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

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| Keyword:                     | sandalwood, authentication, GC-MS, fingerprint analysis, chemometric |
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.

**Key words:** 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 apthous 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 (Burduck, & 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).

Our previous study found that sandalwood in Thai traditional drugstore was available under the names of Chan-thet and Chan-hom. Based on thin-layer chromatography (TLC) method, they were identified as either *S. album*, *S. spicatum* or
S. lanceolatum, (Srisopon, Burana-osot, & Sotanaphun, 2017). TLC’s advantage is simplicity and inexpensive. However, a subjective manner is often conducted. Discrimination between each herb from its closely related species 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 samples 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 subjected to GC-MS analysis.
2.2 Gas chromatography-mass spectrometry

GC-MS analyses were performed on a 6890 gas chromatography, 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 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, position of each peak was calculated as relative retention time (RRT) reference 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 (SA) was calculated as correlation coefficient by Microsoft Office Excel 2003 software (Microsoft Corporation). Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were carried out using the Unscramble X® (Camo Process AS, Norway). Cluster method and distance measure of HCA were Hierachical-linkage and Square Euclidean, respectively.

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 (Figure 2 and 3). The PC1 and PC2 score plots explained for 83% of total variance. The first group (sample 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 (sample 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 excepted for peak no.8. The rest samples (sample 22-23) closely clustered with *S. lanceolatum* at the negative side of PC1 was obviously explained by the peak no.8. These eight common peaks were identified based on their mass spectra and KI as \( \alpha \)-santalol, \( Z-\alpha \)-trans-bergamotol, \( \alpha \)-bisabolol, *E-cis-epi*-beta-santalol, *trans*-farnesol, \( \beta \)-santalol, *trans*-nuciferol and *cis*-lanceol, respectively. Retention times of \( Z-\alpha \)-trans-bergamotol (peak no.2) and \( \alpha \)-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 spicies.

<<Table 2>>

<<Figure 4>>

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 Santatum 
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 (Clarke, 2006). 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-inflammation, anticancer, anti-hyperglycemic, neurological activity (Bommareddy et al., 2017). Then quality of Thai traditional recipe composed with different species of sandalwood should be concerned and 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

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Figure 1. HCA dendrogram of GC chromatograms of all samples (SA = *S. album*, SS = *S. apicatum*, SL = *S. lanceolatum*).  

Figure 2. Score plot of PC1 and PC2 of GC chromatograms of all samples (SA = *S. album*, SS = *S. spicatum*, SL = *S. lanceolatum*).  

Figure 3. Loading plot of (A) PC1 and (B) PC2 of GC chromatograms of all samples.  

Figure 4. Simulative mean GC chromatograms of sandalwood samples (Group 1 = samples 1-3, Group 2 = samples 4-21, Group 3 = samples 22-23).
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Table 1. Similarity analysis of the GC chromatograms of all samples. Data presented as minimum to maximum correlation coefficient values.

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

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<th>Peak no.</th>
<th>RRT*</th>
<th>Compound</th>
<th>% Relative peak area</th>
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<td>Group 1</td>
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<tr>
<td>1</td>
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<td>α-santalol</td>
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<td>2</td>
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<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>E-cis-epi-β-santalol</td>
<td>2.66 ± 0.50</td>
</tr>
<tr>
<td>5</td>
<td>1.07</td>
<td>trans-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>
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<tr>
<td>7</td>
<td>1.09</td>
<td>trans-nuciferol</td>
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<td>8</td>
<td>1.18</td>
<td>cis-lanceol</td>
<td>0.45 ± 0.07</td>
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* Relative retention time