Chemometric Differentiation of Dipterocarpaceae Wood Species Based on Colorimetric Measurements

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The international trade of illegal timber often involves the misdeclaration of the wood species. A simple and reliable means for the differentiation of wood species could contribute to the control of this fraud. In this study, eight (8) commercially important and endangered dipterocarp timber wood species and mahogany were differentiated through colorimetric measurements carried out on hot water and ethanol extracts from the samples. Colorimetric measurements were done using a fabricated colorimeter that measured the absorption of blue, green, and red radiation. Chemometric analysis of the colorimetric data using principal component analysis (PCA) and hierarchical cluster analysis (HCA) revealed clustering, which enabled an efficient differentiation of the wood species.

INTRODUCTION

The development of rapid and accurate methods for the differentiation of wood species presents a challenge. A need has been expressed for methodologies that can be applied for the definitive identification of illegally cut logs (Dormontt et al. 2015). The international trade of illegal timber has promoted the destruction of the world’s forest and threatened the conservation of valuable and endangered woody plants (Li et al. 2008). The control of this trade is hindered by a lack of a reliable wood identification system that can be used in the field since illegal traders often misdeclare their commodities and forest guards cannot determine the correct identity of the wood species being hauled by merchants.

Several techniques have been applied for the determination of wood identity. Presently, the most frequently used method for timber identification is the visual analysis of the wood’s anatomical features. This technique is capable of identification to the genus level only, but often information on the species level is needed (Gasson 2011). DNA-based methods have been developed to determine species identity and provenance of logged timber (Degen and Fladung 2008). Although accurate, these genetic methods involve lengthy analysis time and require expensive materials and equipment.

Chemical methods offer a variety of instrumentation principles that can supply information not obtainable through visual examination within a brief period (Dormott et al. 2015). Most of these techniques are based on the characterization of wood extractives.
Extractives are non-structural components that comprise a secondary fraction of wood. These are low-molecular-weight compounds that can be extracted from wood by solvents and that account for distinct attributes such as odor, color, and durability (Hillis 1971, da Silva et al. 2013). The composition of extractives differs considerably from species to species and presents a basis for species differentiation (Nault and Manville 1997).

Wood identification could be achieved through the characterization of extractives, with or without isolating these molecules from the wood matrix. The analysis of extractives through mass spectrometry (Cabral et al. 2012, Lancaster and Espinoza 2012, Espinoza et al. 2014); Fourier-transform infrared spectroscopy (FT-IR) (Huang et al. 2008, Hobro et al. 2010, Rana et al. 2010, Chen et al. 2010); near-infrared spectrometry (Abedi et al. 2008, Russ et al. 2009, Braga et al. 2011, Pastore et al. 2011); Raman spectrometry (Lavine et al. 2001); colorimetry (Abasolo and Gibe 2015); and gas chromatography-mass spectrometry (Marques et al. 2012, Chen et al. 2015) had been shown to enable differentiation of wood species. Electronic nose technology has likewise been explored for the discrimination of wood species (Garneau et al. 2010, Cordeiro et al. 2012). Differentiation was highlighted through the application of chemometric techniques such as PCA and partial least squares methods.

This paper describes the differentiation of selected dipterocarp wood species through colorimetry coupled with a chemometric analysis of the results obtained from wood extracts. Dipterocarps are commercially important wood species and are highly valued as hardwood timber (Rana et al. 2012). The species have been overutilized because of their superior qualities as plywood and lumber, and as a result they are now considered as critically endangered (IUCN 2018). As such, these species are covered by a logging ban in several countries in the Asia-Pacific region, including the Philippines and Thailand (Durst et al. 2001).

Dipterocarp species have been differentiated through FT-IR spectroscopy (Rana et al. 2010), tree height, wood density, and molecular markers (Rana et al. 2012). In an earlier paper, an analysis of variance carried out on the results of colorimetric measurements from extracts of several wood species – including dipterocarps – indicated that wavelength and solvent had the potential to differentiate wood species (Abasolo and Gibe 2015). Colorimetry presents the advantages of inexpensive instrumentation and simple operation, compared to the other spectroscopic, chromatographic, electronic, and DNA-based methods for species differentiation.

MATERIALS AND METHODS

Wood samples

Selected dipterocarp species belonging to the commercially important Philippine mahogany group were used in this study. Mahogany was also studied to differentiate it from the Philippine mahogany group. Table 1 lists the species investigated, their common name, and their conservation status as assessed by the International Union for Conservation of Nature (IUCN).

The samples were identified and provided by the Forest Products Research and Development Institute of the Department of Science and Technology, and the Department of the Forest Products and Paper Science of the College of Forestry and Natural Resources, University of the Philippines Los Baños. All the samples were chopped to matchstick size and then ground to a powder using a Wiley mill. Three wood samples of each species were used for the measurements.

<table>
<thead>
<tr>
<th>Wood species</th>
<th>Sample code</th>
<th>Philippine common name</th>
<th>Conservation status (IUCN 2018)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipterocarpus grandiflorus Blanco</td>
<td>Dg</td>
<td>A pitong</td>
<td>Critically endangered</td>
</tr>
<tr>
<td>Parashorea malacca (Blanco) Merr.</td>
<td>Pm</td>
<td>Bagtikan</td>
<td>Critically endangered</td>
</tr>
<tr>
<td>Shorea almon Foxw.</td>
<td>Sal</td>
<td>Almon</td>
<td>Critically endangered</td>
</tr>
<tr>
<td>Shorea astylola Foxw.</td>
<td>Sas</td>
<td>Yakal</td>
<td>Critically endangered</td>
</tr>
<tr>
<td>Shorea contorta Vidal</td>
<td>Sc</td>
<td>White lauan</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Shorea negrosensis Foxw.</td>
<td>Sn</td>
<td>Red lauan</td>
<td>Critically endangered</td>
</tr>
<tr>
<td>Shorea polysperma (Blanco) Merr.</td>
<td>Spa</td>
<td>Mayapis</td>
<td>Critically endangered</td>
</tr>
<tr>
<td>Swietenia macrophylla</td>
<td>Spo</td>
<td>Tanguile</td>
<td>Critically endangered</td>
</tr>
<tr>
<td>Mahogany</td>
<td>Sm</td>
<td>Vulnerable</td>
<td></td>
</tr>
</tbody>
</table>

Extraction procedure

A simple extraction method was used which can be performed in the field. Extracts of the powdered wood samples were prepared immediately before analyses, using hot distilled water and ethanol as the solvents. These solvents were chosen to enable the selective extraction of hydrophilic and hydrophobic components. The wood...
samples (10 g) were placed in a vial and the solvent (15 mL) was added. The mixture was stirred vigorously for about 2 min to facilitate the extraction and was set aside to allow the residues to settle completely. The supernatant liquid was transferred to the cuvette for colorimetric measurement.

**Colorimetric measurement**

The colorimetric measurement was carried out using a fabricated colorimeter, which was previously described (Abasolo and Gibe 2015). The colorimeter measured optical absorption of blue (BLU), green (GRN), and red (RED) radiation with peak wavelengths at 468, 520, and 650 nm, respectively. The measurement output was expressed in millivolts (mV), which has been shown to have a linear correlation with the absorbance of the solution.

**Chemometric analysis**

The colorimetric data were analyzed using PCA and HCA through the XLSTAT software package (Addinsoft, USA). PCA reduces the dimensionality of the multivariate data set without losing important information. It transforms the data into components, which are linear combinations of interrelated variables; and the components are graphically illustrated through score and loading plots. HCA groups the data based on calculated Euclidian distance and generates a dendrogram, which clusters variable according to the degree of similarity.

**RESULTS AND DISCUSSION**

**Absorption of radiation by wood extracts**

The extracts exhibited varying colors, ranging from very light yellow to light brown to dark red. In general, the color of the ethanol extracts was darker than that of the hot water extracts. The distinction of the colors was quantified by the colorimeter data obtained using blue (468 nm), green (520 nm), and red (650 nm) light sources are shown in Figure 1. These data represent the absorption of the radiation by the extracts. The extent of optical absorption varies with the concentration and the molar absorptivity of the light absorbing species present in the extract.

Visual inspection of Figure 1 reveals that the extent of absorption of the radiation by the water extracts decreases with the wavelength, with the blue radiation as the most absorbed and the red radiation as the least absorbed. Blue radiation is strongly absorbed by the ethanolic extract, particularly by Dipterocarpus grandifolius, Shorea astylosa, Swietenia macrophylla, and Shorea negrosensis. The higher absorption for these species can be correlated with a higher extractive content. These species have been shown to have an extractive content ranging from about 5 to 17%, while the other species were found to have lower extractive content of about (3%) (Wahlgren and Laundrie 1977, da Silva et al. 2013).

The extracts contain wood extractives that are soluble in the solvent used. The water-soluble extractives are mostly hydrophilic compounds such as phenolic compounds and some carbohydrates (Sjöström and Alén 1998). The ethanol-soluble extractives include both hydrophilic and hydrophobic compounds such as aliphatic and alicyclic compounds, phenols, and carbohydrates (Wahlgren and Laundrie 1977). Some of the extractives have molecular structures such as tannic acids and flavonoids, which are able to absorb radiation in the visible region.

Correlation analysis showed that the absorption data for the water extracts with the blue and green radiation were correlated and that the results for the ethanol extracts with the green and red radiation were also correlated. Correlated properties will provide similar information, and it will be sufficient to consider only one of the properties. As a result, only the data of the water extract with the blue and red radiation and the data of the ethanol extract with the blue and green radiation were considered in the data analysis.

When the normalized data were plotted in a radar graph (Figure 2), different patterns resulted for each of the wood species. These patterns could serve as a fingerprint that will enable the differentiation and identification of the wood species. It can be employed for the authentication of a sample.

The high absorption of blue radiation exhibited by the water extracts of Parashorea malaanonan, Shorea almon, and Shorea palosapis are noticeable in the water plots. The similarity in the behavior of these species can signify a relationship among these three species, which are classified as light density wood species (Brown 1997, Rana et al. 2009). Likewise, the plot highlights the high absorption of blue radiation by the ethanol extracts of Dipterocarpus grandifolius, Shorea astylosa, and Swietenia macrophylla. These three species exhibit medium wood density (Brown 1997, Rana et al. 2009).

**PCA**

In order to emphasize the variation in the behavior of the colorimeter data obtained from the different wood samples, PCA was applied. PCA reduces the set of variables into two principal components (PCs), which can be presented in a score plot to reveal groupings according to the possible similarity in characteristics. These groupings are useful in differentiating the samples.

PCA was initially conducted using a set of six variables.
that consisted of the readings of the water and ethanol extracts with the three radiations. However, analysis of the loading contribution of the variables towards the differentiation of the groups showed similarities between the loadings of the water extract with blue and green radiation, and between the loadings of the ethanol extracts with green and red radiation. Note that these were the same pairs that were identified in the correlation analysis. Consequently, PCA was applied to only four variables: the results obtained from the water extracts with the blue and red radiation, and the data of the ethanol extract with the blue and green radiation.

Figure 3 shows the resulting PCA score plot. A distinct grouping of the data points for the species of the wood species was obtained for the first two components F1 and F2, with a high total variance of 98.07%. The first component F1 differentiated distinctly five species – namely Dipterocarpus grandifolius, Shorea astylosa, Swietenia macrophylla, Shorea negrosensis, and Shorea contorta. The second component F2 contributed to the differentiation of Parashorea malaanonan, Shorea almon, Shorea palosapis, and Shorea polysperma.

The PCA score plot presents a clear separation between mahogany and the wood samples in the Philippine mahogany group. This graphical feature enables the authentication of mahogany and its discrimination from the Philippine mahogany group (dipterocarp species).

Two groupings are recognizable from the plot – those at the right side are heavy and medium-density hardwood
species, and those at the left side are light hardwood species (Wahlgren and Laundrie 1977, da Silva et al. 2013). The species clustered at the left side belong to the genus Shoreae (Rana et al. 2009). Within this cluster, the pair of Parashorea malaanonan and Shorea almon are not well-separated. Similar proximity of the cluster for these two species was also observed when cluster analysis was applied to tree height and wood density data (Rana et al. 2012) and FT-IR spectral data (Rana et al. 2010).

HCA

HCA highlights the dissimilarity relationships between each wood species through the Euclidean distance among the groups. Figure 4 shows the dendrogram that was obtained when HCA was applied to the multivariate data.

Two main clusters can be recognized in the dendrogram: one group includes Dipterocarpus grandifolus, Shorea astylosa and Swietenia macrophylla, which are heavy and medium-density hardwood species; and the other group includes the rest of the samples that are the light hardwood species (Brown 1997, Rana et al. 2009). These main groups are similar to those observed in PCA.

Inspection of the light hardwood cluster reveals two sub-clusters: one for Shorea almon, Shorea palosapis, and Shorea polysperma; and another for Parashorea malaanonan, Shorea negrosensis, and Shorea contorta. The species in the first sub-cluster belong to the genus Shorea sect. Brachypterae (LaFrankie 2010). The species in the second sub-cluster were from different groups – Parashorea, White Meranti, and Shorea negrosensis groups. Parashorea malaanonan and Shorea contorta formed a cluster based on similarity in wood anatomy (Ashton 2004) and were shown to have a close relationship by phylogenetic analysis (Harnelly 2013) and tree traits studies (Rana et al. 2010).

CONCLUSION

Colorimetric measurements were carried out with three radiation sources on the water and ethanol extracts of mahogany (Swietenia macrophylla) and several Dipterocarpaceae species, which are commercially known as Philippine mahogany group. Similarities in the behavior of the colorimetric readings of several wood species were recognized. The differentiation of the sample species was highlighted through the application of chemometric techniques such as PCA and HCA. These pattern recognition methods differentiated not only the individual species but also the heavy and medium-density hardwood and the light hardwood groups. This behavior can be applied to discriminate dipterocarp species and to ascertain the wood identity declared by timber traders. Colorimetry presents a simple, rapid, and inexpensive method that can differentiate effectively timber wood species of the Dipterocarpaceae family.
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