Original Article

Authentication of sandalwood crude drugs using gas chromatography-mass spectrometry and chemometric analysis

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Abstract

Authentication of sandalwood crude drugs available in Thai traditional drugstores was investigated by gas chromatography-mass spectroscopy combined with chemometric methods. Three species of Santalum were discriminated based on GC-MS fingerprint analysis using similarity analysis, cluster analysis and principal component analysis. The major common peaks of the chromatogram were identified by Kovats Index (KI) calculation and by comparing their mass spectra to those in libraries database. All data were compared with those of authentic samples. Most crude drugs were identified as S. spicatum, whereas only some samples were S. album and S. lanceolatum. The result of this study concluded that S. album, the correct species mentioned for medical use of sandalwood, was currently substituted with other Santalum species.

Keywords: sandalwood, authentication, GC-MS, fingerprint analysis, chemometric

1. Introduction

Sandalwood is an important crude drug of Thai traditional medicine. It is used as febrifugal, nerve and skin tonic, anthelmintic, and used for the treatments of aphous ulcer, thirsty, liver disease, pulmonary disease and bile disease (Department for Development of Thai Traditional and Alternative Medicine, 2009). It is also used for genitourinary and bronchial tracts infection, diuretic and expectorant (Sindhu, Upma, Kumar, & Arora, 2010). Its essential oil is mainly used in the perfumery industry and also traditional used for common colds, bronchitis, fever, urinary tract infection and inflammation of the mouth and pharynx (Burdock, & Carabin, 2008). Most textbooks mention that sandalwood used in Thai traditional medicine is originally from the heartwood of Santalum album L. (family Santalaceae). S. album is a native plant widely distribute in southern India, Australia, Timor, Hawaii, etc. Nearly 90% of the natural sandalwood was grown in the southern region of India at Karnataka and Tamil Nadu. Therefore, India was the leader supplier of sandalwood in worldwide market. Sandalwood from India is known as East Indian sandalwood. However, populations of S. album were dramatically decline due to excessive harvesting without replenishment. Currently, most of India sandalwood is substituted supplied by Australia sandalwood (Arun Kumar, Joshi, & Mohan Ram, 2012; Subasinghe, 2013). Six species of Santalum are native plants of Australia and the most exploited being S. spicatum A. DC. and S. lanceolatum R. Br, which are known as Western and Northern Australian sandalwood, respectively (Patricia, 2015).

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Our previous study found that sandalwood in Thai traditional drugstore was available under the names of Chan-theet and Chan-hom. Based on thin-layer chromatography (TLC) method, they were identified as either _S. album_, _S. spicatum_ or _S. lanceolatum_, (Srisopon, Burana-oso, et al., 2017). TLC’s advantage is its simplicity and being inexpensive. However, discrimination between each herb from its closely related species is often conducted in a subjective manner and could be easily error or bias. To confirm and gain more information on sandalwood crude drug in Thailand, this study focused on the chemical fingerprints using gas chromatography coupled to mass spectrometry detector (GC-MS). Multivariate statistical analyses, i.e. similarity analysis (SA), hierarchical cluster analysis (HCA) and principal component analysis (PCA), were the tool used to reduce the subjective decision. Moreover, each main component appearing on the chromatogram was also identified and compared with those of each authentic _Santalum_ species.

2. Materials and Methods

2.1 Crude drug and authentic samples

Twenty-three samples of sandalwood were purchased from Thai traditional drugstores in various regions of Thailand during 2012 to 2013. The authentic samples of _S. album_ were collected from Prachuap-Khiri-Khan Silvicultural Research Station, Royal Forest Department. The authentic samples of _S. spicatum_ and _S. lanceolatum_ were the gifts obtained from Professor Dhanushka S. Hettiarachchi, Wescrop group, Australia. All crude drug samples and authentic were chopped and ground to fine powder. The fine powder (200 mg) of all samples was extracted with n-hexane (2 mL) by sonication for 60 min. The supernatants was filtered with 0.22 µm PVDF filter and subsequently stored at -20°C. Fifty mg of each sample was scanned from 40 to 550 m/z at 1 scans s⁻¹. The chemical constituents were identified based on their Kovats Index (KI), calculated in relation to the retention time of a homologous series of normal alkanes (C8-C20 and C31-C40) as reference products, in comparison with those of the chemical compounds gathered by Adams table (Adams, 2001), the similarity of their mass spectra with those gathered in the MS libraries data (NIST05.LIB version 2002 and Wiley database version 7th edition) provided by the software of the GC-MS system, or reported in the literature. For comparison among fingerprints, the position of each peak was calculated as relative retention time (RRT) referenced to the retention time of the identified α-santalol peak. Quantitative analysis of each component (expressed as area percentage) was carried out by a peak area normalization measurement.

2.2 Gas chromatography-mass spectrometry

GC-MS analyses were performed on a 6890 gas chromatograph, a 5973N mass selective detector (EIMS, electron energy, 70 eV) with a quadrupole analyser, and an Agilent ChemStation data system (Agilent Technologies, U.S.A.). A DB-5MS non-polar fused silica capillary column with a 5 % phenyl-methylpolysiloxane stationary phase (30 m x 0.25 mm id x 0.25 μm film thickness, Agilent Technologies, U.S.A.) was used. The GC settings were as follows: the initial oven temperature was held at 50°C and then heated to 120°C at a rate of 20°C min⁻¹, held for 1 min, and raised at 8°C min⁻¹ to 160°C, held for 2 min, then heated to 170°C at a rate of 2°C min⁻¹, held for 3 min, subsequently increased at 5°C min⁻¹ to 200°C, held for 2 min, and increased at 3°C min⁻¹ to 250°C, held for 3 min, and finally heated to 280°C at 20°C min⁻¹ and held for 20 min. The injector temperature was maintained at 250°C. The n-hexane extract (1 μL) was injected neat, with a splitless mode. The carrier gas was ultra-high purity (99.999%) helium at flow rate of 1.0 mL min⁻¹. Spectra were scanned from 40 to 550 m/z at 1 scans s⁻¹.

The similarity analysis was identified based on their Kovats Index (KI), calculated in relation to the retention time of a homologous series of normal alkanes (C8-C20 and C31-C40) as reference products, in comparison with those of the chemical compounds gathered by Adams table (Adams, 2001). The similarity analysis was calculated as correlation coefficient values among fingerprints. The similarity analysis was calculated as relative retention time (RRT) referenced to the retention time of the identified α-santalol peak. Quantitative analysis of each component (expressed as area percentage) was carried out by a peak area normalization measurement.

2.3 Data analysis

All GC chromatograms (retention time in the range of 16-21 min) were pretreated by Savitzky-Golay smoothing (zero derivative order, zero polynomial and eleven smoothing points) and maximum normalization. The similarity analysis was calculated as correlation coefficient values among GC chromatograms of three authentic _Santalum_ species (r = 0.02-0.54) indicated that their GC chromatograms were dissimilar enough for species discrimination. Three groups of crude drug samples were suggested based on their similarity to each authentic species (Table 1). Samples 1-3 had similar chromatographic patterns to _S. album_ (r = 0.94-0.98), whereas

Table 1. Similarity analysis of the GC chromatograms of all samples. Data presented as minimum to maximum correlation coefficient values.

<table>
<thead>
<tr>
<th></th>
<th><em>S. album</em></th>
<th><em>S. spicatum</em></th>
<th><em>S. lanceolatum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. album</em></td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td><em>S. spicatum</em></td>
<td>0.54</td>
<td>0.49</td>
<td>1.00</td>
</tr>
<tr>
<td><em>S. lanceolatum</em></td>
<td>0.02</td>
<td>0.49</td>
<td>0.05-0.15</td>
</tr>
<tr>
<td>Samples 1-3</td>
<td>0.94-0.98</td>
<td>0.61-0.70</td>
<td>0.35-0.59</td>
</tr>
<tr>
<td>Samples 4-21</td>
<td>0.19-0.74</td>
<td>0.86-0.98</td>
<td>0.89-0.90</td>
</tr>
<tr>
<td>Samples 22-23</td>
<td>0.12-0.13</td>
<td>0.42-0.42</td>
<td>0.42-0.42</td>
</tr>
</tbody>
</table>

3. Results and Discussion

Twenty-three samples of sandalwood crude drugs were collected from Thai traditional drugstores. Their authentication was based on their chemical fingerprints compared with authentic samples. Major constituents of sandalwood are volatile non-polar and could be extracted with n-hexane, then GC-MS was the technique used for this study. All GC chromatograms were extensively analyzed by multivariate data analysis methods, i.e. SA, HCA and PCA. The small similarity calculated as correlation coefficient values among GC chromatograms of three authentic _Santalum_ species (r = 0.02-0.54) indicated that their GC chromatograms were dissimilar enough for species discrimination. Three groups of crude drug samples were suggested based on their similarity to each authentic species (Table 1). Samples 1-3 had similar chromatographic patterns to _S. album_ (r = 0.94-0.98), whereas
samples 4-21 and 22-23 gave high similarity values to *S. spicatum* and *S. lanceolatum*, respectively (r = 0.86-0.98 and 0.89-0.90, respectively). These results were confirmed by HCA (Figure 1) and PCA (Figure 2). Three clusters were indicated from HCA dendrogram and score plot of PCA without overlapping between any three groups identified by SA.

Difference among three sample groups was identified by PCA (Figures 2 and 3). The PC1 and PC2 score plots explained for 83% of total variance. The first group (samples 1-3) which clustered with *S. album* was located on the positive side of PC2 which based on the loading plot mainly corresponded to the peak no.1. The second group (samples 4-21) was the cluster of *S. spicatum*. This group was on the positive side of PC1 of which the important explained variables were all peaks except for peak no.8. The rest of the samples (samples 22-23) closely clustered with *S. lanceolatum* at the negative side of PC1 and were obviously explained by the peak no.8. These eight common peaks were identified based on their mass spectra and KI as α-santalol, Z-α-trans-bergamotol, α-bisabolol, E-cis-epi-β-santalol, trans-farnesol, β-santalol, trans-nuciferol and cis-lanceol, respectively. Retention times of Z-α-trans-bergamotol (peak no.2) and α-bisabolol (peak no.3) were very close. All peaks were further quantified for % relative peak area (Table 2). Mean GC chromatograms of three sample groups are simulated in Figure 4. High content of α-santalol (peak no.1, 53.62 ± 2.91%) in group 1 samples confirmed their identification as *S. album* (Howes, Simmonds, & Kite, 2004; Misra, Das, & Dey, 2013). As same as *S. spicatum*, α-santalol (peak no.1), α-bisabolol (peak no.3) and trans-nuciferol (peak no.7) were predominant in group 2 (Brophy, Fookes, & Lassak, 1991; Valder, et al. 2003). *cis*-Lanceol (peak no.8) was the major compound of samples in group 3 and *S. lanceolatum* (Battaglia, 2016). All of these data corresponded to previous publications for volatile constituents of three authentic Santalum species.

The results of this study confirmed our previous study (Srisopon, Burana-osot, & Sotanaphun, 2017) using TLC technique. Information from Thai Customs Department (2007-2017) showed that sandalwood in Thailand in the last decade was mainly imported from Australia. *S. spicatum* and *S. lanceolatum* are native Santalum species of Australia (Battaglia, 2016). *S. spicatum* is distributed mostly in Western Australia, and then it is known as Western Australian sandalwood. It is an important cultivated economic plant.
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Figure 3. Loading plot of (A) PC1 and (B) PC2 of GC chromatograms of all samples.

Even through *S. album* or East Indian sandalwood is the correct species mentioned in Thai traditional textbooks, lack of this sandalwood species in global market caused its substitution with Australian sandalwood (Arun Kumar, Joshi, & Mohan Ram, 2012; Subasinghe, 2013). However, quality of sandalwood is depended on the content of santalol isomers (Boldovini, Delasalle, & Joulain, 2011; Subasinghe, 2013). Result of this study and previous publication (Brand, Fox, Pronk, & Cornwell, 2007) indicated that the content of these compounds in *S. spicatum* were much lower than *S. album*. Several bioactivities have been reported for santalol isomers, e.g. anti-inflammatory, anticancer, anti-hyperglycemic, neurological activity (Bommareddy et al., 2017). Then quality of Thai traditional recipe composed with different species of sandalwood is of concern and should be further studied.

4. Conclusions

Three *Santalum* species of sandalwood crude drugs currently available in Thai traditional drugstores were identified. Most of them were *S. spicatum*, whereas some were *S. album* and *A. lanceolatum*. GC-MS coupled with chemometric method was the informative analysis method to distinguish among these three species.

Acknowledgements

The authors are thankful to all for providing authentic samples and Faculty of Pharmacy, Silpakorn University for facility support of the research.

Table 2. The relative peak area (RPA) of the major common peaks of GC chromatograms of sandalwood samples (Group 1 = samples 1-3, Group 2 = samples 4-21, Group 3 = samples 22-23) and authentic samples (SA = *S. album*, SS = *S. spicatum*, SL = *S. lanceolatum*).

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>RRT*</th>
<th>Compound</th>
<th>% Relative peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td>1</td>
<td>1.00</td>
<td>α-santalol</td>
<td>53.62 ± 2.91</td>
</tr>
<tr>
<td>2</td>
<td>1.02</td>
<td>Z-α-trans-bergamotol</td>
<td>8.32 ± 1.23</td>
</tr>
<tr>
<td>3</td>
<td>1.02</td>
<td>α-bisabolol</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>1.05</td>
<td><em>E</em>-cis-epi-β-santalol</td>
<td>2.66 ± 0.50</td>
</tr>
<tr>
<td>5</td>
<td>1.07</td>
<td><em>trans</em>-farnesol</td>
<td>4.84 ± 0.82</td>
</tr>
<tr>
<td>6</td>
<td>1.08</td>
<td>β-santalol</td>
<td>18.04 ± 1.86</td>
</tr>
<tr>
<td>7</td>
<td>1.09</td>
<td><em>trans</em>-nuciferol</td>
<td>12.07 ± 5.17</td>
</tr>
<tr>
<td>8</td>
<td>1.18</td>
<td><em>cis</em>-lanceol</td>
<td>0.45 ± 0.07</td>
</tr>
</tbody>
</table>

*Relative retention time
References


