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Comparative Wood Anatomy and Chemical Composition of *Millettia mossambicensis* and *Millettia stuhlmannii* from Mozambique

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The wood anatomy and chemistry of a relatively lesser used wood species, known in Mozambique as nsangala (*Millettia mossambicensis* J. B. Gillett), was compared to overexploited species jambire (*Milletia stuhlmannii* Taub.) to provide diagnostic features for safe discrimination. The anatomical results showed that both species shared several similarities such as intervessel pitting size range (8 µm to 11 µm), rays composed of only procumbent cells, fiber dimensions (average length up to 1359 µm and wall thickness up to 10 µm), and banded axial parenchyma. The extractives and lignin content were higher in jambire, while the carbohydrates and acetyl contents were higher in nsangala. The main anatomical feature separating the two species was the porosity pattern with semi-ring porous wood of nsangala compared to the diffuse-porous structure of jambire. Jambire had wider vessel lumina (200 µm) and up to 3 vessels/mm^2 compared to nsangala vessel lumina of 86 µm and a frequency of 37 vessels/mm^2.

INTRODUCTION

Wood identification based on anatomical features remains a reliable method in the overall control process of legal timber trade across the globe, but often the identification is only possible down to the genus level (Wheeler and Baas 1998; Hermanson and Wiedenhoeft 2011; Johnson and Laestadius 2011). In a country with a range of very similar commercial timbers, the identification process based on general knowledge is not sufficient. Apart from wood anatomy and dendrochronology, several other methods of wood identification are also employed, namely chemical (near infrared spectroscopy, mass spectrometry, detector dogs, stable isotopes, and radiocarbon), genetic (*e.g.*, DNA barcoding, DNA fingerprinting, population genetics), and phylogeography methods (Dormontt *et al*. 2015; UNODC 2016).

Every method has its own merits and limitations, and some are complimentary; therefore an integration of methods is recommended (Dykstra *et al*. 2002; Dormontt *et al*. 2015). Most of the methods previously mentioned above rely on detailed databases from where models and references are used to cross-check unknown wood samples (Beeckman 2003). Examples include large databases of wood collections, and xylariums consulted by experts and law enforcement officers to inspect both the legality and authenticity of traded wood, especially for either endangered or protected timbers under international treaties

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such as Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and World Wide Fund for Nature (WWF) (United Nation Office on Drugs and Crime-UNODC 2016).

Currently, Mozambique's forest authorities are discussing the possibility of banning the export of logs regardless of their commercial class, which means that all harvested wood species have to be processed prior to exportation. Therefore, the high similarity amongst sawn timber boards of many wood species in Mozambique will pose new challenges to both customs and law enforcement officers. The timber of jambire (*Milletia stuhlmannii*) together with a few other species are the most sought after timbers under highly selective logging harvesting regimes that have reduced considerably its growing stock in Mozambique. The forest authorities are also reducing the harvesting quota for this group of species to halt depletion of the growing stock. Thus, the demand for timbers is now extending to relatively lesser-known species, including protected timbers to which very little is known or attention has been paid previously (Uetimane Jr. 2010). Comparative wood anatomy studies of similar species are therefore important to ensure differentiation (Gasson 2011; Maiti *et al.* 2016) as well as allowing customs officials to correctly enforce tax duties and restrict or ban trade of both endangered and protected timbers (Wheeler and Baas 1998).

Timber traders and consumers must be assured on the authenticity of the timbers. It is expected that by reducing both intentional and accidental misclassification of valuable timbers, the government will gain increased revenues of correct duties paid by stakeholders of the timber sector (Dykstra *et al.* 2002). This study is therefore aimed at comparing the wood chemical composition and anatomy of jambire (*Millettia stuhlmannii*) and its closest sister nsangala (*Millettia mossambicensis*). Unlike jambire, nsangala is an endemic tree species (in the central region of Mozambique) that has not been studied apart from dendrological and botanical descriptions by Hyde *et al.* (2017). On the other hand, jambire is widespread across southern Africa, and its wood anatomy and properties are well known and described by several authors (Lemmens 2008; Ali *et al* 2008; Richter and Dallwitz 2000). The same authors reported that the timber of jambire is very dense (720 to 990 kg m^{-3} at 12% moisture content), very durable heartwood, fair workability, dimensionally stable, and mostly used for flooring, framing and luxury furniture. This study is part of a large database project to describe wood anatomical features separating similar timbers to assist with the verification system through a reliable method in the context of reducing the large amount of endangered or protected timbers in the market, thereby prompting conservation of the forests across the country.

EXPERIMENTAL

Materials

The heartwood samples (10×60×200 mm) of *M. mossambicensis* J. B. Gillett were taken from the herbarium Catapu (listed as number 115, identified and catalogued by Meg Coates Palgrave, Caia, Sofala, Mozambique). The natural habitat of *M. mossambicensis* is well-described by Remane and Therrell (2015). Heartwood sample size of 20×140×220 mm (in tangential, radial and longitudinal direction) along with reference permanent microscope slides of *M. stuhlmannii* Taub. were obtained from the Eduardo Mondlane University xylarium (Maputo, Mozambique) listed with numbers 17 to 25-(05/68).

Microscopy Methods

The transverse, radial, and tangential sections (*ca.* 20 to 25-mm-thick) of *M. mossambicensis* and additional microscope slides of *M. stuhlmannii* were prepared using a sliding microtome, bleached in 2 to 3% sodium hypochlorite and stained with 1% w/v aq. Astra blue, followed by 1% safranin in 50% ethanol, dehydrated and mounted in synthetic resin. Fiber and vessel morphological analyses were conducted after maceration using Franklin's method, as modified by Kraus and Arduin (1997). The prepared slides were examined in light microscopy (LM). Scanning electron microscopy (SEM) was used to examine the fibre wall thickness, lumen widths (fibers and vessels), and structure of vestured pits of *M. mossambicensis*. For SEM, semi-thin sections were air-dried, mounted on stubs, coated with gold using a Emitec 5000 (EMITEC, Lohmar, Germany) sputter coater, and observed using a FEI (Philips) XL30 ESEM (Now Thermo Fisher Scientific, Waltham, USA) at 15 kV (Daniel *et al*. 2004). Terminology, definitions, and measurements were performed according to recommendations of the IAWA list of microscopic features (1989).

Micrograph analysis and measurements were processed through Image Pro-Plus Premier software (Media Cybernetics, Inc., version 7, Washington D.C., USA). The microscope slides of *M. stuhlmannii* were mainly used to take micrographs by light microscopy (LM) and cross check the anatomical descriptions previously published (Richter and Dallwitz 2000; Ali *et al.* 2008; Lemmens 2008).

Chemical Analyses

Extractives content

Wood samples were cut from boards and milled to pass a 40-mesh screen. The milled wood was homogenized and 5 g were taken for extraction. Each sample was extracted in a Soxhlet apparatus with a mixture of toluene/ethanol (2/1; v/v) for 6 h, acetone for 6 h, and water for an additional 6 h, and dried at 103 ℃ for 24 h.

Lignin, monosaccharides, and acetyl content

Millettia mossambicensis J. B. Gillett and *Millettia stuhlmannii* Taub. wood samples were analyzed for acid soluble lignin (ASL), acid insoluble lignin (AIL), monosaccharides, and acetyl content (AC) according to the procedure of Sluiter *et al*. (2008). The ASL was determined using a Hitachi U-2910 spectrophotometer (Hitachi, Tokyo, Japan) with an absorptivity of 110 L g^{-1} cm^{-1} at a wavelength of 205 nm. The monomeric carbohydrates were determined using a Chromaster high-performance chromatography (HPLC; Hitachi, Tokyo, Japan) system equipped with an evaporative light scattering detector (ELSD-90; VWR International GmbH, Darmstadt, Germany), and a Metacarb 87P column (300 mm × 6.5 mm; Santa Clara, CA, USA) with a guard column (Metacarb 87P 50 mm × 4.6 mm). The ELSD-90 was operated at 50 ℃, 2.5 bars, and N_2 was used as the nubilizing gas.

The sugars were eluted using ultra-pure water as a mobile phase at a constant flow rate of 0.5 mL min^{-1} and column temperature of 85 ℃. The acetyl content was determined using a diode array detector (DAD; Hitachi, Tokyo, Japan) operated at 210 nm, and a Metacarb 87H column with a guard column (MetaCarb 87H 50 mm × 4.6 mm). The mobile phase was 0.005 mol L^{-1} H_2SO_4 solution (pH 2.1), with a flow rate of 0.6 mL min^{-1} at 30 ℃.

RESULTS AND DISCUSSION

Wood Anatomical Descriptions

Millettia mossambicensis J. B. Gillett

The growth rings' distinct boundaries are invariably marked by both smaller vessels and banded axial parenchyma. The heartwood is distinctively dark compared with the pale sapwood, and the grain of the wood is slightly wavy (Fig. 1B).
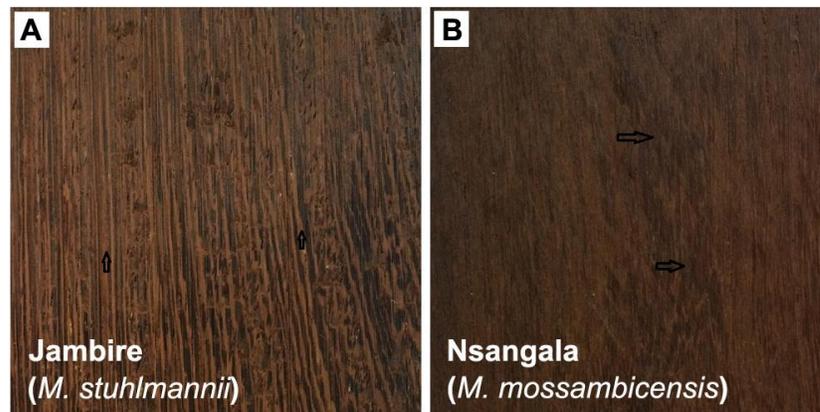

Fig. 1. Macro features of jambire (A-arrows showing yellow stripes of axial parenchyma) and nsangala (B-arrows showing wavy grain)

The vessels were dispersed as diffuse pores, averaging 37 mm^{-2} vessels in transverse sections (range of 23 mm^{-2} to 49 mm^{-2}). The solitary vessel tangential lumen diameter averaged 86 µm (range of 43 µm to 255 µm). There was a semi-ring porosity pattern observed. In transverse sections (TS), there are also multiple vessels along the rays (often clusters of ≥ 4 vessels).
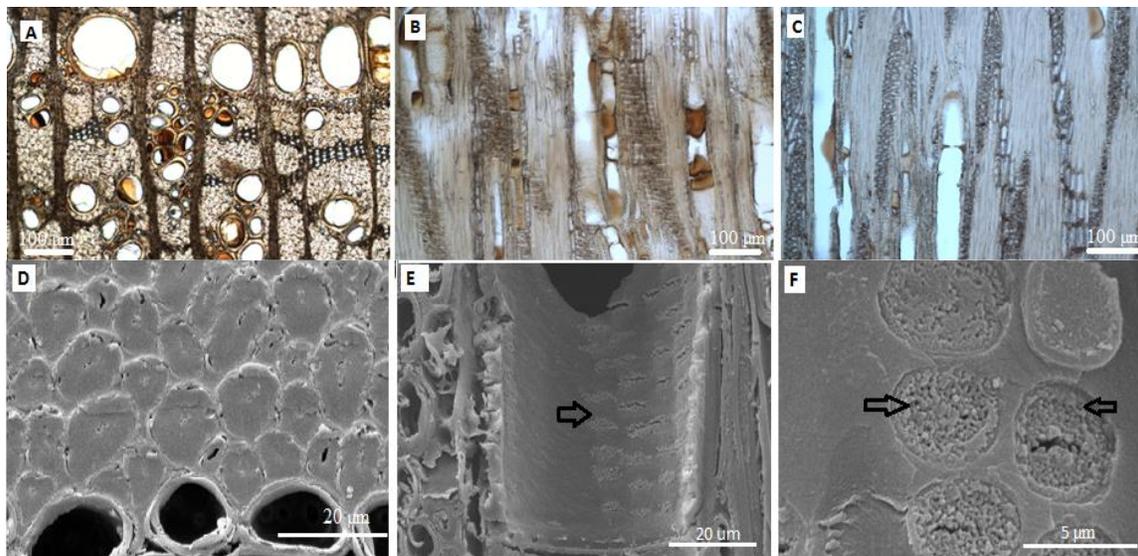

Fig. 2. Micrographs of *M. mossambicensis*: (A) Transverse section view; (B) Radial longitudinal view; (C) Tangential longitudinal view; (D) Thick-walled fibers with almost invisible lumen; (E) and (F) Alternate vestured intervessel pits (arrows). A-B-C (LM micrographs); D-E-F (SEM micrographs).

The outline/shape of vessels varies from rounded to elliptical as seen from transverse sections. The vessels perforation plates are simple while intervessel pits are of minute size averaging 5 µm (range of 4 µm to 9 µm), vestured and arranged in alternating pattern (Fig. 2E). The vessel element length was on average 178 µm (range of 112 µm to 214 µm). The vessel-ray pitting was similar to intervessel pits in shape and size (Figs 4B-D). Gums and other deposits were observed in the heartwood vessels (Figs. 2A and 2B).

The fibers are non-septate, with average length of 1020 µm (range of 620 µm to 1359 µm), with predominantly thick walls averaging 6.93 µm (range of 3.76 µm to 9.58 µm) (Fig. 2D). The fiber pits are mostly simple to minutely-bordered and common in both the radial and tangential walls.

The axial parenchyma is paratracheal, vasicentric, banded, and often reticulate, with two cells per strand. Both the axial and ray parenchyma were filled with extractives (Fig. 2A).

Rays on average counted to 5 mm^{-1} (range of 4 mm^{-1} to 7 mm^{-1}), were scattered in series of 1 to 4 cells in width, with an average height of 449 µm (range of 99 µm to 1078 µm), with a body typically composed of procumbent cells. Prismatic crystals were mixed with extractives in both the radial axial non-chambered parenchyma cells, including fibers.

Milletia stuhlmannii Taub.

The heartwood is typical dark with varying degrees of brownish darks and occasionally along with pale and yellow bands/stripes of axial parenchyma (Fig. 1 A). Growth rings boundaries are hard to detect. At macro scale, both nsangala and jambire are similar dark brown woods.
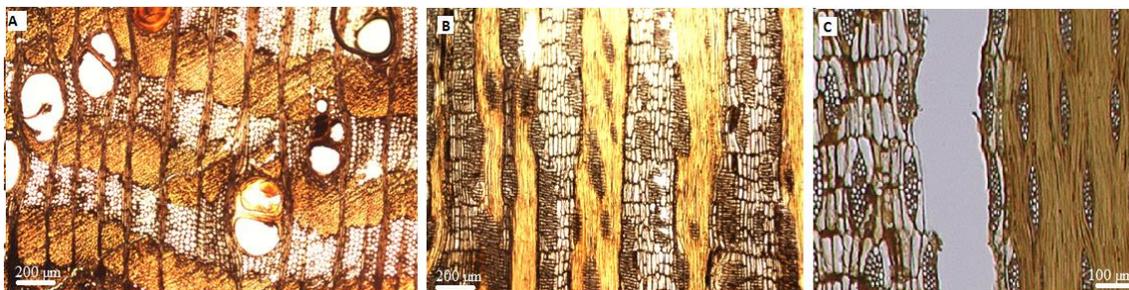
Fig. 3. Micrographs of *M. stuhlmannii*: (A) Transverse section view; (B) Radial longitudinal view; and (C) Tangential longitudinal view

The wood anatomy of *M. stuhlmannii* has also been extensively described by several authors (Richter and Dallwitz 2000; Ali *et al.* 2008; Lemmens 2008). Thus, only its anatomical features are illustrated in Figs. 3-4(A-C). Comparison between the two wood species is discussed in selected categories of both cell morphology and quantitative features summarized in Table 1.

The most striking anatomical features separating both species were the porosity pattern and width of axial parenchyma bands (Figs. 2A and 3A). In tangential sections, there is storied structure in *M. stuhlmannii* but not in *M. mossambicensis* (Fig. 2C and 3C). Comparing the transverse sections, *M. mossambicensis* showed semi-ring porosity and relatively thinner axial parenchyma bands, which is a relatively rare feature in tropical flora (Baas *et al.* 1983); while *M. stuhlmannii* showed regular diffuse pores, wavy and larger bands of axial parenchyma.

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Table 1. Comparison of Some Anatomical Features of Jambire *(M. stuhlmannii)* and Nsangala *(M. mossambicensis)*

Selected Anatomical Features		Jambire *(Milletia stuhlmannii)**	Nsangala *(Milletia mossambicensis)*
Vessels	Porosity pattern	Diffuse	Semi-ring porous
	Density (pores mm^{-2})	1 to 3	37 (23 to 49)
	Tangential lumen diameter (µm)	200 (100 to 310)	86 (43 to 255)
	Element length (µm)	264 (130 to 422)	178 (112 to 214)
	Intervessel pits (µm)	6 (5 to 11)	5 (4 to 9)
Fibers	Wall thickness (µm)	6 (3 to 8)	7 (4 to 10)
	Length (µm)	1102 (669 to 1358)	1020 (620 to 1359)
Axial parenchyma	Typical feature	Paratracheal, marginal bands, aliform winged and confluent	Paratracheal, vasicentric and often reticulate bands mostly filled with extractives
	Band width	3 cells or wider	Nearly the same as the ray width (3 to 4 cells wide)
	Number of cells per axial strand	3 (2 to 4) cells per strand	Mostly 2 to 3 cells per strand
Rays	Width (number of cells)	4 (2 to 6)	3 (1 to 4)
	Average height (µm)	289 (129 to 531)	449 (99 to 1078)
	Cell type and arrangement	Homocellullar (all cells procumbent). Storied structure as seen from tangential view (Fig. 3 C-4A)	Homocellullar (all cells procumbent). Non-storied structure from tangential view (Fig. 2C)
	Number of rays/mm (tangential)	6 (5 to 8)	5 (4 to 7)
Mineral inclusions	Prismatic crystals	Located in chambered axial parenchyma cells. mostly one crystal per chamber	Mixed with extractives in procumbent ray cells and in both non-chambered axial parenchyma cells and rarely in fibers

Range shown in parentheses.
*Richter and Dallwitz 2000; Ali *et al.* 2008; Lemmens 2008 - confirmed by cross checking reference and additional microscope slides.

This is consistent with Gasson *et al.* (2004), who reported variations of both axial parenchyma and vessels distribution within the *Millettia* genus. These authors also observed solitary or paired vessels in most *Millettia* spp. but also clustered vessels in species, such as *M. brandisiana*, in which two distinct sizes of vessels were common. Another example was the semi-ring porosity found in *M. pulchra* and the ring porosity of *M. auriculata* Baker ex. Brand. According to Beeckman (2016), tall trees are expected to have wide vessels at the base of the stem while small trees and shrubs have narrow vessels. This is consistent when comparing the average tangential vessel lumina of jambire (*i.e.*, taller tree-200 *µ*m) to that of nsangala (smaller tree- 86 µm). Intervascular pits were vestured in both species and fell within the *Millettiae* tribe size range (8 μm to 11 μm), which is regarded as a stable feature within the tribe (Gasson *et al.* 2004). In terms of ray features, there were differences in degrees of storeying, but in general, less variability occurs within the *Milletia* genus (Gasson *et al*. 2004). Nsangala and jambire had the same

ray cell composition (*i.e.*, homocellular procumbent cells), which is short and storied (jambire) and mostly 1 to 3 cells wide for both species.
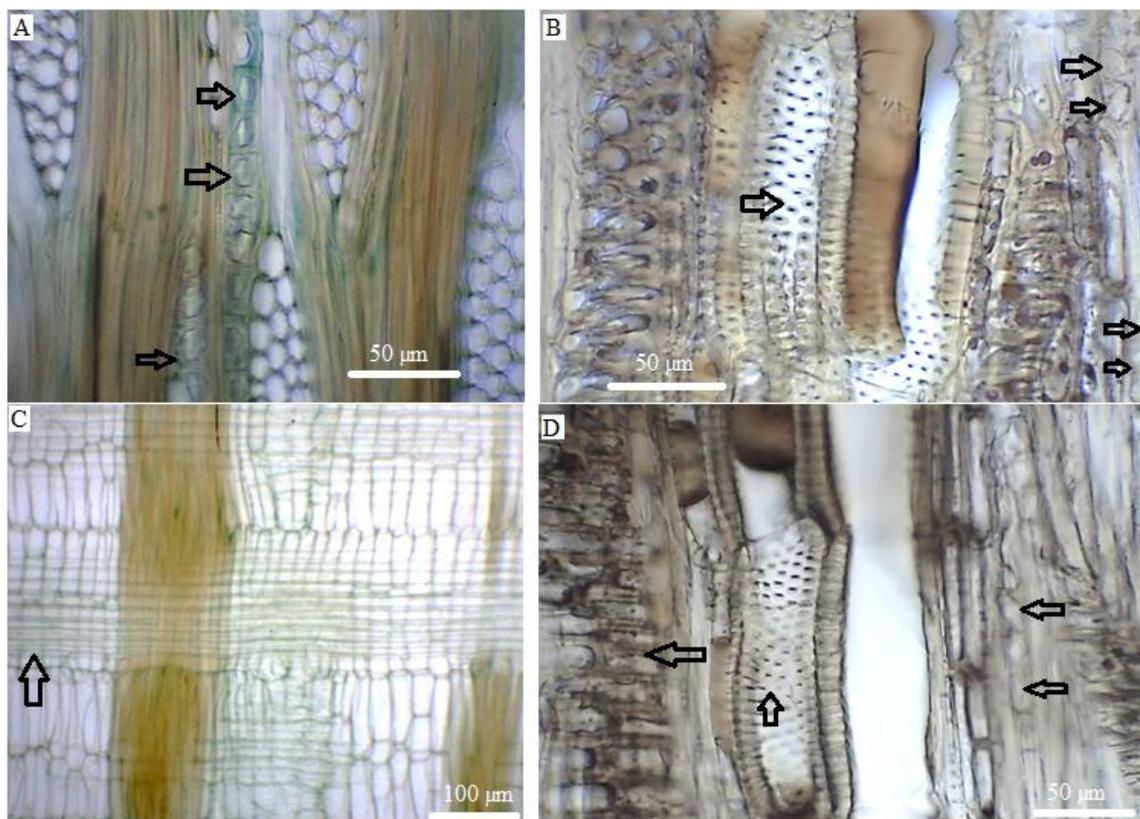

Fig. 4. LM micrographs-Comparative features. (A) Tangential view of *M. stuhlmannii* showing crystals in chambered axial parenchyma cells (arrows); (B) Tangential view of *M. mossambicensis.* Left arrow show intervessel pits and the far right arrows show crystals in axial parenchyma; (C) Radial view of *M. stuhlmannii*-arrow shows procumbent cells in ray body; (D) Radial view of *M. mossambicensis* – left arrow shows procumbent ray cells, middle arrow (vessel ray pits) and the two far right arrows showing crystals in axial parenchyma cells.

The fiber dimensions vary between species of this tribe, especially the fiber length, fiber wall thickness, and total fiber width. In terms of mineral inclusions, prismatic crystals are found in the axial parenchyma of most species of *Millettia* (Gasson *et al*. 2004), including the two species compared in this study. However, prismatic crystals in nsangala were located in ray cells and non-chambered axial parenchyma cells and rarely in fibers (Table 1). These features of *M. stuhlmannii* and *M. mossambicensis* are visualized in Fig. 4A-D).

Chemical Analyses

The results of chemical analyses for jambire and nsangala wood specimens are shown in Table 2.

Using a mixture of toluene/ethanol, acetone, and hot water, jambire exhibited high extractive content (18.5%) compared to nsangala (10.4%). Lhate *et al*. (2010) reported acetone extractive content of up to 5.6% for jambire. Comparing the lignin content, jambire showed a higher total lignin content (39.7%) than nsangala (34.6%). The lignin content of

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jambire reported in this study is at the higher end of those reported in other investigations (Jahan and Mun 2007; Santana and Okino 2007; Lhate *et al.* 2010). The lignin content of some tropical wood species has been reported to range from 29.4% to 40.5% (Fengel *et al.* 1983; Saka 2001; Pastore *et al.* 2004). Santana and Okino (2007) characterized 36 Brazilian Amazon forest wood species and reported a lignin content ranging from 26% to 37%. The difference in lignin content for jambire between the results reported in this study and those reported by Lhate *et al.* (2010) can be explained by the natural variability of wood. Concerning carbohydrate content, nsangala exhibited higher monosaccharides content than jambire, especially for glucose and mannose. Similarly, the acetyl content was higher in nsangala compared to jambire. Compared to the results reported by Lhate *et al.* (2010), the measured monosaccharide content for jambire in this study was slightly higher.

Table 2. Extractives, Lignin and Monosaccharide Compositions of Jambire and Nsangala Wood

Sample	Extractives (%)	Lignins (%)*		Monosaccharides (%)*					AC (%)
		AIL	ASL	Glu.	Xyl.	Gal.	Ara.	Man.	
Jambire	18.5	38.70	0.97	38.16	13.14	1.11	0.59	0.59	3.26
Nsangala	10.4	33.68	0.95	42.66	14.33	0.88	0.43	3.50	4.37
*Extractive-free basis									

CONCLUSIONS

1. This study has provided diagnostic features that can be used to distinguish one of the most commercial timber species in Mozambique from a lesser-known species. A comparative analysis of anatomical features showed that both species shared similar features, such as intervessel pitting, a tendency towards wide tangential bands of axial parenchyma, and rays composed exclusively of procumbent cells that are somewhat storied and composed of mostly up to 3 cells wide.

2. Nsangala possessed relatively wider rays. Fiber morphology cannot be used to separate the two species due to high similarity in the length and wall thickness. The only difference was occasional presence of prismatic crystal in nsangala fibres.

3. The most remarkable anatomical feature separating the two species was the porosity pattern, namely semi-ring porous in nsangala and diffuse porous found in jambire. Another anatomical difference is the ray arrangement in which nsangala rays are non-storied while jambire rays are storied. Vessel size differences were mostly due to their hydraulic architecture, which relates to the tree size.

4. Chemical analyses revealed that the two wood species studied demonstrated considerable variations in extractives, lignin, and carbohydrate content. Compared to nsangala, jambire was rich in extractives and had high lignin content, which are important prerequisites for natural durability. Results of the chemical analysis provide a complete profile of the general characteristics of jambire and nsangala for easy distinguishing between the two wood species.

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