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Morphology and anatomy of rosewood (*Dalbergia cochinchinensis*) and relationship between its elemental components and soil properties for identification of endemic species

Sriudorn, N.[1] and Benchawattananon, R.[2*]

[1]Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand; [2]Integrated Science, Forensic Science, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

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Abstract The species *Dalbergia cochinchinensis*, also known as rosewood or Siam rosewood, is an economically important tree because of the quality of its wood. In spite of being listed in the Appendix II of CITES, illegal logging and the associated international trade in illegally sourced wood products along the borders of Thailand, Laos and Cambodia remain a massive threat to this species. This study was carried out to identify rosewood samples collected from different localities of Thailand, Laos and Cambodia using morphological and anatomical characteristics and FTIR, and to establish relationships between their elemental components and the soil properties for identification of endemic species. The results revealed differences in the FTIR patterns of the leaf and bark samples of rosewood and soil samples collected from different countries, which might be due to the differences in the soil properties, humidity and sunlight intensity. The information obtained from this study could be used as the pieces of evidence in investigating illegal logging suspects and should be at least of assistance in surveillance planning to prevent illegal logging of rosewood.

Keywords: *Dalbergia cochinchinensis*, Illegal logging, Cambodia, Laos, Thailand

Introduction

Dalbergia is a large genus comprising about 100 trees, shrubs, and woody lianas species in the Fabaceae subfamily of the Pea family pantropically distributed. Many of these species have been considered as economic importance due to the quality of their wood and are native to biomes highly threatened by human activities (Buzatti *et al*., 2016; Hartvig *et al*., 2018). Among the economically important species, *D. cochinchinensis*, also known as rosewood and Siam rosewood, is considered as a “first class prime timber” because it is hard, durable, easy to work on and resistant to insects. It is a slow-growing, perennial, non-climbing tree widely distributed in Indochina like

[*] **Corresponding Author:** Benchawattananon, R.; **Email:** rachadaporn@kku.ac.th

Thailand, Vietnam, Laos, and Cambodia. In Thailand, this species occurs in 15 of 20 provinces in Northeast Thailand and its core is used in folk medicine for the treatment of tumor and blood stasis (Liu *et al*., 2016). This species is one of the most valuable timber species in Indochina and is highly sought after for its attractive wood, which is used especially for luxury furniture (Hartvig *et al*., 2018).

Apart from deforestation, illegal logging and associated trade in illegally sourced wood products along the borders of Thailand, Laos and Cambodia are significant contributors to the depopulation of rosewood. International efforts to combat the problem consist primarily of the enactment of laws designed to discourage the trade in illegally sourced timber, and prohibit or limit the trade of specific species or those from specific areas. Trade restrictions are imposed primarily through the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) which lists species in one of three appendices depending on the degree of protection required (Dormontt *et al*., 2015).

Due to the popularity and the exploitation of this species, it is listed as "critically endangered" on the Red List of the International Union for the Conservation of Nature (IUCN) and in the Appendix II of CITES, which lists species that are not currently at threat of extinction, but require controlled trade to avoid over-utilization and future extinction threats, in March 2013 (Zhang *et al*., 2016).

At the forest patch level, the factors that may affect small-scale variations in the topsoil properties include micro- or fine-scale variations in parent rock materials and mineral soil texture, terrain orography, microrelief and pedogenic memory (e.g. effects of snow- and wind-throw, woody debris and soil preparation) (Ayres *et al*., 2009; Paluch and Gruba, 2012). Trees are another important source of variation, which are capable of modifying soil properties in their neighbourhood depending on site conditions, stand density and species-specific architectural and physiological characteristics (Paluch and Gruba, 2012). Thus, in forensic science and other related fields, establishment of the relationships between tree samples and soil or topsoil properties may be useful in providing the useful pieces of evidence for confirming the origin of the trees.

This study was carried out to identify rosewood samples collected from different localities of Thailand, Laos and Cambodia using morphological and anatomical characteristics and FTIR, and to establish relationships between their elemental components and the soil properties for identification of endemic species.

Materials and methods

Materials and sample preparation

The leaf and bark samples of rosewood trees and the rhizosphere soil samples used for the current study were collected from Khon Kaen province of Thailand, Chaiburi province of Laos, and Siem Reap province of Cambodia. The leaf and bark samples were collected from 3-4 trees per site, thereby consisting of 6-8 samples per sampling site. The rhizosphere soil samples were collected by marking a grid of 10 × 10 m^2 area in the sampling sites. The surface of the earth is partially cleaned to remove debris and other foreign contaminations. After that, approximately 500 g of the soil samples were collected from all four corners and one from the center of the area. The soil samples from 1 m of depth horizons were also collected.

The leaf and bark samples were thoroughly cleaned and oven-dried at 60 ℃ for 2-3 days until the weight was stabilized. The samples were then ground to a fine powder and sieved through a 2-mm mesh. On the other hand, the soil samples were air-dried at room temperature for 5 days, then crushed in a mortar and sieved through a 2-mm mesh to remove a large number of stones, leaves and other waste materials. All the samples were stored in plastic bags in a desiccator to avoid any foreign contamination and also to minimize the effect of hot or humid environment.

Apparatus and procedure

The spectra of soil, leaf, and bark samples were recorded on a Spectrum GX FT-IR system (Perkin Elmer) equipped with a DTGS detector. The spectral range from 4000 to 600 cm^{-1} was selected. The sixteen scans per sample with a resolution of 8 cm^{-1} were preferred to avoid the chances of errors/noise in the spectra. The instrument was calibrated with the polystyrene film and the background was done by running the instrument without placing any sample. Each sample was scanned three times and average spectrum value was used as the representative spectra. The repeatability and reproducibility of FTIR instrument were checked by evaluating each of the samples 10 times. The resulted absorbance values of all the 10 samples that gave a standard deviation of ± 0.005 signified the reproducibility of FTIR instrument for these samples.

Results

Morphological characteristics of soil, leaf, and bark samples

The morphological characteristics of the soil, leaf, and bark samples collected from Laos, Cambodia and Thailand are depicted in Figures 1, 2 and 3, respectively. There were differences in the morphology of the soil, leaf and bark samples collected from different three countries. As illustrated in Figure 1, the soil collected from Laos was sandy loam and slightly red in color, which indicates the abundance of iron oxide. Meanwhile, the soils collected from Cambodia and Thailand appeared to be silt loam with dark color, indicating the richness in humic substances.

Figure 1. Morphological characteristics of the soil samples collected from Laos (A), Cambodia (B) and Thailand (C)

The leaf samples collected from Cambodia were larger and thicker with darker green color than those collected from Laos and Thailand (Figure 2). As shown in Figure 3, it was found that the bark samples collected from were blackish-grey, while those collected from Laos and Thailand appeared to be brownish-grey.

Figure 2. Morphological characteristics of the leaf samples of rosewood collected from Laos (A), Cambodia (B) and Thailand (C)

Figure 3. Morphological characteristics of the bark samples of rosewood collected from Laos (A), Cambodia (B) and Thailand (C)

FTIR spectra of soil, leaf, and bark samples

FTIR spectra of the soil, leaf, and bark samples collected from Laos, Cambodia and Thailand are presented in Figures 4, 5 and 6, respectively. The assignments of the bands are given in Table 1, 2 and 3.

As shown in Figure 4 and Table 1, there were some differences in the patterns of FTIR spectra of the soil samples collected from Laos, Cambodia and Thailand. It was found that the most intense peaks at 686 (Si–O quartz), 772 (Si–O quartz), 905 (OH deformation) and 992 (Si–O stretching) cm^{-1} and the presence of peaks at 3618 (Al–O–H stretching) and 3698 (Al–O–H stretching) cm^{-1} could also be used to distinguish the soil samples from Thailand from those from Laos and Cambodia. Meanwhile, the most intense peak at 1638 cm^{-1} (H–O–H bending of water) and the presence of peak at 1412 cm^{-1} (C–H stretching) could be used to differentiate the soil samples from Cambodia from those from Laos and Thailand.

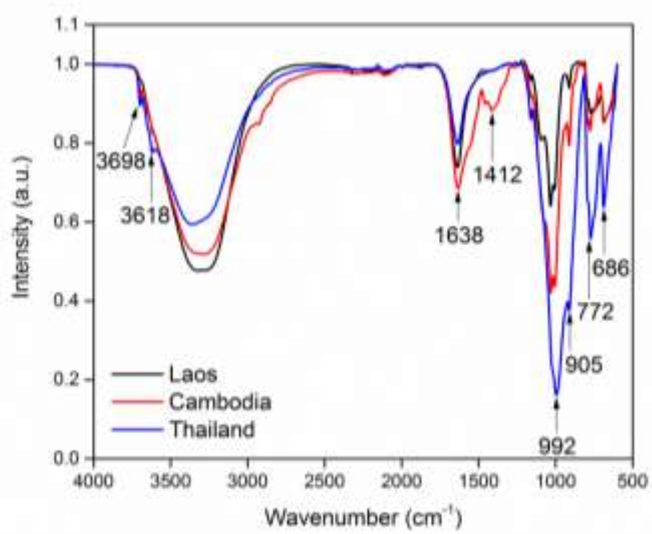

Figure 4. FTIR spectra of the soil samples collected from Laos (A), Cambodia (B) and Thailand (C)

The leaf samples from Thailand could be distinguished from those collected from Laos and Cambodia by the most intense peaks at 1632 (C–O stretching of flavonoids), 1432 ($C–H_2$ scissoring in lignin and carbohydrates), 1318 (C–H stretching in carbohydrates) and 1245 (C–O stretching mainly in cellulose) cm^{-1} (Figure 5 and Table 2). Meanwhile, the most intense peaks at 2912 ($C–H_2$ asymmetric stretching) and 2845 (aliphatic C–H stretching) cm^{-1}

could be employed for differentiating the leaf samples from Laos from those collected from Cambodia and Thailand. On the other hand, the most intense peaks at 1025 (C–O stretching in lignin and holocellulose), 872 (C_1–H deformation in cellulose) and 779 (calcium oxalate) cm^{-1} and the presence of peaks at 1732 (unconjugated C–O stretching in xylans) and 3705 (O–H stretching) cm^{-1} could be used to indicate the leaf samples from Cambodia.

Table 1. The characteristic infrared bands and intensities of the soil samples collected from Laos, Cambodia and Thailand in the region of 4000 – 600 cm^{-1}

Wavenumber (cm^{-1})	**Intensity**			**Band assignment**
	Laos	**Cambodia**	**Thailand**	(Naidja *et al*., 2002; Saikia and Parthasarathy, 2010; Heller *et al*., 2015; Petit and Puskar, 2018; Xing *et al*., 2019)
3698	–	+	+	Al–O–H stretching
3618	–	–	+	Al–O–H stretching
1638	++	+++	+	H–O–H bending of water
1412	–	++	–	C–H stretching
992	+	++	+++	Si–O stretching
905	+	++	+++	OH deformation, linked to $2Al^{3-}$
772	+	++	+++	Si–O quartz
686	++	++	+++	Si–O quartz

Note: –, invisible; +, weak; ++, moderate; +++, strong

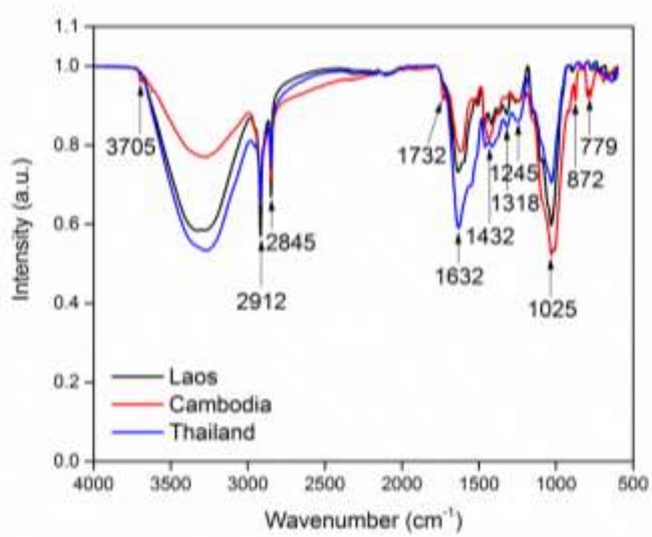

Figure 5. FTIR spectra of the leaf samples collected from Laos (A), Cambodia (B) and Thailand (C)

Table 2. The characteristic infrared bands and intensities of the leaf samples collected from Laos, Cambodia and Thailand in the region of 4000 – 600 cm^{-1}

Wavenumber (cm^{-1})	**Intensity**			**Band assignment**
	Laos	**Cambodia**	**Thailand**	(Zhang *et al*., 2014; Zhang *et al*., 2016)
3705	–	+	–	O–H stretching
2912	+++	+	++	C–H_2 asymmetric stretching
2845	+++	++	+	Aliphatic C–H stretching
1732	–	+	–	Unconjugated C═O stretching in xylans
1632	++	+	+++	C═O stretching of flavonoids
1432	+	++	+++	C–H_2 scissoring in lignin and
1318	–	++	+++	carbohydrates
1245	++	++	+++	C–H stretching in carbohydrates
1025	++	+++	+	C–O stretching mainly in cellulose
872	+	+++	+	C–O stretching (lignin and holocellulose)
779	+	+++	+	C_1–H deformation (cellulose)
				Calcium oxalate

Note: –, invisible; +, weak; ++, moderate; +++, strong

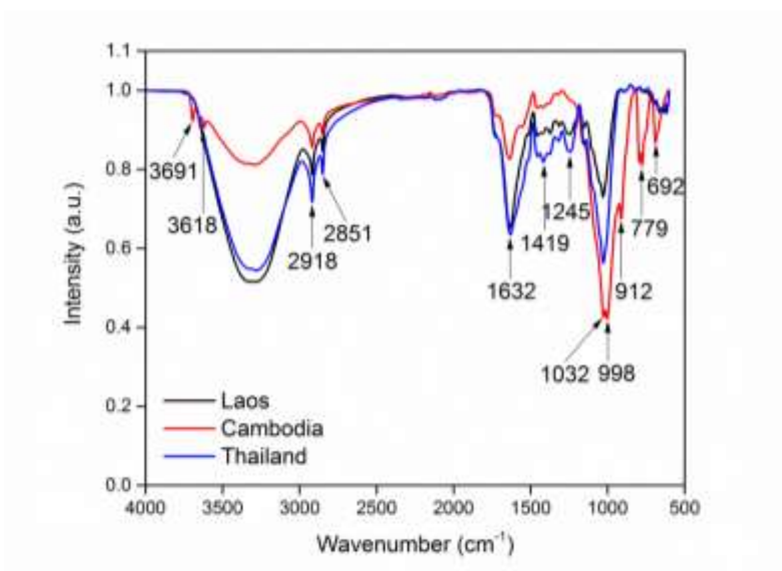

Figure 6. FTIR spectra of the bark samples collected from Laos (A), Cambodia (B) and Thailand (C)

Interestingly, the bark samples from the three countries could be simply distinguished (Figure 6 and Table 3). The most intense peaks at 2918 (C–H_2 asymmetric stretching), 2851 (aliphatic C–H stretching) and 1632 (C–O stretching of flavonoids) cm^{-1} and the presence of peak at 1419 cm^{-1} (C–H_2 scissoring in lignin and carbohydrates) could be used to distinguish the bark samples from Thailand from those collected from Laos and Cambodia.

Meanwhile, the bark samples from Cambodia could be distinguished from those collected from the other countries by the most intense peaks at 1032 (C–O stretching in lignin and holocellulose) and 998 (C–C stretching) cm^{-1} and the presence of peaks at 3691 (O–H stretching), 3618 (O–H stretching), 912 (aromatic C – H out-of-plane deformation), 779 (calcium oxalate) and 692 (aromatic C–H stretching) cm^{-1}.

Table 3. The characteristic infrared bands and intensities of the bark samples collected from Laos, Cambodia and Thailand in the region of 4000 – 600 cm^{-1}

Wavenumber	**Intensity**			**Band assignment**
(cm^{-1})	**Laos**	**Cambodia**	**Thailand**	(Zhang *et al*., 2014; Zhang *et al*., 2016)
3691	–	++	–	O–H stretching
3618	–	+	–	O–H stretching
2918	++	+	+++	C–H_2 asymmetric stretching
2851	++	+	+++	Aliphatic C–H stretching
1632	+++	++	+++	C═O stretching of flavonoids
1419	–	–	+	C–H_2 scissoring in lignin and
1245	+	–	++	carbohydrates
1032	–	+++	–	C–O stretching mainly in cellulose
998	+	+++	++	C–O stretching (lignin and holocellulose)
912	–	++	–	C–C stretching
779	–	++	–	Aromatic C–H out-of-plane deformation
692	–	++	–	Calcium oxalate
				Aromatic C–H stretching

Note: –, invisible; +, weak; ++, moderate; +++, strong

Discussion

As shown in Figures 1-3, the soil samples from different three countries could be most easily distinguished by the anatomical characteristics, followed by the bark samples and leaf samples. The differences in the appearance of these soil samples might be due to the differences in parent rock materials and mineral soil texture, terrain orography, microrelief and pedogenic memory such as woody debris (Ayres *et al*., 2009; Paluch and Gruba, 2012). It is well recognized that trees can influence the biological, chemical and physical properties of soils directly through their deep roots and litter quality and quantity (Day *et al*., 2010). Previous studies on arable land, which has strongly depleted soil organic carbon stocks (Lal, 2004), have shown that afforestation typically results in increased carbon sequestration both above and belowground (Guo and Gifford, 2002).

Even though the appearance and anatomical characteristics of some soils and wood are strikingly similar (Hartvig *et al*., 2015), their chemical compositions may have considerable differences. Accordingly, the soils, and leaf and bark samples of rosewood from different three countries could be easily differentiated according to the discrepancy of their chemical constitutions by FTIR technique. The results showed that all the studied samples could be distinguished by FTIR technique, with the bark samples considered to be the most promising targets for identification of endemic species to trace back the origin of illegally sourced wood products.

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