COMPARING GC×GC-TOFMS-BASED METABOLOMIC PROFILING AND WOOD ANATOMY FOR FORENSIC IDENTIFICATION OF FIVE MELIACEAE (MAHOGANY) SPECIES

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Abstract. Illegal logging and associated trade have increased worldwide. Such environmental crimes represent a major threat to forest ecosystems and society, causing distortions in market prices, economic instability, ecological deterioration, and poverty. To prevent illegal imports of forest products, there is a need to develop wood identification methods for identifying tree species regulated by the Convention on International Trade in Species of Wild Fauna and Flora in Trade (CITES) and other look-alike species. In this exploratory study, we applied metabolomic profiling of five species (*Swietenia mahagoni, Swietenia macrophylla, Cedrela odorata, Khaya ivorensis,* and *Toona ciliata*) using two-dimensional gas chromatography combined with time-of-flight mass spectrometry (GC×GC-TOFMS). We also performed qualitative, quantitative (based on the measurement of vessel area, tangential vessel lumina diameter, vessel element length, ray height, and ray width), and machine-vision aided (XyloTron) wood anatomy on a subsample of wood specimens to explore the potential and limits of each approach. Fifty dried xylaria wood specimens were ground, extracted with methanol, and subsequently analyzed by GC×GC-TOFMS. In this study, the four genera could easily be identified using qualitative wood anatomy and chemical profiling. At the species level, *Swietenia macrophylla* and *Swietenia mahagoni* specimens were found to share many major metabolites and could only be differentiated after feature selection guided by cluster resolution (FS-CR) and visualization using Principal Component Analysis (PCA). Expectedly, specimens from the two *Swietenia* spp. could not be distinguished based on qualitative wood anatomy. However, significant differences in quantitative anatomical features were obtained for these two species. Excluding *T. ciliata* that was not included in the reference database of end grain images at the time of testing (2021), the XyloTron could successfully identify the majority of the specimens to the right genus and 50% of the specimens to the right species. The machine-vision tool was particularly successful at identifying *Cedrela odorata* samples, where all samples were correctly identified. Despite the limited number of specimens available for this study, our preliminary results indicate that GC×GC-TOFMS-based metabolomic profiles could be used as complementary method to differentiate CITES-regulated wood specimens at the genus and species levels.

Keywords: Mahogany, wood identification, GC×GC-TOFMS, wood anatomy, XyloTron.

INTRODUCTION

The Meliaceae family, often known as the mahogany family, comprises 50 genera and greater than 1400 species with approximately 500 species of economic importance, widely distributed in rainforests, mangrove swamps to semideserts (Mabberley et al 1995; Muellner et al 2006). Tree species of the Swietenioideae subfamily (see phylogeny in Muellner et al 2003) are particularly prized for fine furniture and musical instruments, ranking among the most economically significant species in the world (Danquah et al 2019) (Fig 1). Overexploitation is driving true mahogany (*Swietenia* species) toward extinction (White and Gasson 2008), and as

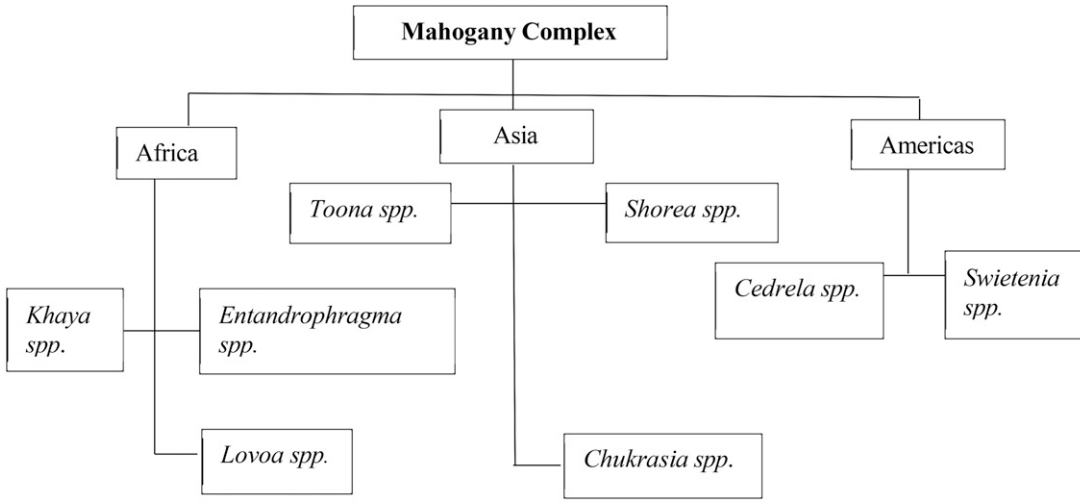

Figure 1. Economically significant mahogany species from Africa, Asia, and Americas (adapted from Danquah et al 2019).

stocks of true mahogany declines in the wild, other similar-looking species are becoming increasingly traded as substitute species, also threatening their survival (Rodan et al 1992; Verissimo et al 1998; Laurance 1999; Gullison et al 2000; O'Neill et al 2001; Kometter et al 2004; André et al 2008). To protect species survival and combat illegal logging, the genera *Swietenia*, *Cedrela* (of the Neotropics), and very recently, *Khaya* have been included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2021): trade in these tropical hardwoods has been restricted by the imposition of specific documentation and permits for importation and exportation of goods. As a result of these essential regulations, other closely related non-CITES species, including *Toona ciliata*, have been introduced as substitutes for these CITES-regulated species. Since Meliaceae species look very similar macroscopically and microscopically, their accurate identification represents a challenge for the application of CITES regulation worldwide (Gasson 2011). Hence, the development and application of efficient wood identification tools are greatly needed to control illegal logging and related trade worldwide.

Wood identification through microscopic analysis of the wood anatomy is the most common, recognized, and enduring method for wood identification available at the moment (Silva et al 2020). This methodology has a long history and is still the most frequently used on the front line for screening purposes (for instance, custom officers, in combination with macroscopic identification aides) and in the laboratory for diagnostic identification (Dormontt et al 2015; Koch et al 2015) in various contexts (archeology, architecture, and art). When the geographic origin and silvicultural history of a sample is known, wood anatomy may successfully determine tree species. If the origin is unknown, the method will allow the identification of wood at the genus or family level. In any case, genus or family confirmation by wood anatomy experts is very important for the development of novel spectrometric and genetic methods for forensic wood identification. As every method has limitations, a combination of approaches and techniques is generally recommended to corroborate identification results (Dormontt et al 2015). Such multidisciplinary approach facilitates the detection of labeling errors of wood specimens, which can occur in any curated xylarium. Indeed, taxonomic uncertainty remains in certain families or genera where species hybridize (eg Finch et al 2019; Bouka et al 2022). Automated wood anatomical analysis (machine-vision) using advanced image capture (Appendix A2) and processing algorithms is another novel area of research that has high potential in the field of wood identification to

identify plants down to the species level (Hermanson and Wiedenhoeft 2011; Hermanson et al 2019). The tool currently uses images captured from transverse wood sections only (end grain), and users need basic knowledge of wood structure to be able to use it as screening tool during field inspections. Confirmatory microscopic observations in the other two wood planes (ie in radial and tangential sections) are generally necessary to support legal cases (forensic evidence).

Apart from anatomy, another very effective approach used in forensic wood species identification is based on metabolite profiling of wood samples. The method is able to characterize species' metabolomes by pairing well-known chromatographic separation methods (ie gas chromatography [GC] and liquid chromatography [LC]) with highly sensitive detection methods using mass spectroscopy (Zhang et al 2019; Shang et al 2020; Brunswick et al 2021). Trees and other plants synthesize compounds called phytochemicals that are often species-specific and/or produced at different levels among species or higher taxonomic groups (Venkataraman 1972; Julkunen-Tiitto 1989). After separation using various chromatographic methods, mass spectrometry ionizes chemical compounds to produce charged molecules whose mass to charge proportions (m/z) that are then quantified and compared with various libraries (ie NIST, "etc.") for metabolite identification (De Hoffmann and Stroobant 2007; Zhang et al 2019). Depending on the natural variation in extractives present in the wood samples, various levels of identification may be possible, including genus and species. Although various mass spectrometric techniques have been used for wood identification (Kite et al 2010; Dormontt et al 2015), the most widely adopted by the research communities are Direct Analysis in Real Time-Time-of-Flight Mass Spectrometry (DART-TOFMS) (Cody et al 2005) and GC×GC-TOFMS (Pierce et al 2006).

DART-TOFMS is one of the most rapid and cost-effective methods for wood identification. However, the technique requires the prior development of an extensive spectral reference database of vouchered wood specimens to identify unknowns. Unlike GC-MS that is extensively used worldwide, only a few countries have currently access to the DART-TOFMS wood identification platform (eg United States and Canada). The DART-TOFMS chemotyping approach has shown potential application for wood identification, for instance; DART-TOFMS was successful in differentiating between two oak species, white oak (*Quercus alba*) from red oak (*Quercus rubra*) (Cody et al 2012), several *Dalbergia* and other commercial species (Lancaster and Espinoza 2012), African Madagascan *Dalbergia* and Asian *Dalbergia* (McClure et al 2015), Araucariaceae species (Evans et al 2017), Meliaceae species (Deklerck 2019), and two Fabaceae species (*Afzelia bipindensis* from *Afzelia pachyloba*) (Kitin et al 2021).

Combination of GC×GC with TOFMS is an important technique, which confirms the presence of large numbers of target metabolites and unknowns in one run (Beens and Udo 2005). GC×GC-TOFMS results in rapid accumulation of spectra, which leads to excellent reproducibility and better signal-to-noise characteristics and make full use of small quantities of samples. The GC×GC technology has been increasingly used for the analysis of petrochemicals and natural products, among others (Wu et al 2004). For example, application of two-dimensional GC-TOFMS method along with the Principal Component Analysis (PCA) in three different species of plants was successful to discover differences among plant samples based on separation of metabolites (Pierce et al 2006). Sun et al (2020) has recently applied two-dimensional GC quadrupole TOFMS to successfully determine and compare CITES-listed agarwood samples from eight different origins.

The objective of the study was to evaluate and compare three wood identification methods; 1) qualitative and quantitative analysis of wood anatomical structure by light microscopy, 2) anatomical analysis using machine-vision aided identification (XyloTron), and 3) wood metabolite profiling using GC×GC-TOFMS followed by feature selection guided by cluster resolution (FS-CR) (Sinkov and Harynuk 2011, 2013; Armstrong et al 2021) to identify and differentiate five easily confused wood species of the Meliaceae family

namely *Swietenia mahagoni*, *Swietenia macrophylla*, *Cedrela odorata*, *Khaya ivorensis,* and *Toona ciliata*. The five similar-looking species belong to the Swietenioideae subfamily and are highly prized for the manufacture of finest furniture products. Except for *Toona ciliata*, the international trade of these endangered species is currently regulated through CITES. A summary, including phylogeny and uses of the four selected genera, is provided as Appendix (A1). For additional information, Danquan et al (2019) wrote a review of the geographic distribution of the economically important Mahogany Complex, highlighting the threats to the sustainability of the species selected in this study.

MATERIALS AND METHODS

As a first step, we described the wood anatomy of 15 selected samples (three samples per species) among 50 available xylaria specimens to validate species' designation and determine the variability of recognized taxonomic characters (ie qualitative and quantitative wood anatomy). Thereafter, we used XyloTron on the same 15 samples as if they all were unknown samples to determine whether current algorithms could identify the wood to the species level. In parallel, we used the untargeted approach of the GC×GC-TOFMS platform to obtain metabolites profiles for all our 50 available xylaria specimens, with the objective to discover genus- or species-specific chemical compounds. When incongruency was detected between the taxonomic identification methods, a second round of verifications was conducted.

Wood Anatomy Using Light Microscopy

Fifteen wood specimens of five tropical hardwood species namely the two closely related species *Swietenia mahagoni* and *Swietenia macrophylla*, *Cedrela odorata* (native to America), *Khaya ivorensis* (native to Africa), and *Toona ciliata* (native to Asia), were sourced from two scientific collections located in Quebec City: the Canadian Forest Service—Canadian Wood Fibre Centre (CFS-CWFC) Xylarium located at the Laurentian Forestry Centre (LFC) and the Centre de recherche sur les matériaux renouvelables (CRMR) Xylarium, located at Université Laval (Table 1). To prepare permanent microscope slides, specimens were first cut into small blocks (1 cm^3) and boiled in water until they were saturated. The softened blocks were then sliced using a rotatory microtome (Leica HistoCore AUTOCUT Model) in cross, radial, and tangential sections at a thickness of 15-30 μm. The wood slices were then stained in laboratory grade, 1% aqueous safranin for 5 min and later with laboratory grade, Astra blue of 90% dye content for 3 min. After staining, slices were dehydrated in alcohol at three different concentrations (50% [v/v], 80% [v/v], and 100% [v/v]) each for 1 min and sections were mounted with Permount™ medium on glass slides. Wood features in the three planes were viewed out under an optical microscope (Nikon Eclipse, E600) connected to a computer where images were stored for subsequent image analysis (Pixel Link).

Anatomical identification of the 15 specimens of known geographic origin (except for sample 543940, see Table 1) to the genus level was performed using IAWA's lists of wood anatomical features (IAWA Committee 1989) through the Internet accessible Inside Wood tool (Wheeler 2011). Five anatomical measurements were further selected for quantitative analysis. Vessel area (μm^2), vessel element length (μm), and tangential vessel lumina diameter (μm) were measured on 30 randomly selected vessels per specimen (one slide per specimen × three specimens per species × five species) with a total magnification of 100× using image processing software Win CELL Pro 2018 e (Regent Instrument Inc., Canada). The tangential diameter of vessel lumina, excluding cell wall width, was measured at the widest part of the opening. The number of vessel elements with a total magnification of 40× were determined by counting the ones present within a field and expressed as a number of vessels per square millimeters (mm^2). All the vessels were counted as individuals, eg radial multiple of three vessels were counted as three individual vessels. For each specimen, the number of rays per linear unit (ie ray frequency per mm) was measured at

Table 1. Information about the 50 wood specimens used in this study. Three wood identification methods were compared on a subsample of 15 specimens (three per species).

Species	Sample Id	WA	XT	GC	Origin	Xylarium
Swietenia mahagoni[a]	539481	—	—	×	Unknown	CFS-CWFC
Swietenia mahagoni	539923	×	×	×	Barbados, North America	CFS-CWFC
Swietenia mahagoni	540152	—	—	×	North America	CFS-CWFC
Swietenia mahagoni	540153	×	×	×	North America	CFS-CWFC
Swietenia mahagoni	541145	—	—	×	Taiwan, Asia	CFS-CWFC
Swietenia mahagoni	543940	×	×	×	Unknown	CFS-CWFC
Swietenia mahagoni	773559	—	—	×	Unknown	CFS-CWFC
Swietenia mahagoni	773560	—	—	×	Unknown	CRMR
Swietenia macrophylla	539581	—	—	×	Central America	CFS-CWFC
Swietenia macrophylla	539857	×	×	×	Mexico, North America	CFS-CWFC
Swietenia macrophylla	539859	×	×	×	Mexico, North America	CFS-CWFC
Swietenia macrophylla	541143	—	—	×	Taiwan, Asia	CFS-CWFC
Swietenia macrophylla	541144	×	×	×	Taiwan, Asia	CFS-CWFC
Swietenia macrophylla	542318	—	—	×	Brazil, South America	CFS-CWFC
Swietenia macrophylla	543938	—	—	×	Central America	CFS-CWFC
Swietenia macrophylla	773561	—	—	×	Unknown	CRMR
Swietenia macrophylla	773562	—	—	×	Unknown	CRMR
Cedrela odorata	542126	×	×	×	Guyana, South America	CFS-CWFC
Cedrela odorata	542084	—	—	×	Unknown	CRMR
Cedrela odorata	542086	×	×	×	Guyana, South America	CFS-CWFC
Cedrela odorata	542085	—	—	×	Unknown	CFS-CWFC
Cedrela odorata	542087	×	×	×	Guyana, South America	CRMR
Cedrela odorata	773566	—	—	×	Unknown	CRMR
Cedrela odorata	773567	—	—	×	Unknown	CRMR
Cedrela odorata	773563	—	—	×	Unknown	CRMR
Cedrela odorata	773564	—	—	×	Unknown	CRMR
Cedrela odorata	773565	—	—	×	Unknown	CRMR
Cedrela odorata	544171	—	—	×	Unknown	CRMR
Khaya ivorensis	540727	—	—	×	Ghana (Gold Coast), Africa	CRMR
Khaya ivorensis	540728	×	×	×	Nigeria, Africa	CRMR
Khaya ivorensis	540729	×	×	×	Africa	CRMR
Khaya ivorensis	540730	×	×	×	West Africa, Africa	CRMR
Khaya ivorensis	540768	—	—	×	West Africa, Africa	CFS-CWFC
Khaya ivorensis	544225	—	—	×	Gabon, Africa	CRMR
Khaya ivorensis[b]	544226	—	—	×	Central African Republic, Africa	CRMR
Khaya ivorensis[b]	544227	—	—	×	Central African Republic, Africa	CRMR
Khaya ivorensis	539546	—	—	×	East Africa, Africa	CRMR
Khaya ivorensis	539547	—	—	×	East Africa, Africa	CFS-CWFC
Khaya ivorensis	773554	—	—	×	Unknown	CRMR
Khaya ivorensis	773555	—	—	×	Unknown	CRMR
Toona ciliata	539508	—	—	×	India, Asia	CFS-CWFC
Toona ciliata	541768	—	—	×	India, Asia	CFS-CWFC
Toona ciliata	543406	×	×	×	Papua New Guinea, Oceania	CFS-CWFC
Toona ciliata	541871	×	×	×	Pakistan, Asia	CFS-CWFC
Toona ciliata	543555	—	—	×	Thailand, Asia	CFS-CWFC
Toona ciliata	542956	—	—	×	Australia, Oceania	CFS-CWFC
Toona ciliata	541491	×	×	×	India, Asia	CFS-CWFC
Toona ciliata	541725	—	—	×	India, Asia	CFS-CWFC

(*continued*)

Table 1. Information about the 50 wood specimens used in this study. Three wood identification methods were compared on a subsample of 15 specimens (three per species). (cont.)

Species	Sample Id	WA	XT	GC	Origin	Xylarium
Toona ciliata	773557	—	—	×	India, Asia	CRMR
Toona ciliata	773558	—	—	×	Unknown	CRMR

[a]Note: Specimen 539481 initially labeled as *Swietenia mahagoni* and later verified through microscopic wood anatomy was found to belong to *Khaya* genus.

[b]Specimens 544226 and 544227 initially labeled as *Khaya ivorensis* and later verified through microscopic wood anatomy were found to belong to *Entandrophragma* genus. They were excluded from the chemical analysis.

WA denotes wood anatomy by light microscopy; **XT** denotes wood anatomy by means of XyloTron, a computer-aided wood identification tool used on the end grain surface of wood specimens; and **GC** refers to GC×GC-TOFMS, ie two-dimensional gas chromatography combined with time-of-flight mass spectrometry, to obtain metabolomic profiles.

10 different locations using a magnification of 40×, whereas for ray height (μm) and ray width (μm), 30 randomly selected rays were manually determined from tangential section at a magnification of 100×.

Statistical analysis was performed with SPSS (version 25) (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). The mean values of five anatomical features for five mahogany species were compared using an analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences between means were considered significant when the *p*-value of the ANOVA was less than 0.05. Data were represented as Mean ± SD. For Duncan's multiple range test, the means carrying different letters indicate significant difference.

Machine-Vision Wood Anatomy Using XyloTron

The same 15 wood specimens identified to the genus level with Inside Wood were further evaluated using the XyloTron machine-vision tool (Hermanson et al 2019; Ravindran et al 2020, see Appendix (A2)) The computer vision system comprises multiple tree species-specific algorithms, which calculate a probability that an unknown specimen belongs to a given species present in the XyloTron reference database (Hermanson et al 2019; Ravindran et al 2020). To this end, the transverse surface of the selected wood specimens was sanded by grit sandpapers (120 [fine], 240 [very fine] and 1000 [super fine]), using compressed air and adhesive tape to remove the dust from the cell lumina between each grit. The imaging of sanded wood surfaces was done using a macroscopic handheld camera (10×). Rays of the wood were aligned in vertical position, as shown in Figs A2-2 to A2-6. Each image (with dimensions 2048 × 2048 pixels) from XyloTron represents 6.35 × 6.35 mm of wood tissue.

Metabolomic Profiling Using GC×GC-TOFMS

A total of 50 wood specimens of five tropical hardwood species were used for chemical analysis (Table 1). The heartwood part of each specimen was ground with a wood file at CFS-LFC. The powder was placed in 2 mL polypropylene tubes (Supplier: Eppendorf™ 022363352; Fisher Scientific, Canada) and sent to The Metabolomics Innovation Centre (TMIC) located at the University of Alberta, Edmonton, Canada, for chemical analysis. At the TMIC research platform, a quantity of 30 mg of wood powder was weighed in a 2 mL Eppendorf™ plastic centrifuge tube. And 1 mL of methanol containing 1% (v/v) formic acid was added to the centrifuge tube. Extraction solvent was prepared by dissolving an internal standard (IS) (20 mg/L, *n*-pentadecane-d32, CDN Isotopes) and 1% (v/v) formic acid (98%, Millipore-Sigma, Canada) in HPLC grade methanol (>99.9%, Millipore-Sigma, Canada). Tubes were vortexed for 10 min. Samples were left at room temperature and allowed to extract overnight (18.0 ± 0.5 h). After extraction, sample tubes were

centrifuged for 10 min at 10,000× g. An aliquot of 200 μL of the supernatant was transferred to a GC insert vial (Chromatographic Specialties, CA) for GC×GC-TOFMS analysis. Two replicate aliquots (R) and two replicate samples (RS) were also analyzed.

All GC×GC-TOFMS untargeted analyses were carried out on a LECO Pegasus 4D system (Leco Instruments, St. Joseph, MI) equipped with a four-jet dual stage modulator. The first-dimension column was a 60 m × 0.25 mm × 0.25 μm Rtx-5 and the second-dimension column was a 1.6 m × 0.25 mm × 0.25 μm Rtx-200MS (Chromatographic Specialties). Two-dimensional chromatographic separations were conducted with a constant flow rate of 2.0 mL/min utilizing helium as the carrier gas and a modulation period of 2.5 s. A GERSTEL MPS Autosampler was used for automated injection of 1 μL of sample. The oven was at first held at 40 °C for 4 min and warmed at 3.5 °C/min to a final temperature of 315 °C. The ultimate temperature was held for 10 min. The secondary oven and modulator temperature offset were constant at +10 °C relative to the GC oven temperature and +15 °C relative to the secondary oven temperature, respectively. Mass spectra were collected at an acquisition rate of 200 Hz over a mass range between 40 and 800 m/z. A relative voltage offset of 200 V was selected as the optimized detector voltage with an electron impact energy of −70 eV. The ion source temperature was 200 °C with a transfer line temperature of 250 °C. The total analysis time was 92.57 min.

Data processing was performed using Chroma TOF® (v.4.72; LECO), a commercial software from LECO. For the processing method, the baseline offset was set to 0.9 and the expected peak widths throughout the entire chromatographic run was set to 10 s for the first dimension and 0.12 s for the second dimension. The peak finding threshold of S/N was set to 50:1 with the minimum S/N ratio for subpeaks to be retained set at 6. All chromatographic peaks were searched against the NIST and Wiley MS libraries (2017). Peaks were tentatively identified based on forward and reverse mass spectral matches greater than 700 and retention index matching (±15 Kovat's RI).

Retention time shift of metabolites is common in metabolomics studies. ChromaTOF® Statistical Compare was performed for aligning the peak tables using retention times and mass spectral match. Tolerances for retention time shift were ±10 modulation period (PM = 2.5 s) in the first-dimension separation, and tolerances for the second-dimension separation were set to 0.2 s to account for the possible retention time shift across all samples. The minimum similarity for mass spectral match was set at 600 to combine if the peaks have a match score of 600 or greater for all m/z values with abundances greater than 10%. The Statistical Compare result was exported as a comma-separated values file (csv) for further data analysis. The exported aligned data from ChromaTOF® was imported in MATLAB® R2017a, Windows 64-bit version (The Mathworks Inc., Natick, MA). With the obtained results, multivariate statistical analysis, ie PCA were performed using PLS_Toolbox (Eigenvector Research, Manson, WA).

In this study, FS-CR was applied to reduce the number of variables required to model differences between the five species. Afterward, 45 different compounds were selected as the most significant and used to visualize the grouping of each species. A heat map was constructed in MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/MetaboAnalyst/home.xhtml) using the 45 selected variables.

RESULTS AND DISCUSSION

Efficient tools and techniques are essential to distinguish listed CITES species and their relatives to fight illegal logging and deforestation. In this study, we evaluated and compared three wood identification methods based either on wood anatomy or metabolic profiles. The latter approach is promising but it should be complemented by traditional wood anatomy method based on light microscopy to build reliable reference database.

Table 2. Qualitative wood anatomical characteristics and mean specific gravity of five Meliaceae species considered in this study.

	America			Africa	Asia
Anatomical features	*Swietenia mahagoni*	*Swietenia macrophylla*	*Cedrela odorata*	*Khaya ivorensis*	*Toona ciliata*
Growth rings	Distinct	Distinct	Distinct	Indistinct/absent	Distinct
Vessel distribution	Diffuse-porous	Diffuse-porous	Semiring porous	Diffuse-porous	Semiring porous
Vessel frequency per mm^2	7 to 10	5 to 7	2 to 3	3 to 4	3 to 4
Perforation plates	Simple	Simple	Simple	Simple	Simple
Intervessel pitting	Alternate	Alternate	Alternate	Alternate	Alternate
Axial parenchyma	Diffuse, scanty paratracheal, vasicentric, marginal bands	Diffuse, scanty paratracheal, vasicentric, marginal bands	Diffuse, scanty paratracheal, vasicentric, marginal bands	Scanty paratracheal, vasicentric, marginal bands (variable/rare)	Diffuse, diffuse-in-aggregates, vasicentric, marginal bands
Prismatic crystals	Present	Present	Present	Present	Present
Rays in radial section	Heterocellular	Heterocellular	Partially heterocellular	Heterocellular	Heterocellular
Ray width (number of cells)	Multiseriate 1-6	Multiseriate 1-6	Multiseriate 1-4	Two distinct sizes: short uniseriate and multiseriate 1-8	Multiseriate 1-6
Rays structure	Storied	Storied	Unstoried	Unstoried	Unstoried
Average ray frequency per mm	6 (4-10)	5 (4-8)	5 (3-6)	5 (4-7)	5 (4-7)
Specific gravity	0.66 ± 0.85	0.50 ± 0.06	0.45 ± 0.01	0.49 ± 0.03	0.42 ± 0.13

Note: Density (specific gravity) is also an important factor for wood identification. According to consensus on phylogeny at the angiosperm family level, strong phylogenetic signals are recorded in wood density (Chave et al 2006) which means that the most closely related species share a more similar wood density value rather than low closely related species. In this study, all five closely related species have a medium wood density value and fall within the range 0.3-0.7. Hence, they cannot be separated based on this criterion.

Wood Anatomy Using Light Microscopy

The observations of taxonomic/anatomical wood features of all five species of interest (subsample of three specimens per species) are summarized in Table 2, whereas the quantitative analysis of anatomical features and statistical results are shown in Tables 3 and 4.

Swietenia mahagoni. Figure 2(a) revealed that the wood samples of *Swietenia mahagoni* showed a tendency to diffuse porosity, contained distinct growth rings with apotracheal axial parenchyma banded and marginal. Vessels were solitary and in radial multiples of up to five (usually two and three); they were round or slightly oval in transverse view and often contained red gum deposits. Apotracheal axial parenchyma was diffused, whereas paratracheal axial parenchyma was scanty and occasionally vasicentric. In the radial section, rays were heterocellular as shown in Fig 2(b). Prismatic crystals were observed in axial parenchyma and in upright or procumbent ray cells. In tangential view, uniseriate rays were rare, whereas multiseriate rays were three to six cells wide as in Fig 2(c). The range of values reported for different quantitative wood anatomical characters for *Swietenia mahagoni* (Table 4) were similar to the values reported by Panshin (1933), except for vessel element length (150-500 μm vs 49-229 μm in this study).

Table 3. Analysis of variance for selected parameters among five different species of Meliaceae family.

ANOVA					
Dependent variables	Sum of squares	df	Mean square	*F* value	*p*-Value
Vessel area	68,944,541,090	4	17,236,135,273	67	0.000[a]
Tangential diameter of vessel lumina	728,259	4	182,065	80	0.000[a]
Vessel length	743,599	4	185,899	69	0.000[a]
Ray's height	1,068,812	4	267,203	14	0.000[a]
Ray's width	21,949	4	5487	13	0.000[a]

[a]Means are significantly different at 0.05 level of significance.

Swietenia macrophylla. The qualitative wood anatomical characters of *Swietenia macrophylla* (Fig 3) were similar to that of *Swietenia mahagoni* (Fig 2). Hence, *Swietenia mahagoni* and *Swietenia macrophylla* were indistinguishable based on qualitative wood anatomy (Table 2). Panshin (1933) detailed that "it is nearly impossible to isolate the woods of the *Swietenia* species anatomically." Donaldson (1984) reported roughly comparable values for vessel element lengths (100-600 μm), tangential vessel lumina diameter (29-126 μm), eight vessels/mm^2 (range 3-16 vessels/mm^2), ray's height (100-600 μm), and five rays/mm (2-9 rays/mm), respectively.

Cedrela odorata. According to Fig 4(a), vessels were predominantly solitary but also in radial multiples up to four. The wood showed tendency to have semi-ring porosity, vessels were round-shaped in transverse view. Apotracheal axial parenchyma were banded and diffuse, whereas paratracheal axial parenchyma were scanty and vasicentric (Fig 4[a]). Rays were slightly heterocellular consisting mainly of procumbent cells as shown in Fig 4(b). Multiseriate rays (Fig 4[c]) were generally two to four cells in width. The vessel measurement results shown in Table 4 were found to be in close proximity to the research conducted by Richter (2000).

Khaya ivorensis. Figure 5(a) revealed that the wood of *Khaya ivorensis* was diffuse-porous, with indistinct growth rings and absence of marginal parenchyma band. These characteristics were similar

Table 4. Quantitative wood anatomical characters of the five Meliaceae species achieved by Duncan's multiple range tests (the means carrying the same letter along a column are not significantly different at 0.05 level of significance). The first line represents the mean value followed by standard deviation, the second line gives the standard error, and the third line gives the range of values (minimum-maximum).

Species	Vessel area (μm^2)	Tangential diameter of vessel lumina (μm)	Vessel element length (μm)	Ray's height (μm)	Ray's width (μm)
Swietenia	12,374 ± 7129[a]	115 ± 40[a]	144 ± 40[a]	343 ± 112[a]	58 ± 28[b]
mahagoni	752	4	4	12	3
	1006-25,321	32-191	49-229	162-797	19-121
Swietenia	20,159 ± 9096[b]	148 ± 35[b]	172 ± 38[b]	417 ± 150[b]	65 ± 26[c]
macrophylla	959	4	4	16	3
	5037-38,956	53-236	68-254	91-1086	29-190
Cedrela odorata	48,873 ± 24,799[d]	236 ± 64[d]	260 ± 69[e]	369 ± 128[a]	49 ± 13[a]
	2614	7	7	13	2
	6510-115,713	76-400	90-408	133-785	22-76
Khaya ivorensis	31,465 ± 12,503[c]	182 ± 33[c]	206 ± 37[c]	484 ± 163[c]	67 ± 19[c]
	1318	4	4	17	2
	2754-66,071	83-254	96-309	219-987	27-107
Toona ciliata	35,722 ± 20,616[c]	185 ± 58[c]	225 ± 64[d]	379 ± 127[ab]	67 ± 14[c]
	2173	6	7	13	2
	5172-106,512	40-322	89-403	167-788	36-98

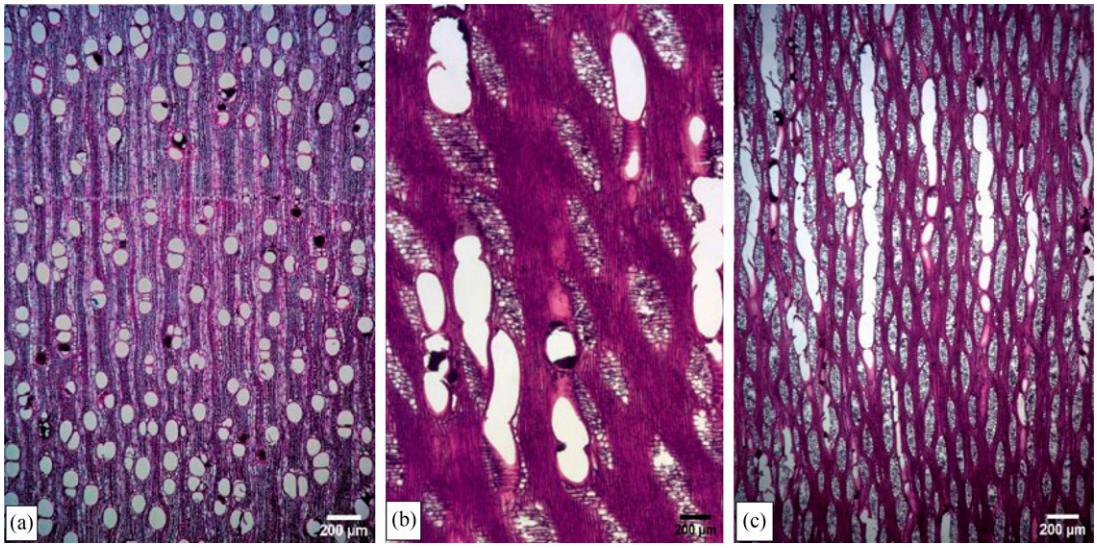

Figure 2. *Swietenia mahagoni* (Sample Id_539923). (a) Transverse section, 20× magnification, showing marginal parenchyma band, and scanty/vasicentric paratracheal axial parenchyma. (b) Radial section, 20× magnification, and heterocellular rays. (c) Tangential section, 20× magnification, multiseriate, and mostly storied rays.

to those reported by Inside Wood (2004 and onwards). Prismatic crystals were observed in upright and/or square ray cells, which is in agreement with Panshin (1933) and White and Gasson (2008). Paratracheal axial parenchyma were mainly vasicentric and also scanty. Rays were heterocellular in radial view (Fig 5[b]) and of two distinct sizes (ie uniseriate and multiseriate up to eight cells wide) in tangential view (Fig 5[c]). Two specimens initially labeled as "*Khaya ivorensis*"

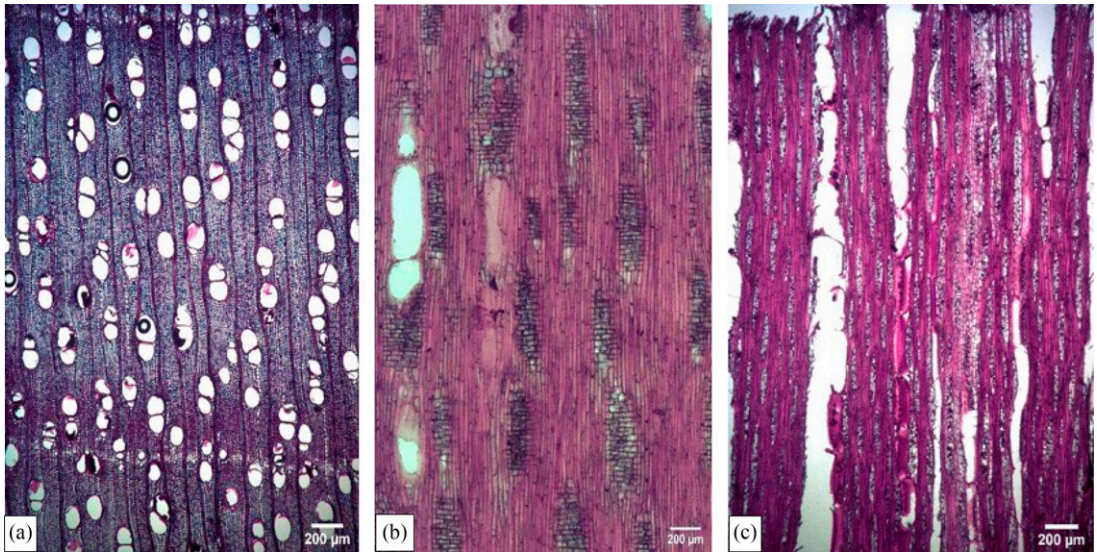

Figure 3. *Swietenia macrophylla* (Sample Id_539859). (a) Transverse section, 20× magnification, showing marginal parenchyma band, scanty/vasicentric, and diffuse axial parenchyma. (b) Radial section, 20× magnification, and heterocellular rays. (c) Tangential section, 20× magnification, multiseriate (≥tetraseriate), and mostly storied rays.

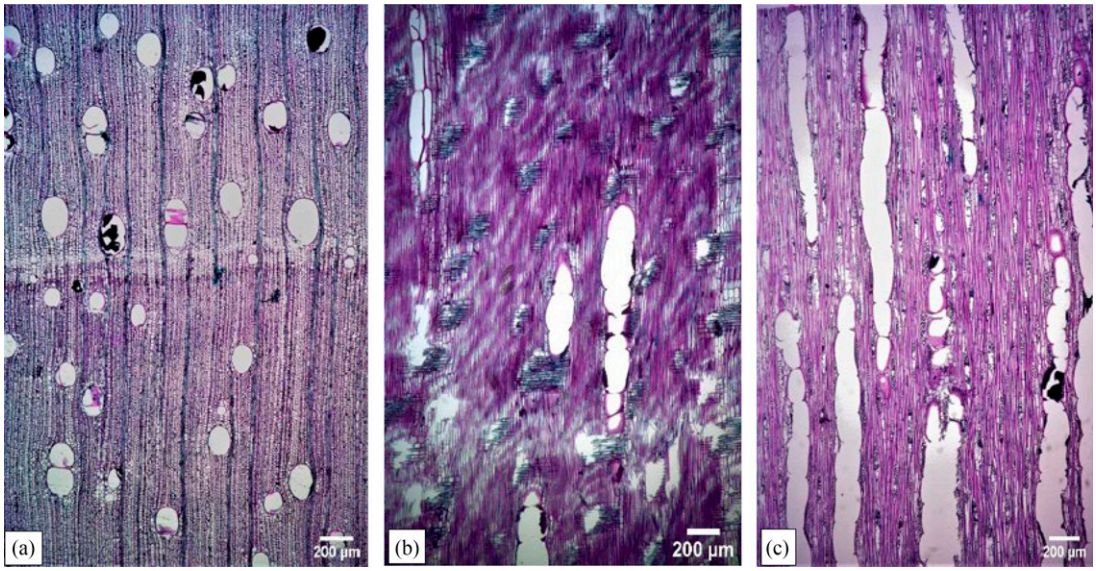

Figure 4. *Cedrela odorata* (Sample Id_542126). (a) Transverse section, 20× magnification, semiring-porous wood, marginal parenchyma band, diffuse apotracheal axial parenchyma, and vasicentric paratracheal axial parenchyma. (b) Radial section, 20× magnification, weakly heterocellular rays consisting mainly of procumbent cells, and simple perforation plate. (c) Tangential section, 20× magnification, commonly biseriate to triseriate rays.

were later found to belong to closely related *Entandrophragma* genus (based on microscopic wood anatomy observations). They were, therefore, removed from subsequent chemical analyses. This result highlights the importance of meticulous curation and the need for species verification using complementary techniques.

Toona ciliata. *Toona ciliata* was characterized by semiring-porous vessel distribution, with distinct growth ring boundaries delineated by marginal parenchyma bands and large earlywood vessels (Fig 6[a]). Perforation plate was simple, intervessel pit was alternate type, rays were heterocellular in radial section (Fig 6[b]), prismatic crystals were observed in axial parenchyma and ray cells (Fig 6[b]), rays were multiseriate in tangential view (Fig 6[c]), one to six cells in width, apotracheal axial parenchyma were diffused to diffuse-in-aggregates, and paratracheal axial parenchyma were vasicentric. Our quantitative results were similar to a previous study by Wood (2004), where mean tangential vessel lumina diameter of *Toona ciliata* was reported as 100-200 µm, mean vessel element length was less than or equal to 350 µm, vessel frequency per mm^2 was 5-20, and the average number of rays per mm was 4-12.

Our microscopic observations for the five Meliaceae species studied confirmed a close relationship among them as they share a large number of similar wood anatomical characteristics (Table 2). The characteristics agreed with previous studies (Kribs 1930; Pennington and Styles 1975; White and Gasson 2008; Oyedeji-Amusa et al 2020). Anatomical results were also consistent with the features documented in Inside Wood (2004 onwards) and Description Language for Taxonomy (DELTA) (https://www.delta-intkey.com), except for two features of *Cedrela odorata.* According to Inside Wood, the mean tangential vessel lumina diameter of five species falls within the range of 100-200 µm and this corresponds to our study except for *Cedrela odorata* (mean 236 µm). Further, in our study, the result for vessel element length (mean 260 µm) of *Cedrela odorata* complies with Delta intkey, however, slightly differs with Inside Wood (350-800 µm). These differences could be explained by age and environmental conditions of the sample tree, which are unknown for our xylaria samples.

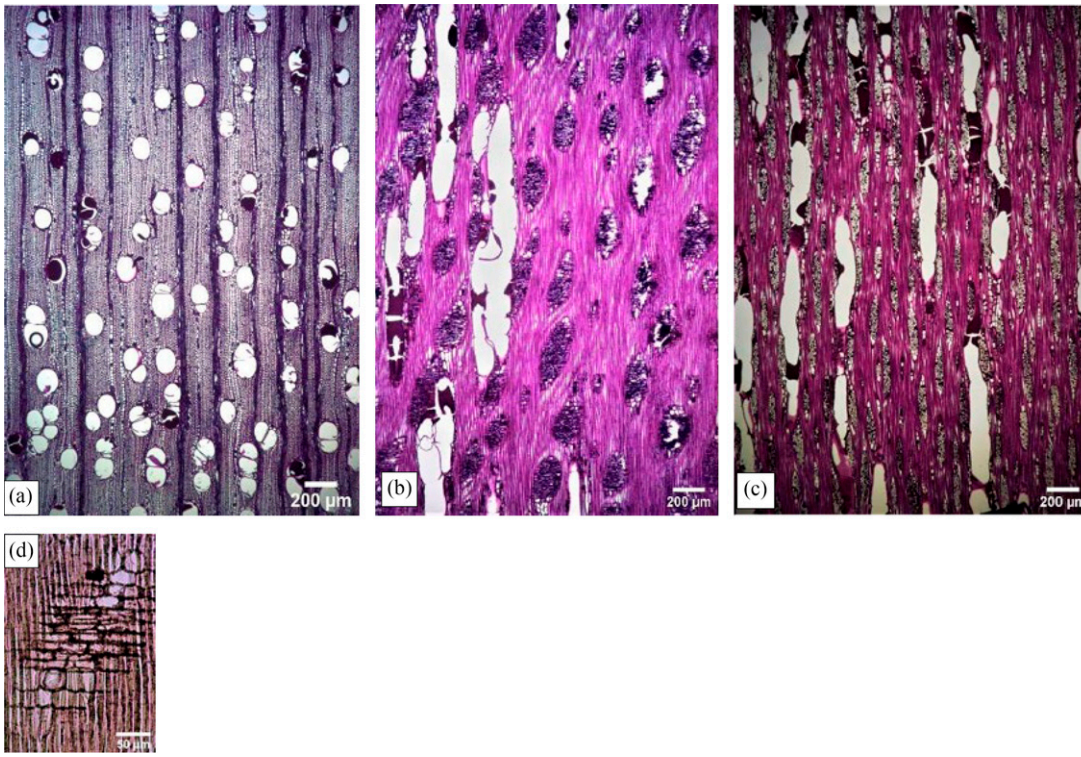

Figure 5. *Khaya ivorensis* (Sample Id_ 540728). (a) Transverse section, 20× magnification, and scanty/vasicentric paratracheal parenchyma. (b) Radial section, 20× magnification, heterocellular rays, and simple perforation plate. (c) Tangential section, 20× magnification, rays of two distinct sizes, uniseriate and multiseriate rays, and not storied. (d) Radial section, 40× magnification, prismatic crystals present in upright ray cells.

Species comparisons. According to our study, *Khaya ivorensis* can successfully be separated from the other four species by the presence of prismatic crystals present only in upright cells, and rays of two distinct sizes. The lack of banded apotracheal axial parenchyma in *Khaya* spp. also helps to differentiate it from *Swietenia* spp. as reported by White and Gasson (2008). *Cedrela odorata* and *Toona ciliata* can be distinguished from *Swietenia* species and *Khaya ivorensis* by their vessel distribution: *Cedrela odorata* and *Toona ciliata* have semiporous vessel arrangement while the other studied species have diffused porous vessel distribution. In addition, *Cedrela* and *Toona* woods are highly aromatic with a strong cedar-like scent, whereas the other species are much less aromatic in their chemical structure and thus, relatively odorless.

ANOVA among the five Meliaceae species showed that species are statistically different ($p < 0.05$) based on five measured wood anatomical characteristics (Table 3). The Duncan's multiple range test revealed that *Swietenia mahagoni* was significantly different from *Swietenia macrophylla* based for each of the five wood anatomical characteristics (Table 4). Moreover, *Cedrela odorata* was significantly different from *Toona ciliata* on the basis of four wood anatomical characteristics namely vessel area, tangential vessel lumina diameter, vessel element length, and ray's width (ie except for ray's height). *Khaya ivorensis* was not significantly different from *Toona ciliata* based on vessel area, tangential vessel lumina diameter, and ray's width (except for vessel element length and ray's height). However, *Khaya ivorensis* was significantly different from *Swietenia*

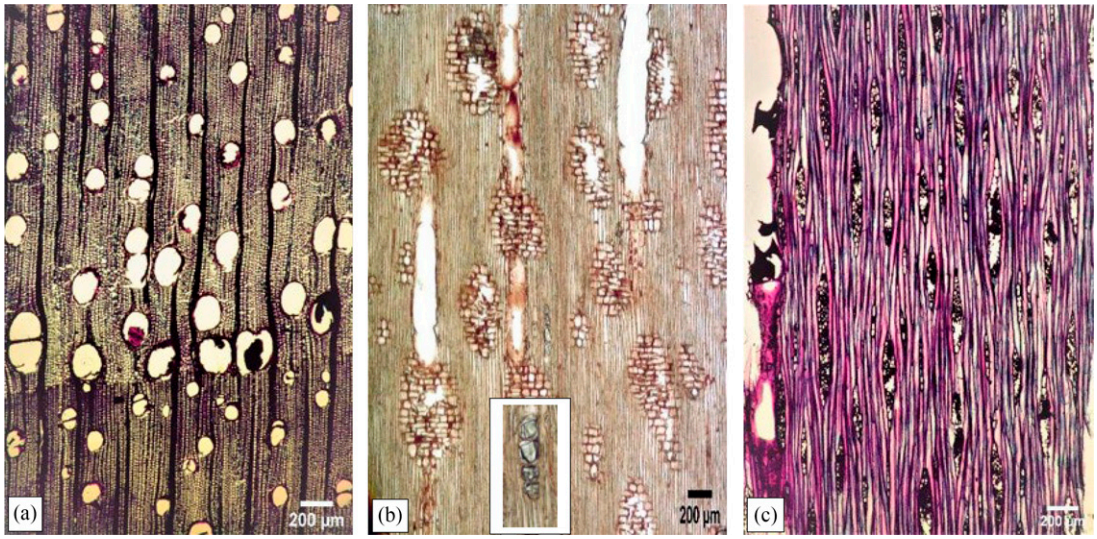

Figure 6. *Toona ciliata* (Sample Id_543406). (a) Transverse section, 20× magnification, semiring-porous wood, vasicentric axial paratracheal parenchyma, and diffuse and diffuse-in-aggregates apotracheal axial parenchyma. (b) Radial section, 20× magnification, heterocellular rays, and presence of prismatic crystals (top right). (c) Tangential section, 20× magnification, multiseriate rays, and simple perforation plate.

mahagoni, *Cedrela odorata*, and *Swietenia macrophylla* (except for ray's width).

White and Gasson (2008) reported that unless the geographic origin is known, differentiating *Cedrela odorata* from *Toona ciliata* is probably impractical. In our study, *Cedrela odorata* and *Toona ciliata* could not be separated based on qualitative wood anatomy, but they could be separated based on quantitative analysis (except for ray's height, Table 4). Similarly, our qualitative wood anatomical observations could not separate the two *Swietenia* species; however, our quantitative results could. Given the small number of wood specimens used in this study and the presence of substantial within species variability depending on environmental conditions and genotype (Baas and Miller 1985; Kite et al 2010; Gasson 2011; Tsumura et al 2011), this result should be taken with caution.

Machine-Vision Wood Anatomy Using XyloTron

A total of 15 wood samples were tested through XyloTron (Table 5). The three wood specimens of *Swietenia mahagoni* were identified as *Swietenia macrophylla* while two wood specimens of *Swietenia macrophylla* were identified as *Cedrela odorata* and only one wood sample was identified as *Swietenia* spp. All three wood specimens of *Cedrela odorata* were accurately identified. The two specimens of *Khaya ivorensis* were assigned to *Khaya ivorensis* with probability score of more than 50%, while the third one was assigned to *Swietenia macrophylla*. However, the tool associated *Toona ciliata* wood anatomy to *Cedrela odorata*, which is correct as these two species cannot be distinguished anatomically unless the geographic origin is known (White and Gasson 2008). At the time of analysis, the XyloTron image database comprised between 21 and 79 reference wood specimens per species (Table 5 personal communication with J. Hermanson 2021). There was no algorithm specifically developed for *Toona ciliata* (no images were available in the reference database). Nevertheless, we presented our 15 wood samples as "unknowns" to the XyloTron (Table 5) to evaluate if identification responses would be similar to that obtained through traditional wood anatomy. At present, the open-source system is in development and additional images of

Table 5. Wood specimens tested through the XyloTron machine-vision system of CFS (and traditional wood anatomy). For each sample, the XyloTron algorithms provided a conformity score (%) with the anatomically most similar tree species found in the image reference database.

Species	Sample Id	XyloTron taxonomic prediction (probability in %)	*N*
Swietenia mahagoni	539923	*Swietenia macrophylla* (98%)	21
Swietenia mahagoni	540153	*Swietenia macrophylla* (87%)	
Swietenia mahagoni	543940	*Swietenia macrophylla* (93%)	
Swietenia macrophylla	539857	*Swietenia macrophylla* (63%)	59
Swietenia macrophylla	539859	*Cedrela odorata* (78%), *Swietenia macrophylla* (3.5%), and species of other family (18.5%)	
Swietenia macrophylla	541144	*Cedrela odorata* (76%), *Swietenia macrophylla* (2.6%)	
Cedrela odorata	542126	*Cedrela odorata* (68%)	41
Cedrela odorata	542086	*Cedrela odorata* (98%)	
Cedrela odorata	542087	*Cedrela odorata* (95%)	
Khaya ivorensis	540730	*Swietenia macrophylla* (82%), *Cedrela odorata* (16%) and *Khaya ivorensis* (2%)	79
Khaya ivorensis	540729	*Khaya ivorensis* (53%)	
Khaya ivorensis	540728	*Khaya ivorensis* (74%)	
Toona ciliata	543406	*Cedrela odorata* (91%)	0[a]
Toona ciliata	541871	*Cedrela odorata* (97%)	
Toona ciliata	541491	*Cedrela odorata* (64%)	

[a]Note: *Toona ciliata* was not included in the XyloTron reference database when the test was carried out.
N indicates the current number of reference wood specimens used by the XyloTron algorithm for species identification (reference: Dr. J. Hermanson, XyloTron developer, pers. comm. 2021).

reference (vouchered) wood samples are being continuously acquired by different laboratories around the world to improve existing models and expand their ability to identify new species. The tool is also being tested for field inspections. With a larger number of reference wood specimens, XyloTron would be more well-suited to capture macroscopic images of wood sample with interesting macroscopic variation. Indeed, it is desirable to have a minimum of 20-30 samples for wood identification purposes (personal communication with J. Hermanson 2021), but the larger the number of individuals sampled, the better the wood identification will be.

Metabolomic Profiling Using GC×GC-TOFMS

The GC×GC-TOFMS analysis revealed the presence of nearly 1831 chemical compounds in the wood samples of five Meliaceae species (data not shown). Considering the diversity of biological compounds, there is a need for multivariate statistical analysis to separate chemically similar wood species.

Figure 7 shows the scores plot and biplot obtained from PCA. The PCA model was built using 45 selected features and allowed the visualization of the differences among clusters of *Swietenia mahagoni*, *Swietenia macrophylla*, *Cedrela odorata*, *Khaya ivorensis*, and *Toona ciliata* (Fig 7). The list of compounds of the biplot are shown in Table A1, whereas the heat map of the relative abundance of these compounds is shown in Fig 8. Results revealed the presence of two main groups (see cladogram at the top of Fig 8): on the left, Group I that includes the *Khaya* (green cell) and *Swietenia* species (dark blue and light blue cells); and on the right, Group II with the *Toona* (pink cell) and *Cedrela* (orange cell) species. This cluster is congruent with the phylogeny of Meliaceae, which is based on chloroplast and nuclear DNA regions (Muellner et al 2003). Effectively, the two groups of species (Group I and Group II)

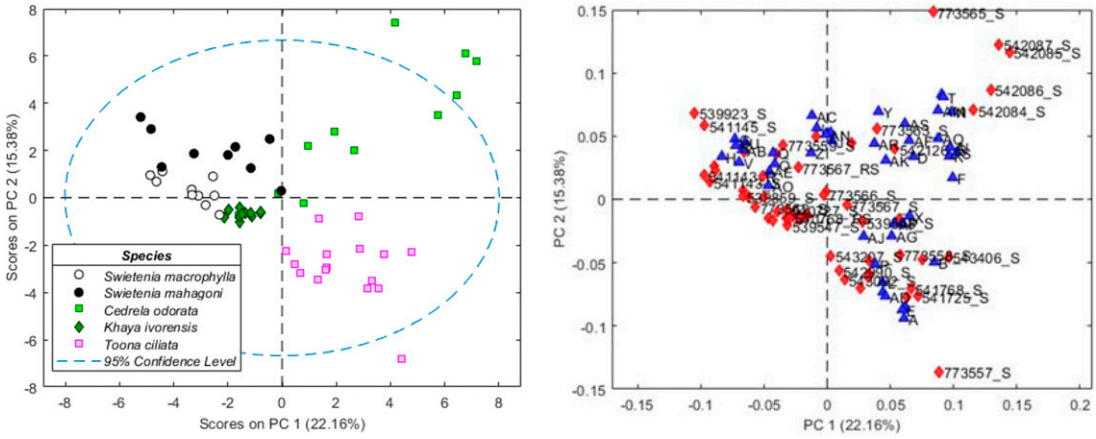

Figure 7. Scores plot (left) and biplot (right) obtained from principal component analysis of 51 wood samples (S), two replicate aliquots (R), and replicate samples (RS). The PCA model was built using 45 selected features to visualize the differences between clusters of *Swietenia mahagoni, Swietenia macrophylla, Cedrela odorata, Khaya ivorensis*, and *Toona ciliata*.

observed in our study and based on metabolic profiles correspond well to the two Swietenioideae subgroups. The compounds that distinguish the two groups are Sesquiterpenol C and alpha-Muurolene.

Chemical Profiles Among Genera

Group I (Khaya vs Swietenia). We observe that *Swietenia* samples comprised much larger amounts of triterpenoid (B, C, and E) and C28 sterol A compared with *Khaya* samples. Other metabolites (ie Metabolites 173 and 1273) were also more abundant in *Swietenia*, and may be selected as potential markers for future identification. One sample initially labeled as *Swietenia* (ie 539481 in Table 1) grouped as *Khaya* based on metabolite profile. We verified the wood anatomy of the specimen using light microscopy and Inside Wood (2004 and onwards) and confirmed its belonging to the *Khaya* genus (ie sample was mislabeled). Gasson (2011) detailed that customs officers usually have trouble distinguishing a shipment of reddish-brown-colored wood and mark it as *Swietenia* sp., *Khaya*, or *Entandrophragma* from Africa or a *Dipterocarp* from Southeast Asia

Group II *(Toona* vs *Cedrela).* We observe that *Toona* samples comprised much larger amounts of "C28 sterol C" and "C27 sterol B" compared with *Cedrela*. In this study, triterpenoid A was very abundant in *Toona* (practically unique) and could potentially be used to distinguish from *Cedrela* (and from the other study species). According to Muellner et al (2003), morphological, phytochemical, and molecular studies indicate a close relationship between the genera *Cedrela* and *Toona*, leading to establish a sister taxon forming a monophyletic clade within Swietenioideae, justifying their positioning within the same tribe.

In comparison with *Toona ciliata*, *Cedrela odorata* was found to be rich in sesquiterpenes, sesquiterpenols, sesquiterpene oxides, and sterols (see example in Figs A3-3 and A3-5). Suarez et al (2018) also stated that although the chemical compositions of *Cedrela odorata* are remarkably different, they all are dominated by sesquiterpene hydrocarbons regardless of the plant tissue or geographical location. Sesquiterpenes are secondary metabolites mainly found in essential plant oil. Further, several studies have revealed that sesquiterpenes were found in abundance in oil composition of *Cedrela* species (Campos et al 1991; Nogueira et al 2020).

Cedrela odorata wood specimens were scattered suggesting the presence of an outlier (Fig 7 left).

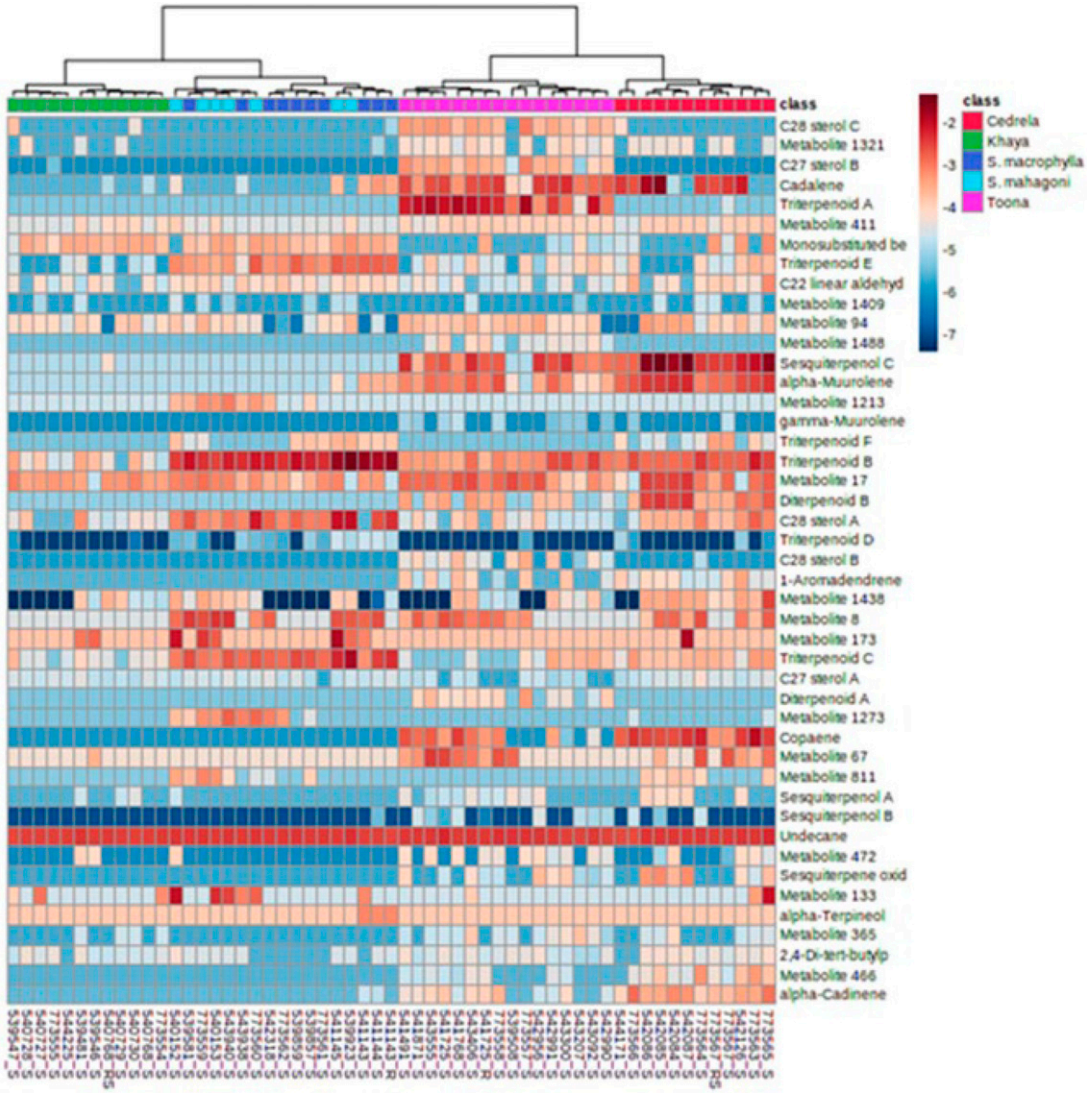

Figure 8. Heat map using 45 selected features from chemical profiles of *Swietenia mahagoni*, *Swietenia macrophylla*, *Cedrela odorata*, *Khaya ivorensis*, and *Toona ciliata*. Data were log-scaled to emphasize differences in the relative amounts of compounds across different wood species.

We verified the wood anatomical characteristics and found them similar to *Cedrela odorata* and *Cedrela fissilis* according to Inside Wood (2004 and onwards). However, we found that the *Cedrela odorata* specimen had been mistakenly sampled in sapwood (light beige) instead of heartwood (brown color), which may explain the difference in chemical signatures. It has been reported that heartwood contains more extractives than sapwood (Hillis 1971; Miranda et al 2006) although the content may decrease from the outer to the inner heartwood (Wilkes 1984). Another possible explanation is that some of these outliers could be other closely related species or hybrids between the various *Cedrela* species inhabiting these regions (see S1).

Table 6. Comparison of resolution (genus vs species) and degree of convergence between the analytical methods tested in this wood identification study.

Species	Can wood anatomy results differentiate species? (Yes/No)		Correspond to Inside Wood description? (Yes/No)	Can XyloTron differentiate species? (Yes/No)	Can major chemotypes alone differentiate species? (Yes/No)	Can chemotypes (PCA) analysis differentiate species? (Yes/No)
	Qualitative evaluation	Quantitative evaluation[a]				
Swietenia mahagoni	No, similar to *Swietenia macrophylla*	Yes, significantly different from *Swietenia macrophylla* on basis of vessel area, tangential vessel lumina diameter, vessel element length, ray's height, and ray's width	Yes (except vessel element length)	No, only at the genus level	No, only to genus level (almost similar chemotype to *Swietenia macrophylla*)	Yes
Swietenia macrophylla	No, similar to *Swietenia mahagoni*, both are diffuse porous	Yes, significantly different from *Swietenia mahagoni* on basis of vessel area, tangential vessel lumina diameter, vessel element length, ray's height, and ray's width	Yes (except vessel element length)	Yes, but occasionally	No, only to genus level	Yes
Cedrela odorata	No, similar to *Toona ciliata*	Yes, significantly different from *Toona ciliata* on basis of vessel area, tangential vessel lumina diameter, vessel element length, and ray's width	Yes (except tangential vessel lumina diameter and vessel element length)	Yes	Yes, richer in sesquiterpenes, sesquiterpene oxides than other four species	Yes

(*continued*)

Table 6. Comparison of resolution (genus vs species) and degree of convergence between the analytical methods tested in this wood identification study. (cont.)

Species	Can wood anatomy results differentiate species? (Yes/No)		Correspond to Inside Wood description? (Yes/No)	Can XyloTron differentiate species? (Yes/No)	Can major chemotypes alone differentiate species? (Yes/No)	Can chemotypes (PCA) analysis differentiate species? (Yes/No)
	Qualitative evaluation	Quantitative evaluation[a]				
Khaya ivorensis	Yes, *Khaya ivorensis* differs from other species by presence of indistinct growth rings and prismatic crystals only in upright cells	Yes, significantly different from *Swietenia mahagoni*, *Cedrela odorata* on basis of vessel area, tangential vessel lumina diameter, vessel element length, ray's height, and ray's width; *Swietenia Macrophylla* on basis of vessel area, tangential vessel lumina diameter, vessel element length, and ray's height	Yes (except vessel element length)	Yes, but occasionally	No, usually to genus level	Yes
Toona ciliata	No, similar to *Cedrela odorata*, both are semiporous	No, not significantly different from *Khaya ivorensis* on basis of vessel area, tangential vessel lumina diameter, and ray's width	Yes	No[a] (reference samples not available in XyloTron)	No, usually to genus level	Yes

[a]Vessel area, tangential vessel lumina diameter, vessel element length, ray's height, and ray's width.

The chemical composition of any plant species is known to be influenced by the genotype, environmental, and agronomic conditions (Rota et al 2008; Kizil 2010). This highlights the importance of collecting physical, photographic and accurate geolocation data for each reference sample that are used to build the databases. Even with accurate sample data, multiple techniques might be necessary to correctly identify a wood sample, since despite all care, collection specimens may have been mislabeled during the acquisition or curation process.

Chemical Profiles Within Genus

Swietenia mahagoni vs *Swietenia macrophylla*. Figure 8 indicates that the two *Swietenia* species are chemically closely related. The heat map reveals that Metabolite 1213 and Metabolite 1273 could potentially be used to separate *Swietenia mahagoni* from *Swietenia macrophylla*. But the separation is not as clear as for the above "between genera" comparisons. Indeed, we see that the groupings of samples are variable, as illustrated by dark blue samples (*Swietenia mahagoni*) intermingled with light blue samples (*Swietenia macrophylla*). This can be attributed to the fact that *Swietenia* species can potentially hybridize. As such, species delineation becomes more difficult and the notion of species might be revised in a context of CITES enforcement (meaning that it might not always be possible to achieve species-level identification because the definition of species is diffuse). The within-genus similarity in the main chemical profiles is also illustrated in Figs A3-1 and A3-2. In this study, these two species were successfully separated through GC×GC-TOFMS with PCA visualization acquiring a total variance of 44.48% as represented (not shown). The PC1 and PC2 captured 30.69% and 13.79% variance, respectively, allowing for wood species separation.

Deklerck (2019) reported that *Swietenia mahagoni* and *Swietenia macrophylla* represented a similar chemotype. According to our study, cycloeucalenol acetate (9,19-cycloergost-24[28]-en-3-ol, 4,14- dimethyl-, acetate, [3β,4α,5α]) is one of the dominant compounds found in *Swietenia* species and represents between 0.3% and 18.75% of the total spectra in *Swietenia mahagoni* and between 0% and 11.2% in *Swietenia macrophylla* (data not shown). Amorós-Marín et al (1959) stated that cycloeucalenol was isolated for the first time from West Indian Mahogany wood through petroleum ether extraction process. Other chemical compounds detected from heartwood samples of *Swietenia mahagoni* were ketone (2-acetyl-3-methoxyphenol); aldehyde (eg syringaldehyde), and so on and this result was supported by Asmara (2018). Moreover, chemical constituents extracted from *Swietenia macrophylla* were ketones (eg 2-acetyl-3-methoxyphenol) and sterols (eg β-Sitosterol) (not shown).

The untargeted metabolomics approach that was applied on five mahogany species of interest aimed to identify as many chemical compounds as possible for taxonomic identification. However, in the context of operational wood identification, it is not necessary to identify all chemical compounds present in wood, but only those that are discriminately consistent for identifying a tree species. This approach using a GC×GC-TOFMS paired with cluster analysis has the potential to increase the number of metabolites measured due to its sensitivity. This strategy could lead to the discrimination of species using a targeted approach for some species of interest. To this end, there is a need to continue the research with additional reference samples to strengthen the link between various analytical identification methods, including untargeted and targeted chemotyping approaches and improve identification efficiency. An overview of the results obtained through the various analytical methods used in this wood identification research project can be found in Table 6.

CONCLUSIONS

In this study, we compared traditional wood anatomy with machine imaging (XyloTron) and chemical (metabolite chemotyping) identification methods. Fifty wood samples were sourced from two xylaria and 15 of them were analyzed with the three methods. Microscopic wood anatomy,

which is the most widely used method for wood identification, was conducted by observing and measuring qualitative and quantitative wood anatomical characteristics, respectively. Evaluation of qualitative anatomical features alone failed to show clear differences among *Cedrela odorata* and *Toona ciliata* and the two *Swietenia* species. *Khaya ivorensis* could be differentiated from the other four species by the presence of rays of two distinct sizes, and the presence of prismatic crystals only in upright cells.

Quantitative anatomy analysis could distinguish *Swietenia mahagoni* from *Swietenia macrophylla* and *Cedrela odorata* from *Toona ciliata*. However, *Khaya ivorensis* was not significantly different from *Toona ciliata* for three of the five measured anatomical features (vessel area, tangential vessel lumina diameter, and ray's width). These quantitative results are counterintuitive knowing that traditional wood anatomy can easily identify trees to the genus level. The considerable variations observed in the morphology of vessel area, tangential vessel lumina diameter, vessel element length, ray's height, and ray's width through quantitative evaluations may not always be genus- or species-specific, ie be more reflective of contrasted growth environments. Overall, the value of additional quantitative features remains uncertain when applied to only a few samples per species.

XyloTron analysis of macroscopic end grain images achieved species-level identification for *Cedrela odorata* and for *Khaya ivorensis* in some cases. Similar to the traditional wood anatomy method, the machine-vision method could not separate *Swietenia mahagoni* from *Swietenia macrophylla.* The fact that XyloTron identified the *Toona ciliata* specimens (not included in the image database) as *Cedrela odorata* (included in the database) makes sense since the two species are closely related and cannot be distinguished with certainty using traditional wood anatomy (White and Gasson 2008). Hence, knowing the geographic origin becomes very important in a context of forensic analysis.

Chemical profiling using GC×GC-TOFMS was also used in this study for taxonomic identification. Separating closely related taxa based on chemical profile was challenging since some species shared similar chemotype, especially between the two congeneric species *Swietenia mahagoni* and *Swietenia macrophylla*. However, GC×GC-TOFMS, analyzed by cluster analysis and expressed using multivariate statistical analysis (PCA) was found to be an effective approach to separate these five closely related species. Hence, the GC×GC-TOFMS and PCA visualization method, in combination with wood anatomy, shows good potential to produce forensic identification of CITES-listed species, in support of law enforcement officials verifying the legality of timbers in trade. This research may contribute to the global effort to curb illegal logging and trade of forest products.

Limitations and Recommendations of Study

Taxonomically validated and geographically referenced wood standards are essential for wood forensics. In this study, we had a very limited number of reference collection samples for each species and some samples were only geo-referenced at the country level. Lack of accurate geo-referencing information may limit the interpretation of the presence of outliers amongst the samples tested, whose presence could be due to genetic factors (such as hybridization between closely related species) and/or environmental factors. Hence, there is a need for more research projects to support the continuation of wood collections (e.g. Williams et al 2020), updating botanical information of the xylarium specimens, coding the specimens according to the criteria of the International Association of Wood Anatomists, improving microscopic and macroscopic image material for identification keys and to assess the wood density, and its variability based on xylarium specimens. Finally, there is a need to develop adequate and complete reference databases including anatomical and metabolite profiling approaches that are supported by molecular phylogeny or population genetics approach. Then, this information can be widely used to combatting illegal logging by empowering law enforcement officers to make easy field triage of suspected wood shipments.

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APPENDIX

A1 DESCRIPTION OF THE FOUR GENERA AND FIVE SPECIES CONSIDERED IN THIS STUDY

Swietenia

The *Swietenia* genus is widely distributed in the Neotropics (Figueroa Colon 1994) and comprises three species: *Swietenia mahagoni*, *Swietenia macrophylla*, and *Swietenia humilis* and two natural hybrids (Pennington 1981). ***Swietenia macrophylla***, also known as big-leaf mahogany, has a wide geographic range from the southern tip of Florida extends through the southern parts of Mexico and Central America passing across the southern Amazon basin of Bolivia and Brazil (Pennington 1981; Danquah et al 2019). Since, the late 1700 s, *Swietenia macrophylla* has been heavily exploited (Rodan et al 1992). In Central America, populations have declined by 80% in the last 50 yr, and some are already extinct (Navarro et al 2003). In 2003, due to the high risks to population viability related to overexploitation and habitat destruction, *Swietenia macrophylla* was listed on CITES Appendix II (Grogan and Barreto 2005). ***Swietenia mahagoni***, also known as American mahogany, Cuban mahogany, or small-leaf mahogany, is widely distributed in Southern Florida in the United States and islands in the Caribbean, including the Bahamas, Cuba, Jamaica, and the Dominican Republic and Haiti (Mayhew and Newton 1998; Danquah et al 2019).

Cedrela

The genus *Cedrela* P. Browne is native to Neotropical region of America (Pennington 1981; Patiño Valera et al 1996; Estrada-Contreras et al 2016; Danquah et al 2019). The genus recognizes about 17 species and two of the 17 species (*Cedrela odorata*, *Cedrela fissilis*) are widespread lowland species occurring in both rainforests and drier areas, whereas the majority is either in wet mountainous forest or seasonally dry forest species scattered through Central and South America (Muellner et al 2009). *Cedrela odorata* wood is eminent for its magnificence, durability, and pest resistance. The heartwood of *Cedrela odorata* contains an aromatic and insect-repelling resin, which makes it resistant to insects, termite, and rot. *Cedrela* timber has been utilized for numerous finishes in colonial buildings in different countries of South America (Pennington et al 2010). Due to deforestation and the selective logging of *Cedrela odorata* individuals, the species has declined by 28.8% over the last 100 yr and it is estimated that it will decrease by 40.4% over the next 100 yr (CITES 2018). Selective overharvesting of *Cedrela odorata*, targeting the tallest and healthiest trees, has been shown to reduce populations across their range and genetic diversity (Llerena et al 2012). The *Cedrela* genus (populations of the Neotropics) has been placed in CITES Appendix II in August 2020. Finch et al (2019) recently described the genetic structure of *Cedrela*.

Khaya

The genus *Khaya*, traded as African mahogany, comprises about five species: *Khaya ivorensis*, *Khaya anthotheca*, *Khaya grandifoliola*, *Khaya senegalensis*, and *Khaya Madagascariensis*, which are native from Africa and the surrounding islands (Louppe et al 2008; Sexton et al 2015). *Khaya ivorensis*, also known as Lagos mahogany, is a very large evergreen tree attaining a height between 40 and 50 m. The wood is highly valued for a variety of uses, including boatbuilding and the making of furniture and musical instruments (Louppe et al 2008). The high-value timber of *Khaya* makes it, like many other tree species in tropical Africa, a victim of illegal logging (Hansen and Treue 2008). Throughout their ranges, natural regeneration after logging is seriously hindered by the low density of adult trees and low regeneration rates (Louppe et al 2008; IUCN 2015). Today, *Khaya* genus is under heavy exploitation pressure. Consequently, at the 19th meeting of the Conference of the Parties on CITES (CITES CoP19) held in November 2022 in Panama, the whole *Khaya* genus was added to CITES Appendix II, effective in February 2023.

Toona

Toona is a genus is native to Afghanistan moving southwards to India and east to North Korea, Papua New Guinea, and Eastern Australia (Mabberley 2017). It consists of six major commercially important timber species namely *Toona calantas* (Philippine mahogany), *Toona ciliata* (*Toona australis*—Australian red cedar or Indian mahogany), *Toona sinensis* (Chinese mahogany), *Toona sureni* (Vietnamese mahogany; Indonesian mahogany), and *Toona fargesii* (Danquah et al 2019). *Toona ciliata* is a timber tree species that is native from Southeast Asia. In the past, the genus was often incorporated within a wider circumscription of the related genus *Cedrela*, but that genus is now restricted to species from the Americas. *Toona ciliata* is red in color, easy to work, and very highly valued. The wood characteristics of *Toona ciliata* are similar to that of *Cedrela odorata* and its cultivation may reduce the harvesting pressure on *Cedrela odorata* in native forests (Dordel et al 2010). *Toona ciliata* is currently recorded as Least Concern by IUCN red list (IUCN 2021).

A2 LIST OF IMAGES OF FIVE MELIACEAE SPECIES ACQUIRED THROUGH XYLOTRON (MAGNIFICATION 10×)

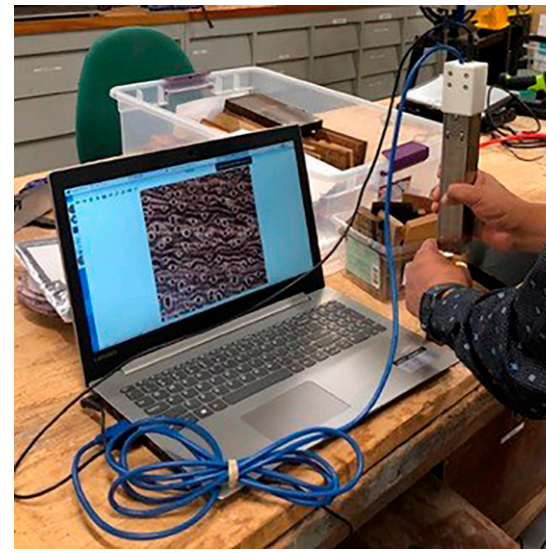

Figure A2-1. XyloTron is a machine-vision system for wood species identification (www.xylotron.org) (Hermanson et al 2019).

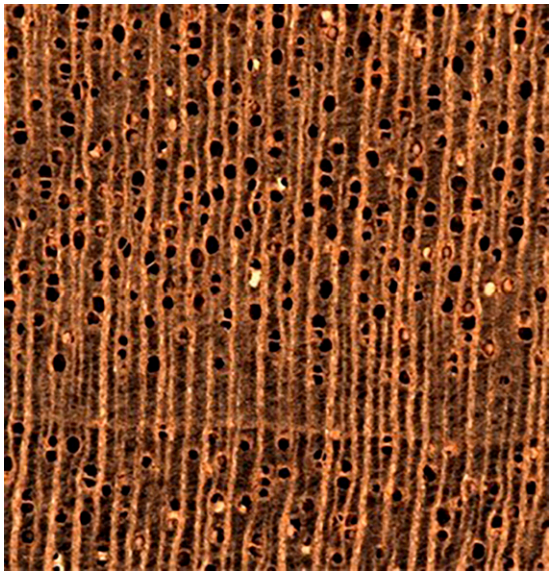

Figure A2-2. Macroscopic image of *Swietenia mahagoni* (Sample Id_539923).

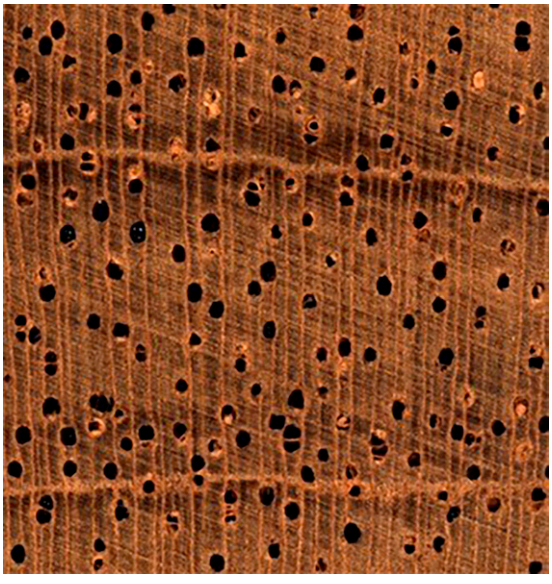

Figure A2-3. Macroscopic image of *Swietenia macrophylla* (Sample Id_539857).

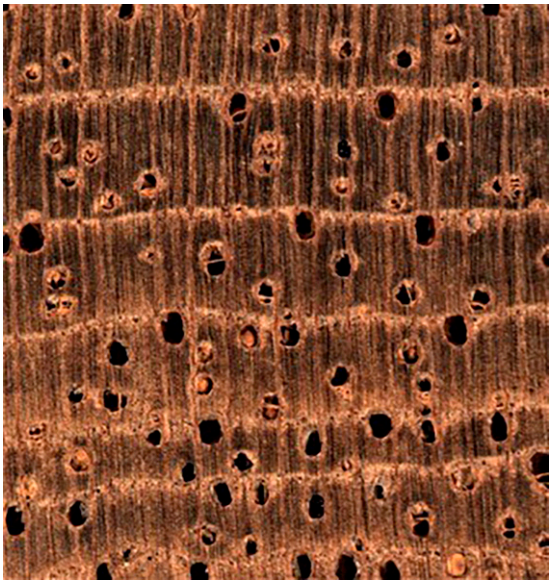

Figure A2-4. Macroscopic image of *Cedrela odorata* (Sample Id_542086).

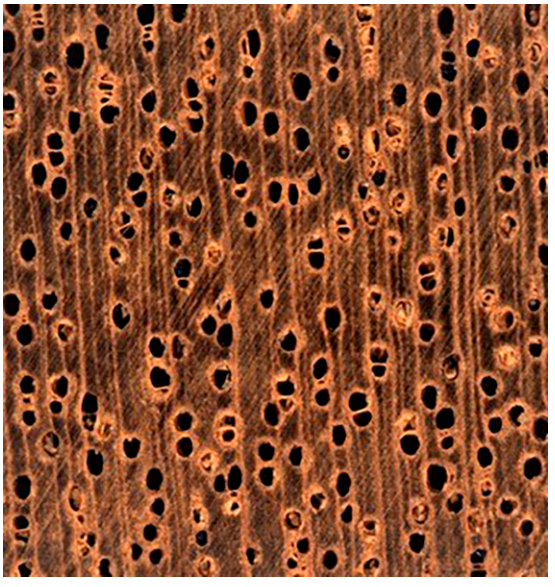

Figure A2-5. Macroscopic image of *Khaya ivorensis* (Sample Id_540729).

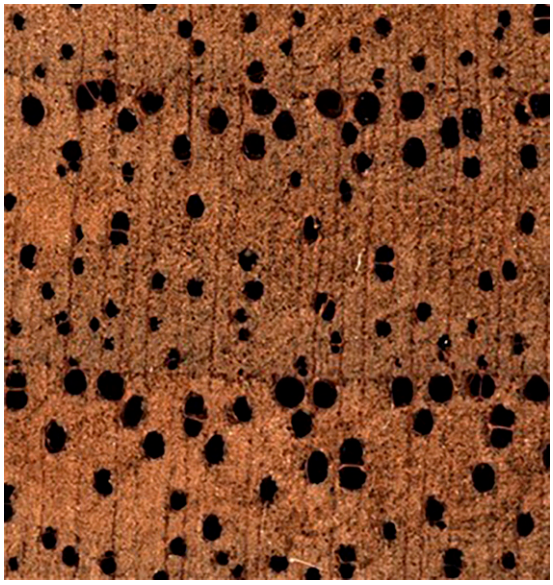

Figure A2-6. Macroscopic image of *Toona ciliata* (Sample Id_543406).

A3 LIST OF CONTOUR PLOTS/CHROMATOGRAPHIC PEAKS OF MELIACEAE SPECIES OBTAINED FROM GC×GC-TOFMS ANALYSIS

The following figures represent contour plot/chromatographic peak obtained after GC×GC-TOFMS analysis. The *x*-axis indicated first-dimension retention time, whereas *y*-axis indicated second-dimension retention time. Each colored "blob" or contour represents a peak and the color scale shows the intensity of the peaks. It can be observed that the two *Swietenia* species have fewer peaks and quite similar numbers of sterols, whereas *Cedrela odorata* and *Toona ciliata* have a greater variety of detected compounds and increased peak intensities compared with other species. Further, it is noteworthy that *Cedrela odorata* is found to be dominant in sesquiterpenes, sesquiterpenols, sesquiterpene oxides, and sterols.

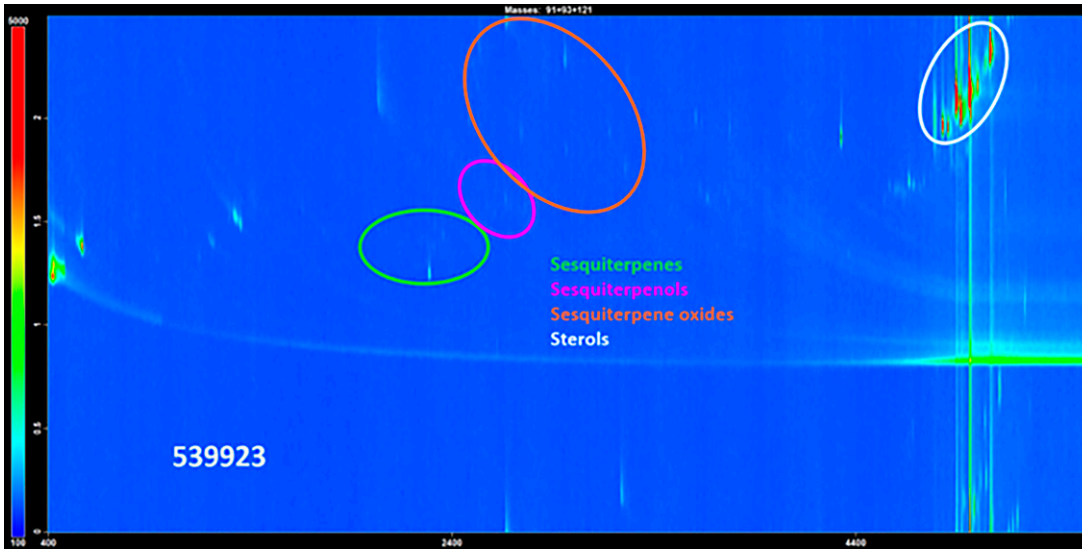

Figure A3-1. *Swietenia mahagoni* (Wood sample_Id: 539923).

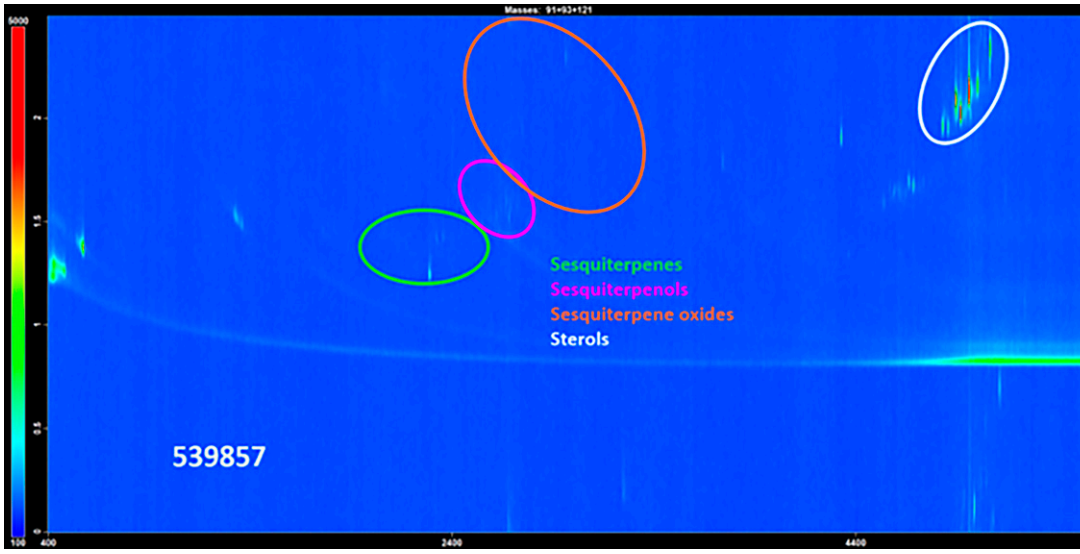

Figure A3-2. *Swietenia macrophylla* (Wood sample Id: 539857).

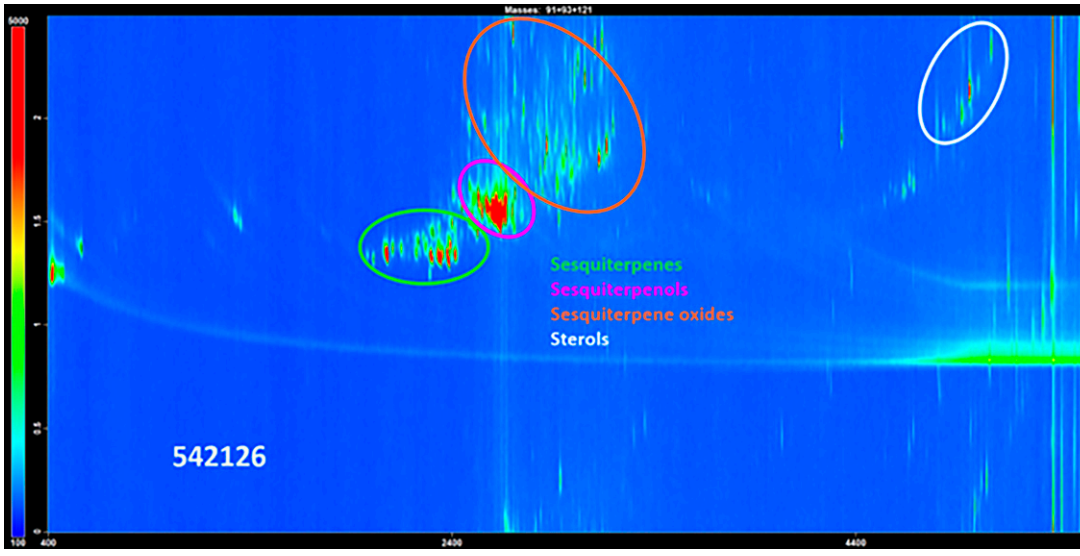

Figure A3-3. *Cedrela odorata* (Wood sample Id: 542126).

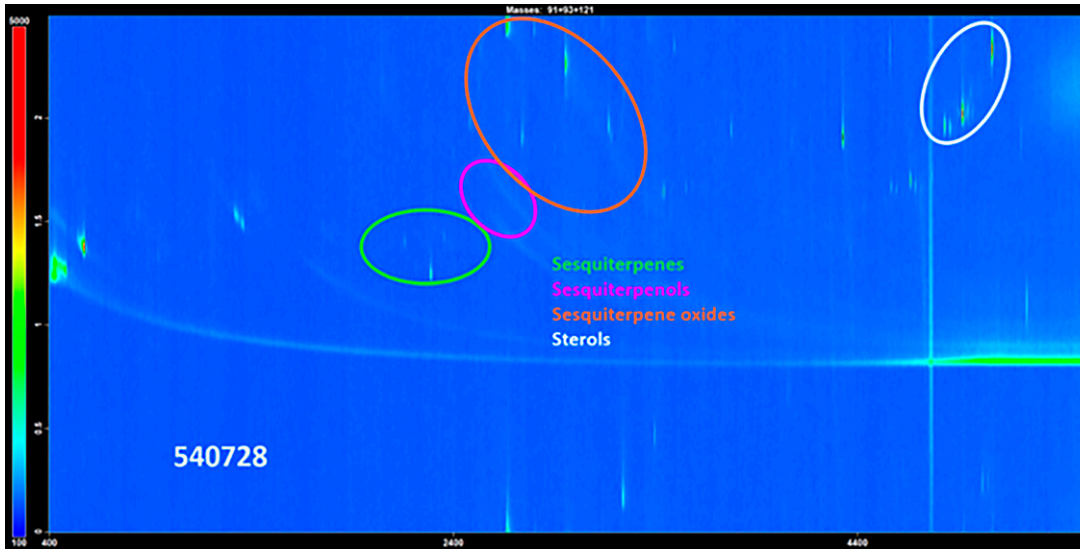

Figure A3-4. *Khaya ivorensis* (wood sample_ Id: 540728).

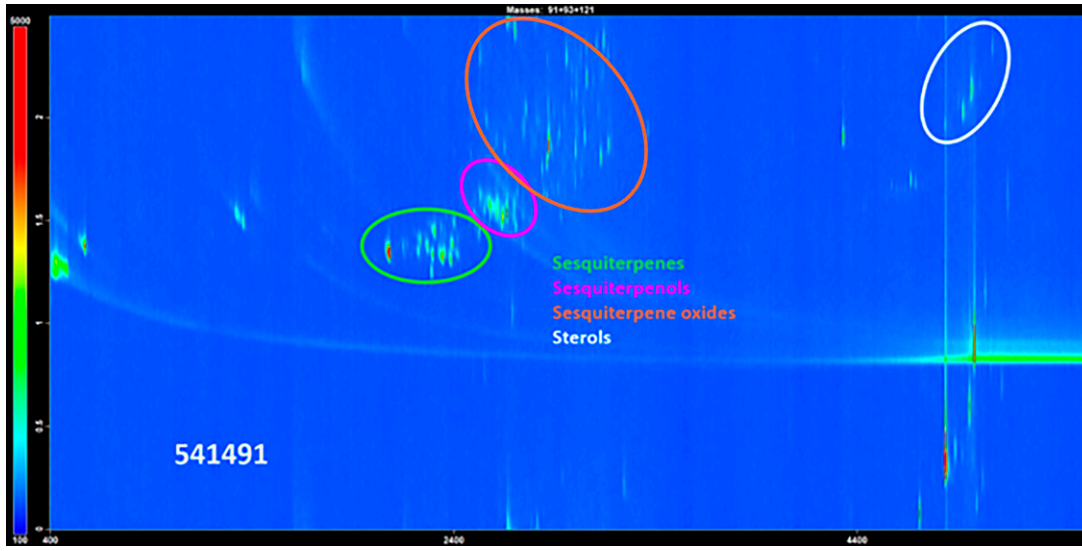

Figure A3-5. *Toona ciliata* (wood sample_Id: 541491).

Table A1. Selected features for principal component analysis of *Swietenia, Cedrela, Khaya,* and *Toona* samples.

Variable label	Compound name	Variable label	Compound name
A	C28 sterol B	AA	Metabolite 173
B	Metabolite 1321	AB	Triterpenoid C
C	C27 sterol B	AC	C27 sterol A
D	Cadalene	AD	Diterpenoid A
E	Triterpenoid A	AE	Metabolite 1273
F	Metabolite 411	AF	Copaene
G	Monosubstituted benzaldehyde	AG	Metabolite 67
H	Triterpenoid E	AH	Metabolite 811
I	C22 linear aldehyde A	AI	Sesquiterpenol A
J	Metabolite 1409	AJ	Sesquiterpenol B
K	Metabolite 94	AK	Undecane
L	Metabolite 1488	AL	Metabolite 472
M	Sesquiterpenol C	AM	Sesquiterpene oxide A
N	alpha-Muurolene	AN	Metabolite 133
O	Metabolite 1213	AO	alpha-Terpineol
P	gamma-Muurolene	AP	Metabolite 365
Q	Triterpenoid F	AQ	2,4-Di-tert-butylphenol
R	Triterpenoid B	AR	Metabolite 466
S	Metabolite 17	AS	alpha-Cadinene
T	Diterpenoid B	—	—
U	C28 sterol A	—	—
V	Triterpenoid D	—	—
W	C28 sterol B	—	—
X	1-Aromadendrene	—	—
Y	Metabolite 1438	—	—
Z	Metabolite 8	—	—