Comprehensive two-dimensional gas chromatography analysis of commercial essential oils from different sandalwood species

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Abstract

Essential oils (EOs) are often used for aromatherapy. With the rise of alternative medicine, demand for EOs is expected to continue growing and currently has an estimated global market value of $10 billion. This study uses comprehensive two-dimensional gas chromatography with flame ionization detection and quadrupole mass spectrometry (GC×GC-qMS/FID) to characterize the components of three commercial sandalwood EO products from species *Santalum paniculatum*, *Santalum spicatum*, and *Santalum album*. Results demonstrated about 27 major components within sandalwood EO. Sesquiterpene compounds dominated the sandalwood EO profile. The main components of all three species were α-santalol (17-55%) and β-santalol (7-21%), but other compounds varied across species (e.g. lanceol, farnesol, santalene, and bisabolol). GC×GC-qMS/FID was a powerful tool to elucidate subtle but important changes in these complex chemical profiles of natural products and could be applied in numerous similar applications where discrimination of products is desired.

Background

Various species of Sandalwood (Genus: Santalum) are grown in different regions of the world, including Hawaiian Sandalwood (*Santalum paniculatum*), Australian Sandalwood (*Santalum spicatum*) and East Indian Sandalwood (*Santalum album*). Sandalwood is commonly extracted into essential oil (EO) form such that it can be used for therapeutic purposes [1]. Sandalwood oil is typically used in capsule or emulsion form, but is also widely found in cosmetics, fragrance products, and aromatherapy products [2]. The major 'claims to fame' on packaging of sandalwood EO is its ability to reduce inflammation and provide benefits for the throat and airways. It has also been claimed as a medicine to treat sub-acute and chronic infections of mucous tissues, specifically in treating ailments such as ghonorrhea, bronchitis, diarrhea, bladder inflammation, and pyelitis [2, 3]. Sesquiterpenes such as α-santalol and β-santalol are the main constituents of sandalwood oil. Terpenes and sesquiterpenes are naturally occurring compounds responsible for familiar plant aromas and flavors. Many of these volatile compounds are known to have therapeutic benefits, which make sandalwood EOs promising for aromatherapy purposes.

The International Organization for Standardization (ISO) recently released a new version of ISO 3518[4], which specifies the characteristics to assess sandalwood oil (*Santalum album*) quality. This document replaces prior versions dating back to 1979. The original version requires measurements of α-santalol and β-santalol, while the newest version requires characterization of two additional compounds (*Z*-lanceol, *E,E*-farnesol). Developing a comprehensive method to profile these compounds and other additional components may improve understanding of sandalwood oil use and quality, and provide a means to monitor commercial products for other components such as contaminants or allergens.

No published work has investigated sandalwood oil (*Santalum album* or others) using multidimensional chromatography. Comprehensive two-dimensional gas chromatography (GC×GC) is a great technique for

EO characterization due to the high separation power of the instrument in resolving complex mixtures. In GC×GC, two columns are connected via a modulator junction that traps and releases short plugs from the primary onto the secondary column. The two columns provide independent mechanisms of separation for analytes to improve the separation of complex mixtures. This study demonstrates a method for the analysis of sandalwood oil using GC×GC with flame ionization detection (FID) and quadrupole mass spectrometry (qMS). The objective was to compare sandalwood oils from different species based on their GC×GC-qMS/FID profile, and investigate how the differing profiles may contribute to claimed aromatherapy benefits. The chemometric analysis of commercial sandalwood oils was performed via a tile-based feature selection software to determine similarities and differences between samples.

Experimental

The three products used in this experiment were sourced online from the supplier Eden's Garden. The cost of a 5 mL vial ranged from $48-62 and the relative cost of each oil is listed in Table 1, along with packaging information. Each of the commercial EOs were diluted to 0.25% in HPLC grade Methanol and a fragrance allergen mix was also run at 15 ppm.

Table 1. Commercial sandalwood oils purchased from Eden's Garden. Oils were listed as 100% pure and species were self-reported by manufacturer. Provenance was not verified by other means.

SPECIES REPORTED	LOCATION REPORTED	RELATIVE COST	PACKAGING CLAIMS
Santalum paniculatum (*S. paniculatum*)	Hawaii	High	Clears airways, Aids acne, Soothes sunburn
Santalum spicatum (*S. spicatum*)	Australia	Low	Inflammation relief Relieves sore throat Mental clarity Refreshes surfaces
Santalum album (*S. album*)	East India	Medium	Improves skin appearance Relieves sore throat Reduces frustration and stress

Five replicates of each sandalwood species were run using automated liquid injection and analyzed using GC×GC-qMS/FID. This generated a volatile organic compound (VOC) profile for the sandalwood oils measured. Chromcompare+ (SepSolve Analytical) was used to visualize the complex profiles, as well data processing. The method of analysis is listed below in Table 2.

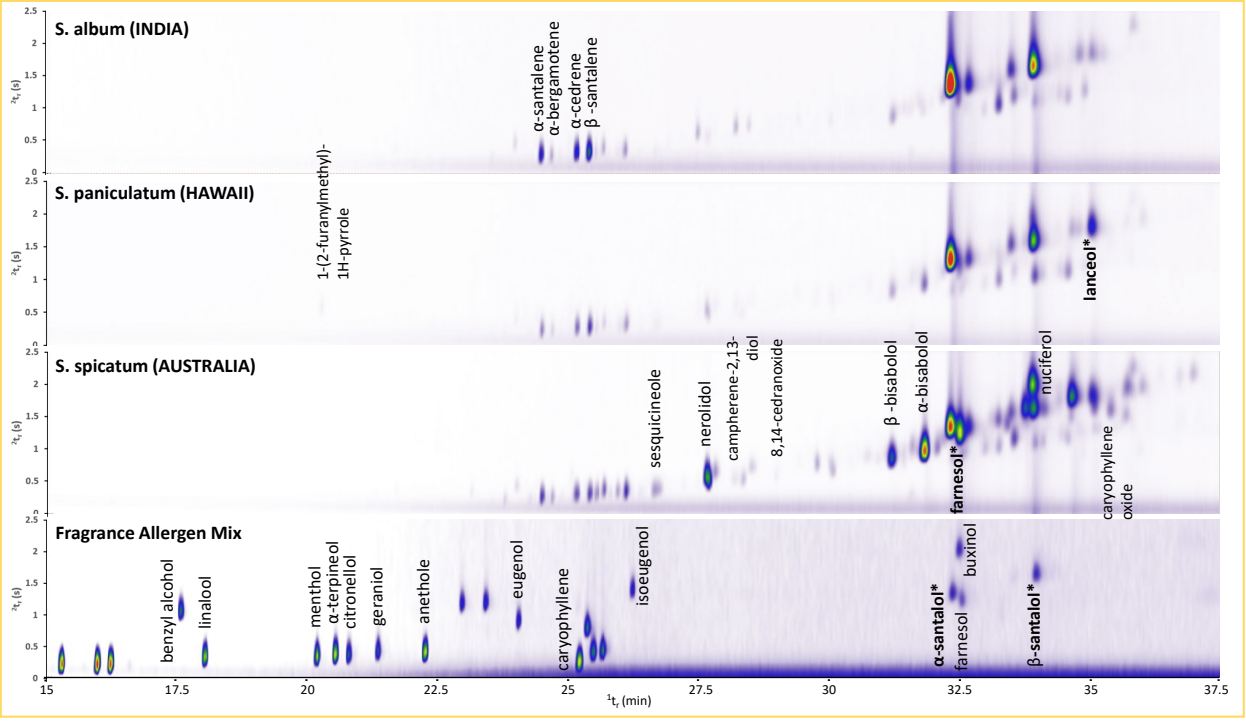
Table 2. Method parameters for the analysis of commercial essential oils.

GC×GC-qMS/FID METHOD	
Instrument Configuration	Trace 1300 GC/FID and ISQ 7000 Single Quadrupole MS
Carrier gas	Ultra high purity helium (1mL/min ^{1}D, 20 mL/min ^{2}D)
Inlet	Split flow: 20 mL/min; purge flow: 5 mL/min; 250°C
1st Dimension Column	Rxi-624Sil MS: 30 m x 0.25 mm ID x 1.4 µm (Restek)
2nd Dimension Column	Stabilwax: 5 m x 0.25 mm x 0.25 µm (Restek)
Modulator (SepSolve Analytical)	INSIGHT Reverse Fill/Flush; modulation period: 2.5s; flush time: 100ms
Loop Dimensions	0.53 mm ID × 1133 mm; loop volume: 25 µL
GC Oven	80°C (for 1 min), 8/min to 250°C (for 19 min)
Ion Source & Transfer Line Temp	280°C
Mass Range	40-300 amu
Acquisition Rate	120 Hz (FID), ~41.5 scans/s (qMS)

Results and Discussion

As seen in Figure 1, the three different species of sandalwood EO shared similar compositions; however, the GC×GC-qMS/FID system can easily reveal the differences between species at a first glance.

Figure 1. GC×GC-qMS contour plots for the three sandalwood essential oil species.



*Compounds monitored in ISO 3518.

The contour plots illustrate the relative complexity of the EO profiles. Select compounds of interest are labeled for the sandalwood species, along with the four compounds monitored in the ISO 3518 standard (*). *Santalum spicatum* had the most diverse aroma profile. The nontargeted nature of this method allows monitoring of key quality indicators (α-santalol, β-santalol, farnesol, and lanceol), while also allowing other components to be detected, including potential fragrance allergens. This could be helpful as more nontargeted methods for personal care product monitoring appear to be under development in recent years. The fragrance allergen plot served as a reference to identify α-santalol and β-santalol and farnesol components in the sandalwood species. The plot comparisons indicate the absence of allergen contaminants in the sandalwood EOs, other than farnesol in *Santalum spicatum*.

This study used Chromcompare+ software for an automated data processing workflow to facilitate the profile compilation of different sandalwood EO species, to determine any differences and similarities, and to provide a means to differentiate the species. The principal component analysis (PCA) scores plot in Figure 2A shows the comparison of the three species. *S. album* (from India) and *S. paniculatum* (from Hawaii) appeared to be most similar in composition as they cluster closely on the PCA plot. *S. spicatum* (from Australia) appeared to be more distinct from the other two species. The volcano plots also shown in Figure 2 highlight some of the discriminating compounds between species. Compounds such as α-santalol and 1-furfurylpyrrole were upregulated and more abundant in the Indian and Hawaiian sandalwood species compared to the Australian species. The Australian species contained high amounts of β-curcumen-12-ol, farnesol, sesquicineole, and caryophyllene oxide compared to the Hawaiian and

Indian species. Volcano plots are an effective means to investigate relevant features for class differentiation. The data displayed in the PCA and volcano plots are consistent with the differences observed in the Figure 1 contour plots.

Figure 2. GC×GC-qMS data comparing the sandalwood species: principal component analysis (PCA) score and volcano plots.

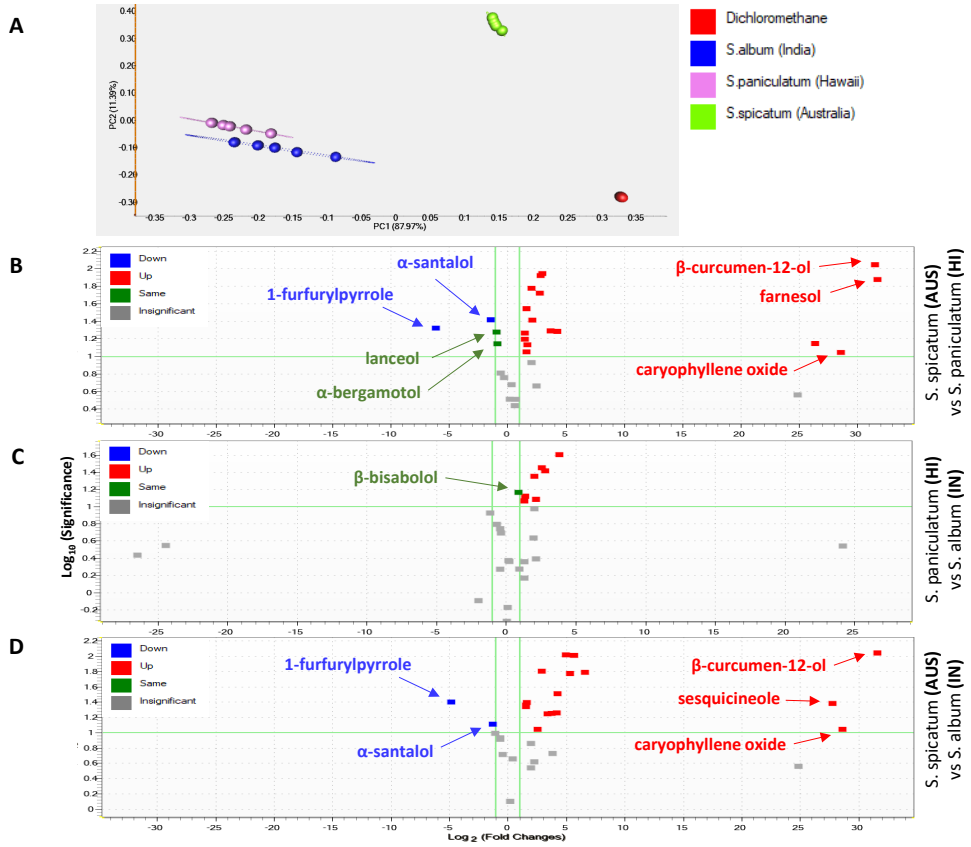
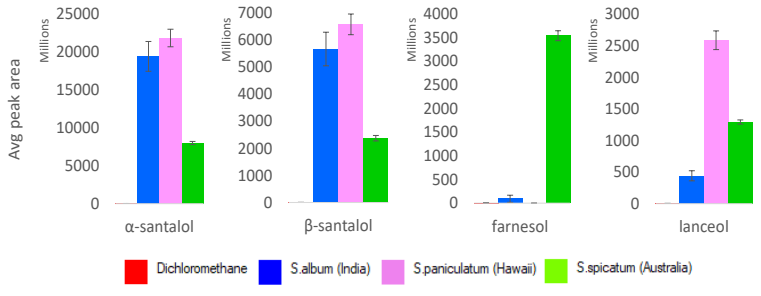


Figure 3 displays data regarding the four compounds actively measured for quality in the ISO 3518 *S. album* method. The amount of these compounds represented by their peak areas in Figure 3A varied between species. While α-santalol and β-santalol present the same trend for each species, farnesol and lanceol diverged from that trend. In reference to the ISO document, the data shown in Figure 3B confirmed that the *S. album* oil in this study meets quality requirements specified for an oil obtained from *S. album*, with the α- and β-santalol content falling in the upper ranges of the required percentages. *S. paniculatum* also contained high levels of α- and β-santalol (>70%). *S. paniculatum* and *S. spicatus*

Figure 3. Comparisons of select compounds monitored in the three versions of sandalwood species

A



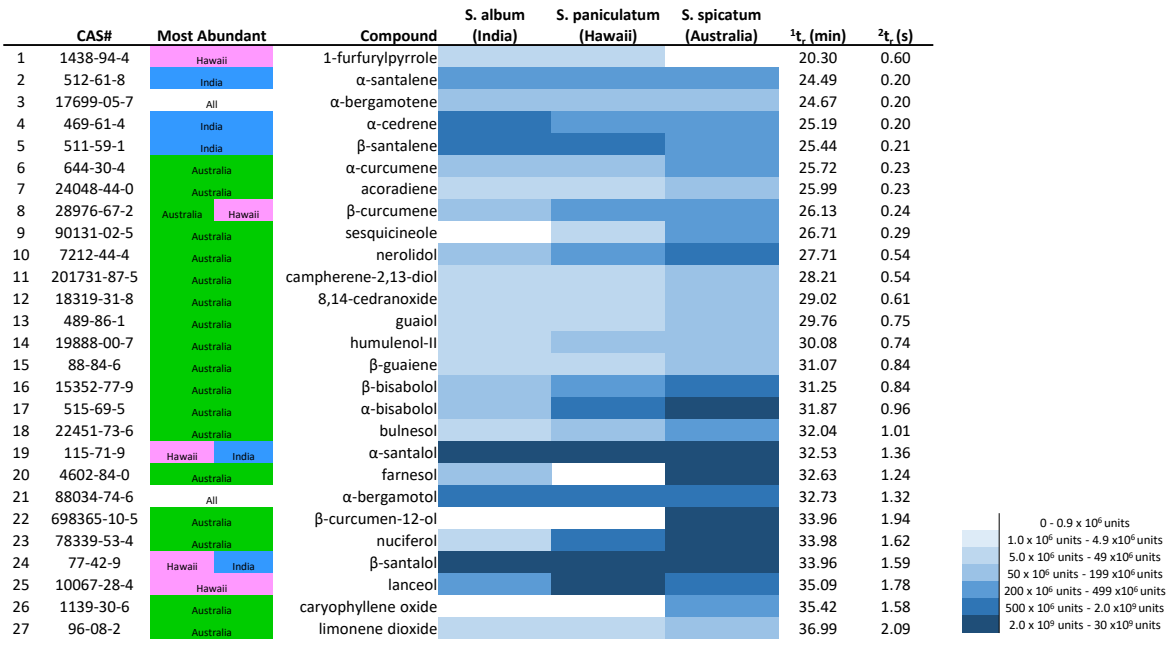
B

	% of total average peak areas			
	α-santalol	β-santalol	farnesol	lanceol
ISO method (S. album)	41-55	16-24	0-1	0-2
S. album (India)	55	21	0.3	1.2
S. paniculatum (Hawaii)	50	22	---	8.4
S. spicatum (Australia)	17	7.0	11	3.9

displayed different proportions of some compounds monitored, indicating they are not oils from *S. album*. *S. paniculatum* included high amounts of lanceol and no detection of farnesol. However, an abundance of farnesol in *S. spicatum* contributes to a different and increased fragrant profile, which possesses calming, antibiotic, anti-inflammatory, disinfectant, analgesic, neuroprotective, and anticancer properties [3, 5]. Farnesol and is also considered an allergen, which may make the Hawaiian and Indian sandalwood oils safer options for some people.

Figure 4 provides a select list of compounds detected and a heat map to compare compound abundance between species. Based on peak visualizations in Figure 1 and similar first dimension retention times (^1t_r) of multiple compounds in Figure 4, co-eluting peaks in a 1D GC system would have likely been hidden and many of these compounds would be disregarded. This could potentially prevent a clear differentiation between species, especially since the *S. panticulatum* and *S. album* species are quite similar. The sandalwood EO profile predominately consists of sesquiterpene and sesquiterpenoid compounds. Many of the sequiterpenes listed in Figure 4 have been studied and are associated with therapeutic benefits [5]. For example, santalol compounds elicit calming, anti-inflammatory, antifungal and anticancer benefits [1].

Figure 4. Select compounds of interest with heat map of compound average peak areas.



	CAS#	Most Abundant	Compound	^1t_r (min)	^2t_r (s)
1	1438-94-4	Hawaii	1-furfurylpyrrole	20.30	0.60
2	512-61-8	India	α-santalene	24.49	0.20
3	17699-05-7	All	α-bergamotene	24.67	0.20
4	469-61-4	India	α-cedrene	25.19	0.20
5	511-59-1	India	β-santalene	25.44	0.21
6	644-30-4	Australia	α-curcumene	25.72	0.23
7	24048-44-0	Australia	acoradiene	25.99	0.23
8	28976-67-2	Australia Hawaii	β-curcumene	26.13	0.24
9	90131-02-5	Australia	sesquicineole	26.71	0.29
10	7212-44-4	Australia	nerolidol	27.71	0.54
11	201731-87-5	Australia	campherene-2,13-diol	28.21	0.54
12	18319-31-8	Australia	8,14-cedranoxide	29.02	0.61
13	489-86-1	Australia	guaiol	29.76	0.75
14	19888-00-7	Australia	humulenol-II	30.08	0.74
15	88-84-6	Australia	β-guaiene	31.07	0.84
16	15352-77-9	Australia	β-bisabolol	31.25	0.84
17	515-69-5	Australia	α-bisabolol	31.87	0.96
18	22451-73-6	Australia	bulnesol	32.04	1.01
19	115-71-9	Hawaii India	α-santalol	32.53	1.36
20	4602-84-0	Australia	farnesol	32.63	1.24
21	88034-74-6	All	α-bergamotol	32.73	1.32
22	698365-10-5	Australia	β-curcumen-12-ol	33.96	1.94
23	78339-53-4	Australia	nuciferol	33.98	1.62
24	77-42-9	Hawaii India	β-santalol	33.96	1.59
25	10067-28-4	Hawaii	lanceol	35.09	1.78
26	1139-30-6	Australia	caryophyllene oxide	35.42	1.58
27	96-08-2	Australia	limonene dioxide	36.99	2.09

Santalene compounds are known to have antioxidant and anti-inflammatory effects. The favorable properties of santalol and santalene could contribute to the skin, lung, and throat benefits claimed in the packaging. Many of the compounds detected in this study overlap with similar or additional benefits. *S. panticulatum* and *S. spicatum* contain an abundance of lanceol, as shown in Figures 1, 3, and 4. Lanceol is an isomer of santalol and contributes to the high value of sandalwood oil [3]. Other santalol isomers discovered in the oils studied include α-santalene and β-santalene, which were found in relatively higher concentrations in *S. album*, as well as α-bergamotol, which was found across the three species. *S. spicatum* contained higher levels of α-bisabolol and β-bisabolol, which boosts additional anti-inflammatory, antianxiety, antibiotic, analgesic, neuroprotective, and anticancer effects and impedes the progression of atherosclerosis [3]. This supports the inflammation relief claim for the *S. spicatum* species of this brand.

Conclusion

This study has shown that:

- GC×GC was a powerful tool to elucidate subtle but important changes in these complex chemical profiles.
- Nontargeted analysis allowed the investigation of compounds that are quality indicators according to standard methods simultaneously with other product components that could be relevant to monitoring these products.
- Monitoring additional compounds within the profile may assist in understanding their different therapeutic benefits by tracking a component to its known pharmacology.
- The Hawaiian and Indian sandalwood oils analyzed here are unique for their high concentrations of α- and β-santalol (>70%) and low allergen profile. Australian sandalwood oil encompassed a different profile with several other compounds and still contained a significant amount of α- and β-santalol (~24%), which offers slightly different unique therapeutic benefits.

This was applied here for essential oil investigation, but could be applied in numerous similar applications where discrimination or characterization of natural products is desired.

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