and initially developed by Brighente *et al*. (2007) [3]. This colorimetric method uses aluminum trichloride ($AlCl_3$) as a reagent. It is based on the fact that the trichloride establishes a dative bond with the free doublets of the oxygen of the OH groups of the flavonoids, producing a complex of yellow color. The coloring of the complex will then be measured by UV spectrometry under the same conditions as the total phenols, but this time the maximum absorbance is measured at a wavelength of 415 nm. The flavonoid content in the ethanolic extract will then be calculated from a calibration curve presented in appendix 4 and whose standard used is quercetin, a flavonol. The results are expressed in mg quercetin equivalent/g of dry plant matter. Proanthocyanidins Proanthocyanidins are more or less cross-linked polyphenols also called condensed tannins. They consist mainly of a more or less complex assembly of catechin and epicatechin. The method for assessing their content uses acidified butanol as a reagent (Diouf *et al*. 2009, St. Pierre, 2012) [7, 26]. This method is based on the transformation of proanthocyanidins into red-colored anthocyanidins by breaking interflavanic bonds in an acid medium followed by oxidation, in the presence of Fe^{3+} as a catalyst. The anthocyanidins in the form of cyanidins will be used for the quantification of the proanthocyanidins present in the aqueous extract attributed to cinnamic acids by UV-visible spectrometry. The absorbance is measured at the wavelength of 550 nm. The results are expressed in mg equivalent of cyanidin chloride/g

of dry plant matter by referring to the calibration curve. Hydroxycinnamic acids Hydroxycinnamic acids are low molecular weight polyphenols derived from the biosynthesis of lignin. The method for evaluating their content in the aqueous extract is that of Singh (1987) [23]. This method consists of successively adding to 1 ml of the aqueous extract (1 mg/l), 1 ml of 0.5 MolHCl, 1 ml of Arnow's reagent (sodium nitrate 10% m/v + sodium molybdate 10% m/v in water), 1 ml of 2.125 MolNaOH and 1.5 ml of distilled water. The solution is then stirred and then using a UV-visible spectrometer then its absorbance is read at 525 nm. Unlike other polyphenols, the blank of the aqueous extract is produced, which contains all the reagents except Arnow's reagent. The latter is replaced by distilled water. A subtraction between the absorbance of the solution containing Arnow's reagent and its blank makes it possible to calculate approximately the real absorbance of the aqueous extract attributed to the cinnamic acids. Chlorogenic acid which is a hydroxycinnamic acid was used as a standard molecule. The results are expressed in mg chlorogenic acid equivalent/g of dry matter by referring to the calibration curve.

3. Results and discussions

Total extract content Yields of extracts soluble in ethanol-toluene and hot water

The results reported in Table 1 represent the contents obtained in each of the two trees by level of height and radial position for a total number of 36 repetitions. Fengel and Wegener (1984) [10] believe that hot water extraction dissolves alkaloids, proteins, tannins, carbohydrates, coloring matter, etc. While the ethanol-toluene mixture also extracts coloring matters, tannins, stilbenes as well as flavonoids and anthocyanins. The results in Table 1 show that in the heartwood of tree 1, the yields of the soluble extracts from the ethanol-toluene extraction vary from 3.7% to 6.6%, whereas those of the hot water extraction 3.9% to 5.9%. For the sapwood of the tree1, the yields vary from 3.3 and 4.1% to ethanol-toluene and from 3.1 and 4.5% to hot water. The average contents of total extracts in tree 1 are 10.1% in the heartwood and 7.5% in the sapwood. In the heartwood tree 2, the ethanol-toluene extracts vary from 3.6% to 6.9% and those with hot water between 3.7% and 5.7%. The sapwood of this tree gives yields varying between 2.7% and 4.2% for the azeotropic mixture and between 2.8 and 5.1% for hot water. The average in total extracts found in tree 2 is similar to that of tree 1, i.e., 9.8% in the heartwood and 6.8% in the sapwood. The statistical analysis in Table 2 shows a significant difference in the content of hot water-soluble extracts in the radial position at mid-height (P = 0.02 and F = 11.2). However, there is no height effect (P = 0.645 and F = 0.48). The extracts soluble in the ethanol-toluene mixture do not indicate any significant effect either in the radial or axial positions. Even if the content of extracts in Wamba wood is very high compared to wood from temperate zones, it is however lower than those reported by Saha (2015) [22] in the heartwood of Moabi (Baillonellatoxisperma). The author measured extract yields of 7.8% in ethanol-toluene versus 8.4% in hot water. The same is true for Tali (*Erythriphleumivorense*) for which the author indicates a solubility in extractables of 8.5% in the azeotropic mixture and 10% in hot water. This is not the case in the heartwood of Movingui (*Distemonanthusbenthamaianus)* where the yields were 8.7% with ethanol-toluene and 3.8% with hot water.

Table 1: Variation in the content of total extracts (%) in Wamba wood Extract content (%)

	Tree 1			Tree2		
Height	Ethanol/toluene	Hot water	Total 1	Ethanol/toluene	Hot water	Total 2
SCD	3,7	5,9	9,6	4,1	5,5	9,6
MTD	4,4	5,9	10,3	3,6	5,7	9,3
HPD	6,6	3,9	10,5	6,9	3,7	10,6
Average	4,9	5,2	10,1	4,9	5,0	9,8
Standard deviation	1,5	1,2	0,5	1,8	1,1	0,7
COV (%)	31	22	5	37	22	7
SCA	4,1	4,5	8,6	4,2	5,1	9,3
MTA	3,3	3,1	6,4	2,7	2,8	5,5
HPA	3,6	3,9	7,5	2,7	3,0	5,7
Average	3,7	3,8	7,53,2	3,2	3,6	6,8
Standard deviation	0,4	0,7	1,1	0,9	1,3	2,1
COV (%)	11	18	15	27	35	31

Table 2: Statistical analysis of the variation in total extract content in Wamba wood

	Extracts soluble in hot water (%)				Ethanol-toluene soluble extracts (%)			
Height	Sapwood	Heartwood	P Rd	FRd	Sapwood	Heartwood	P Rd	F Rd
SC	4.21	5.90	0.123^{ns}	3.44	3.86	5.26	0.561^{ns}	0.39
MT	3.02	6.09	0.02^{ns}	11.26	3.01	3.99	0.303^{ns}	1.32
HP	4.75	5.53	0.431^{ns}	0.73	3.39	3.99	0.160^{ns}	2.71
Ht x Rd	0.296^{ns}	1.58			0.779^{ns}			0.26
P Ht	0.645^{ns}	0.02		12.26	0.256^{ns}	0.105^{ns}		3.90
F Ht	0.48				1.81			

Cellulose content

Cellulose content results are shown in Table 3. Cellulose content for Tree 1 ranges from 46.1% to 46.4% in the sapwood and from 45.8% to 48.7% in the heartwood. For this tree, the average cellulose yields are 46.3% in the sapwood and 46.8% in the heartwood. In tree 2, the cellulose content is between 46.3% and 47.8% in the sapwood and between 46.9% and 48.5% in the heartwood.

The average cellulose content in Tree 2 is 47.2% in the sapwood and 47.6% in the heartwood. These results show that for the two trees, the cellulose levels vary little for the radial and longitudinal positions. The statistical test carried out for this purpose and whose results are recorded in Table 4 shows no significant difference in the radial position (P = 0.413 and F = 0.80) between the different height levels (P = 0.357 and F = 1.27). Furthermore, the cellulose content of Wamba wood is significantly higher than that found in most African tropical woods. To this end, Gérard *et al*. (1998) [12] indicates that the heartwood of Bilinga (*Naucleadiderricchii*) would have a cellulose content of 42.1%, that of Izombé (*Testuelagabonesis*) would be 35.2% and Limba (*Terminaliasuperba*) 41.7%. Doat (1972) found 39.8% cellulose content for Azobé (*Lophiraalata*). Our results are rather close to that of MinkuéM'Ény (2000) [17] with averages of 49.42% in the sapwood and 50.43% in the heartwood of Okoumé wood (*Aucoumeaklaineana*). Bruchert and Gardiner (2006) [4] explain that the rate of cellulose which is a little higher in the upper part of the tree would allow the latter to fight against environmental constraints, in particular the wind, the influence of which is variable over time and intensity.

Table 3: Variation in the proportion of cellulose in the two trees

Height	Cellulose content (%) Tree 1		Cellulose content (%) Tree 2	
	Sapwood	Heartwood	Sapwood	Heartwood
SC	46,4	48,7	47,8	48,5
MT	46,1	45,9	46,3	47,5
HP	46,3	45,8	47,5	46,9
Average	46,3	46,8	47,2	47,6
Standard deviation	0,2	1,6	0,8	0,8
COV(%)	04		2 2	

Table 4: Statistical analysis of cellulose variation in Wamba wood

Cellulose (%)				
Heigth	Sapwood	Heartwood	P Rd	FRd
SC	47.1	48.6	0.679 ^{ns}	0.19
MT	46.2	46.7	0.948^{ns}	0.00
HP	46.9	46.4	0.114 ^{ns}	3.67
Ht x Rd			0.305^{ns}	1.53
P Ht	0.37^{ns}		0.413^{ns} 0.80	
F Ht	1.27			

Hemicellulose (pentosans) content

The proportion of hemicelluloses obtained by the hydrolysis of hydrochloric acid gives a furfural index which, multiplied by 1.874, transforms the xylans into pentosans. Table 5 summarizes the results obtained on the variation of the pentosan content. This table indicates a more abundant concentration of pentosans in the sapwood. Note that in tree 1, the pentosan content varies from 18.0% to 21.9% in the sapwood and 16.2% and 17.7% in the heartwood. These results obtained in tree 1 are similar to those of tree 2 with a content varying from 17.3% to 19.9% in the sapwood and 16.4% and 18.4% in the heartwood. For the two trees, the average pentosan content is 19.2% in the sapwood against 17.3% in the heartwood. The results also indicate a VOC variation ranging from 8% to 11% in the sapwood against 5% to 6% in the heartwood. The statistical test performed (Table 6) shows that the pentosan content is significantly more abundant in the sapwood, but only in the wood close to the crown (P = 0.04 and F = 8.46). This result is consistent with the biology of the sapwood since it contains reserve substances such as starch, thus helping to make it less resistant to biological agents. Concerning the height in the tree, the variation in pentosans is not significant. These yields of pentosans in *Tessmaniaafricana* species are in the order of magnitude of those found in a wide range of tropical woods. To this end, MinkuéM'Ény (2000) [17] reports percentages of 15.2% in the sapwood and 15.1% in the heartwood of Okoumé (*Aukoumeaklaineana*). Gerard *et al*. (1998) [12] indicate percentages of 17.9% in Tola (*Gossweilerodendronbalsamiferum*), and 18.1% in Gombé wood (*Didelotiaafricana*) then 14.0% in Izombé (*Testuelagabonesis*).

Table 5: Variation in pentosan content in the two trees

Height	Pentosan content (%) Tree1 Heartwood	Sapwood	Pentosan content (%) Tree 2 Heartwood	Sapwood
SC	17.3	21.9	17.3	19.8
MT	17.7	18.3	16.4	17.3
HP	16.2	18.0	18.4	19.9
Average	17.1	19.4	17.4	19.0
Standard deviation	0.9	2.2	1.0	1.5
COV(%)	5	11	6	8

Table 6: Statistical analysis of pentosan variation in Wamba wood

Hemicelluloses (Pentosans)				
Height	Sapwood	Heartwood	P Rd	FRd
SC	20.82	17.40	0.04^{ns}	8.46
MT	17.82	16.99	0.514^{ns}	0.49
HP	19.00	16.99	0.149^{ns}	2.90
Ht x Rd		0.370^{ns}	1.22	
P Ht	0.211^{ns}	0.028^{ns}	9.41	
F Ht		2.27		

Total lignin content

The results relating to the variation of the total lignin content (Table 7) come from the addition of the Klason lignin and the soluble index. The concentration of total lignin varies little from sapwood to heartwood and from base to top of tree trunks. Thus, in tree 1 for example, the total lignin varies between 27.0% and 29.7% in the heartwood against 26.7% and 28.9% in the sapwood. In this tree, the average total lignin content remains similar with 28.3% in the heartwood and 28.2% in the sapwood. The coefficients of variation are also close with respectively a VOC of 5% in the heartwood against 4% for the sapwood. We find almost the same trend of variation in tree 2 in which the average concentration of total lignin is 28.1% in the heartwood against 27.8% in the sapwood. The VOCs are 6% in the heartwood and 2% in the sapwood. Student's test indicates neither a height effect (P = 0.537 and F= 0.71) nor a radial effect (P = 0.323 and F= 0.528) of lignin yield as reported in Table 8. The lignin contents presented in Table 7 are similar to those reported in the literature, in particular for tropical woods. Nuopponen *et al*. (2006) [19] report that the lignin content of tropical hardwoods can exceed that of softwoods to be between 29% and 41%. Along the same lines, Gerard *et al*. (1998) [12] indicate that the lignin content of Tola wood (*Gossweilerodendronbalsamiferum*) is 28.3% while MinkuéM'Ény (2000) [17] reveals that in Okoumé (*Aukoumeaklaineana*), the percentage of lignin is around 30%. in the heartwood and 28% in the sapwood. Barton and McDonald (1971) [2] report that in western cedar heartwood the lignin content is 29.0% at the butt end of the tree trunk, 28.1% at mid-height and 28.3% at the end end while in the sapwood, the lignin content was 32.3% at the large end, 31.4% at the mid-height and 29.4% at the end end. Muller *et al*. (1987) [18] indicate that the lignin concentration in plant tissues varies little within the same species. It is good to note that lignification is necessary for all the tissues which serve for the conduction of the sap. Lignin also contributes to the rigidity of cell walls and ensures the erect habit of plants (Panshin and de Zeeuw 1980, Stevanovic and Perrin 2009) [20, 25]. It can be noted that in general, the variation in the yield of each of the structural chemical constituents in the Wamba wood does not show a significant difference between sapwood and heartwood as well as the three height levels.

Table 7: Variation in lignin yield in Wamba wood

	Lignin content (%) Tree 1			Lignin content (%) Tree 2		
Heigth	Lignin Klason	Soluble acid	Total lignin	Lignin Klason	Soluble acid	Totallignin
SCD	27	1.1	28.2	25.9	1.5	27.4
MTD	25.7	1.4	27.0	27.8	2.1	29.9
HPD	28.4	1.3	29.7	25.3	1.7	27.0
Average	27.0	1.3	28.3	26.3	1.8	28.1
Standard deviation	1.4	0.2	1.4	1.3	0.3	1.6
COV (%)	5	12	5	5	17	6
SCA	27.1	1.9	29	26.1	1.9	28.0
MTA	24.9	1.8	26.7	25.4	1.6	26.8
HPA	27.5	1.2	28.7	26.0	1.8	27.9
Average	26.5	1.5	28.2	26.1	1.8	27.8
Standard deviation	1.4	0.4	1.2	0.4	0.2	0.7
COV (%)	5	25	4	1	8	2

Table 8: Statistical Analysis of Lignin Variation in Wamba Wood

Total lignin (%)				
Heigth	Sapwood	Hearwood	P Rd	FRd
SC	28.5	28.0	0.612^{ns}	0.29
MT	26.6	28.4	0.142^{ns}	3.03
HP	28.3	28.2	0.980^{ns}	0.00
Ht x Rd		0.323^{ns}	1.43	
P Ht	0.537^{ns}	0.528^{ns}	0.46	
F Ht		0.71		

Table 9: Summary of yields of chemical indices at breast height

Chemical indices (%) Tree 1			Tree 2	
	Sapwood	Heartwood	Sapwood	Heartwood
Cellulose	46.1	45.8	47.5	46.7
Pentosans	18.0	16.2	19.9	18.4
Total lignin	28.7	29.7	27.9	27.0
Total extracts	7.5	10.5	5.7	10.6
Total (%)	100.3	102.2	101.0	102.7

Table 10: Summary of chemical index returns at mid-height

Chimical indices (%)	Tree 1		Tree 2	
	Sapwood	Heartwood	Sapwood	Heartwood
Cellulose	46.1	45.9	46.3	47.5
Pentosans	18.3	17.7	17.3	16.4
Total lignin	26.7	27	26.8	29.9
Total extract	6.4	10.3	5.5	9.3
Total (%)	97.5	100.9	95.9	103.1

Table 11: Summary of chemical indices near the crown

Chemical indices (%)	Tree1		Treee 2	
	Sapwood	Heartwood	Sapwood	Heartwood
Cellulose	46.4	48.7	47.8	48.5
Pentosans	21.9	17.3	19.8	17.3
Totale lignin	28.9	28.2	28	27.4
Total extracts	8.6	9.6	9.3	9.6
Total (%)	105.8	103.8	104.9	102.8

Content of phenolic compounds

Total phenols examination of Table 12 shows that in the sapwood of tree 1, the total phenols vary from 211 mg to 348 mg gallic acid equivalents/g of dry extract. In heartwood, total phenols range from 248 to 434 mg gallic acid equivalents/g dry extract. In tree 2, the yield in total phenols is between 125 and 290 mg gallic acid equivalents/g of dry extract in the sapwood against 281 and 397 mg gallic acid equivalents/g of dry extract in the heartwood. With one exception, the total phenol content is significantly higher in heartwood than in sapwood. We also observe that the maximum values are found at mid-height except for the sapwood of tree 1. The Student test in Table 13 indicates a very significant difference (P = 0.01 and F = 20.67) in the concentration of total phenols between the sapwood and the heartwood. Statistical analysis also reports a very significant height effect (P = 0.001 and F = 13.76) on total phenols. In addition, the effect of height position is related to the effect of radial position (P = 0.001 and F = 14.38). The total phenol contents of ethanol-soluble *Tessmaniaafricana* wood are of the same order of magnitude as the results of St. Pierre (2012) [26] who found concentrations of 203 ± 25 mg gallic acid equivalents/g (AGE/g) in the heartwood of yellow birch (*Betulaallenghaniensis*) and 296 ±27 mg gallic acid equivalents/g dry extract in heartwood of sugar maple (*Acer saccharum*). Compared to tropical woods, the wood of *Tessmaniaafricana* gives significantly higher yields than those published for some African tropical woods from Cameroon. Indeed, Saha (2015) [22] reports contents of 163 mg gallic acid equivalents/g of the acetone extract against 120 mg gallic acid equivalents/g of the dry ethanol-toluene extract in the heartwood of Movingui (*Distemonanthusbenthamaianus*), and 165 mg gallic acid equivalents/g dry extract in Padouk heartwood (*Pterocarpussoyauxii*).

Table 12: Variation in the concentration of total phenols in the two trees

	Tree 1		Tree 2	
Height	Sapwood (AGE/g)	Heartwood (AGE/g)	Sapwood (AGE/g)	Haetwood (AGE/g)
SC	348	248	278	281
MT	309	434	290	397
HP	211	349	125	315

Table 13: Test student of variation of total phenols

Total phenols (mg eqgallicacid/g de PS)				
Heigth	Sapwood	Heartwood	P Rd	FRd
SC	264	313	0.159^{ns}	2.74
MT	299	415	0.01	15.56
HP	168	332.20	0.001	31.10
Ht x Rd			0.001	14.38
P Ht	0.001		0.001	20.63
F Ht		13.76		

Flavonoids

Table 14 presents the flavonoid content in the two Wamba trees. In the heartwood of tree 1, the flavonoids vary between 9 and 13 mg quercetin equivalents per g of dry extract (mg eqQu/g PS) and from 3.5 to 11 mg eqQu/g PS in the sapwood. In tree 2, the flavonoid content varies from 12.0 to 22.0 mg eqQu/g SP in the heartwood and from 10.0 to 19.0 mg eqQu/g SP in the sapwood. As with total phenols, flavonoid contents in *Tessmaniaafricana* are also higher than those found in yellow birch and sugar maple (St. Pierre 2012) [26]. Picture The Student test carried out in Table 15 on the concentration of flavonoids for the two trees combined reveals no significant difference in the radial position (P = 0.625 and F= 0.27) and in the height of the tree trunks (P = 0.877 and F = 0.13). The radial and longitudinal variation of flavonoids in tree 1 does not seem to have any particular pattern. However, the flavonoid content in tree 2 shows an increasing progression from base to crown for sapwood and heartwood.

Table 14: Variation in flavonoid content in the two trees

	Tree1 (mg eqQu/g PS)		Tree2 (mg eqQu/g PS)	
Height	Sapwood	Heartwood	Sapwood	Heartwood
SC	11	9	19	22
MT	11	13	14	16
HP	31.5	11	10	12

Table 15: Student test of flavonoid content

Flavonoids (mg eqquercetin/g de PS)				
Height	Sapwood	Heartwood	P Rd	FRd
SC	15	15.6	0.944^{ns}	0.01
MT	12.5	14.3	0.822^{ns}	0.05
HP	20.7	11.5	0.281^{ns}	1.46
HtxRd		0.572^{ns}	0.63	
P Ht	0.877		0.625^{ns}	0.27
	F Ht		0.13	

Hydroxycinnamicacids

The results relating to the content of hydroxycinnamic acids in the wood of *Tessamaniaafricana*are recorded in Table 16. This content of hydroxycinnamic acids varies between 112.6 and 193.1 mg of chlorogenic acid equivalent per g of dry extracts (mg AC eq /g PS) in the heartwood against 82 and 153.5 (mg AC eq/g PS) in the sapwood for tree 1. In tree 2, the contents of these chemical substances vary between 103.6 and 166 .8 (mg AC eq/g PS) in the heartwood against 81.4 and 179.2 (mg AC eq/g PS) in the sapwood. Although visibly more concentrated in the heartwood, the Student test performed on the variation of hydroxycinnamic acids and presented in Table 17 shows no significant effect in the radial (P = 0.145 and F= 2.98) and longitudinal (P=0.329 and F=1.40). The hydroxycinnamic acid contents of Wamba are much higher than those found by Perez (2010) in balsam fir wood (*Abiesbalsamea* L. Mill.) With hydroxycinnamic acid yields varying between 94 and 117 mg AC eq/g PS . St. Pierre (2012) [26] indicates levels ranging from the order of 62 mg of chlorogenic acid equivalents per g of dry extract in the heartwood of sugar maple.

Table 16: Variation of hydroxycinnamic acids in the two trees

	Tree 1(mg AC eq/g PS)		Tree 2 (mgACeq/g PS)	
Height	Sapwood	Heartwood	Sapwood	Heartwood
SC	147.9	112.6	98.6	103.5
MT	153.5	193.1	179.2	166.8
HP	82.0	160.4	81.4	151.4

Table 17: Student's test of the variation of hydroxycinnamic acids

Hydroxycinnamicacids (mg AC eq/g de PS)				
Height	Sapwood	Heartwood	P Rd	FRd
SC	125	109	0.652^{ns}	0.23
MT	132	175	0.260^{ns}	1.61
HP	82	157	0.079^{ns}	4.83
Ht x Rd		0.251^{ns}	1.85	
P Ht	0.329^{ns}		0.145^{ns}	2.98
F Ht		1.40		

Variation of proanthocyanidins or condensed tannins

The variation in proanthocyanidin concentration expressed in mg of cyanidin chloride equivalent per g of dry extract (mg Chl C eq /g PS) is presented in Table 18. The levels obtained vary from 43.6 to 93.6 mg Chl C eq /g PS for the heartwood and from 6.4 to 63.3 mg Chl C eq /g PS for the sapwood of the tree1. In tree 2, these compounds vary between 17.6 and 68.0 mg Chl C eq /g PS in the heartwood and between 17.6 and 44.8 mg Chl C eq /g PS in the sapwood. Like the previous polyphenols, the part of the heartwood presents better concentrations with a predominance for the mid-height both in the sapwood and in the heartwood. The results of the statistical analysis reported in Table 19 show an effect of the height of the proanthocyanidin content (P = 0.01 and F = 8.32) while the difference between sapwood and heartwood does not appear. The effect of height is also easily seen in Figure 3.14. Perez (2010) reports concentrations of 13.73 mg Chl C eq /g PS in black spruce (Piceamariana. Mill.), 17.08 mg Chl C eq /g PS in jack pine (*Pinusbanksiana*. Lamb) and 12.89 mg Chl C eq /g PS in balsam fir (*Abiesbalsamea* L. Mill.) The results of Hillis (1987) [14] on the main sources of commercial condensed tannins reported by Stevanovic and Perrin (2009) [25] showed that the yields of some temperate zone plants in the heartwood and sapwood as well as in the bark did not exceed 55 mg AC eq/g PS. Finally, the dosage of polyphenols has just shown that Wamba wood is a tropical species rich in phenolic compounds. Most of these compounds are more concentrated in the heartwood and mid-height. This is the case for total phenols, proantocyanidins and hydroxycynamic acids. These results translate the well-known difference between sapwood and heartwood from the point of view of their chemical composition. Thus, it is known that the sapwood, of pale color, which represents the external part of the trunks of trees contains reserve substances like starch. Stevanovic and Perrin (2009) [25] indicate that in this part of the wood, there are only living cells involved in metabolism, nutrient storage and transport of water and minerals to the upper part of the tree. These authors conclude that during the aging of the tree towards the central part, the structure of the wood changes as well as its chemical composition. The heartwood is generally darker than the sapwood. According to Dirol and Xavier (2001) [8], heartwood is a wood that has undergone chemical transformations. As a result, it acquires chemical substances such as alkaloids, tannins and other coloring matters which act as a natural antiseptic against biological agents. Still according to Stevanovic and Perrin (2009) [25], duraminization is an active process undertaken by the tree in order to increase the natural durability of the wood. These authors report that increased production of polyphenols is typically found in the heartwood. Apart from their importance in the biological, pharmaceutical, cosmetic and nutraceutical fields, the presence of these substances also

plays an important role in wood technology. Their presence in cell walls and in cell cavities contributes to the improvement of physico-mechanical properties such as shrinkage, density and in some cases the modulus of elasticity in axial compression. Luxford (1931) [15] quoted by (Hernández 1989) [13] already reported that the maximum stresses in longitudinal compression are more affected by the effect of extractables than the stresses in bending failure.

Table 18: Variation in proanthocyanidins in the two trees

	Tree 1 (mg CCheq/g PS)		Tree 2 (mg CCheq/g PS)	
Height	Sapwood	Heartwood	Sapwood	Heartwood
SC	6.3	43.6	17.6	17.6
MT	74.8	93.6	44.8	68.0
HP	6.4	46.5	19.2	45.7

Table 19: Student's test of the variation in proanthocyanidins

Proanthocyanidins (mg CCheq/g de PS)				
Height	Sapwood	Heartwood	P Rd	FRd
SC	31	40	0.549^{ns}	0.41
MT	60	81	0.226^{ns}	1.90
HP	13	47	0.079^{ns}	4.86
Ht x Rd		0.213^{ns}		2.14
P Ht	0.01	0.150^{ns}		2.88
F Ht		8.32		

4. Conclusion

Yield measurements of chemical constituents showed that the cellulose content varies little in Wamba wood. The average value of this compound is 46.8% in the heartwood against 46.3% in the sapwood with very little variation from tree to tree. Hemicelluloses through pentosans gave an average content of 17.3% in the heartwood against 19.2% in the sapwood. The higher concentration of pentosans in the sapwood seems consistent with the physiological role of the latter. The results relating to the variation of the total lignin content come from the addition of the Klason lignin and the soluble index. The average total lignin content in tree 1 is 28.3% in heartwood and 28.2% in sapwood. The corresponding values for tree 2 are 28.1 and 27.8%. Apart from the pentosans which indicated a radial effect in the wood near the crown, the Student's test performed on each of the macromolecular constituents of Wamba wood did not report any significant difference in their content in the radial and longitudinal positions. But this is not the case for the content of total extracts soluble in ethanol-toluene and then in hot water. The overall average after summation of the ethanol-toluene and hot water extracts in tree 1 gives 10.1% in the heartwood and 7.5% in the sapwood. Very similar values were measured in tree 2. Statistical analysis showed a significant difference in the content of hot water-soluble extracts in the wood at mid-height. The extracts soluble in the ethanol-toluene mixture, however, did not indicate any significant effect in either the radial or axial position. The contents of different classes of polyphenols were determined by visible UV spectroscopy. In the heartwood of tree 1, total phenols vary from 248 to 434 mg gallic acid equivalents/g of dry extract and are more abundant in the heartwood, particularly at mid-height. The Student test also indicated a very significant difference in the concentration of total phenols between the sapwood and the heartwood. A very significant effect of height on total phenols was also detected. The flavonoid content showed a variation which increased from the base of the trunk towards the crown in the heartwood, but the Student's test showed no significant difference either in radial position or in height. The content of hydroxycinnamic acids varies between 112.6 and 193.1 mg of chlorogenic acid equivalent per g of dry extract in the heartwood against 82.0 and 153.5 mg of chlorogenic acid equivalent per g of dry extract in the sapwood of tree 1, and similar values for tree 2. Like total phenols, hydroxycinnamic acids show a higher concentration in the heartwood than in the sapwood and maximum values at mid -height in the sapwood as in the heartwood. The variation in proanthocyanidin concentration was expressed in mg of cyanidin chloride equivalent per g of dry extract. The results of the statistical analysis showed an effect of the height of the concentration of proanthocyanidins. Finally, the dosage of polyphenols showed that Wamba wood is a tropical species rich in phenolic compounds. Most of these compounds are more concentrated in the heartwood and mid-height. In addition to their importance in the biological, pharmaceutical, cosmetic and nutraceutical fields, the presence of these substances also plays an important role in wood technology.

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