**Little Things Matter: Effect of Minor Constituents on Aroma Profile of Indian Sandalwood Oil**

**Hettiarachchi, Dhanushka** [1*]; Brown, Andrew [1]; Mettetal, Alexandra[1]; Eatt, Jonathan [1]; Barrow, Judith[1]

[1]Quintis Sandalwood, West Perth, Western Australia, Australia

*Quintis Sandalwood, Level 1/87, Colin Street, West Perth, WA 6005 Australia.

+61419793136 danny@qunitis.com.au

## Abstract

**Background:** Indian sandalwood is historically acclaimed for its aroma and wellness benefits on skin. The complexity and comforting warmth of sandalwood is highly desired, which is evident by its presence in over half of the fragrances created for perfume, cosmetic and personal care products. While most of the sandalwood notes used in cosmetics are synthetic bio-mimics or biotechnological isolates, only natural Indian sandalwood can produce the authentic complex aroma. Indian sandalwood oil (ISO) is composed of over 125 molecules. The sweet, creamy and woody aroma of sandalwood is attributed to beta-santalol, while the main constituent alpha-santalol plays a therapeutic role.

**Methods:** Samples were obtained from the sustainable sandalwood industry and other authentic commercial products. Further separations were conducted by fractional distillation and liquid chromatography. Chemical analysis was conducted by gas chromatographic mass spectroscopical analysis; constituents were identified by standard databases and published data. Isolated fractions were also subjected to olfactory classification with the help of a professional perfumer and a trained sensory panel.

**Results:** Major constituents of sandalwood are of sesquiterpene alcohol origin, which includes the alpha and beta santalols. These major constituents showed a uniform composition across samples of different origin, without altering the odour profile. However, minor constituents such as sequiterpene hydrocarbons, monoterpenes, oxidised sesquiterpenes, organic acids and wood by-products varied significantly in chemical composition and olfactory profile. These minor constituents account for less than 10% of total oil mass of ISO, but variations in their composition make significant change to the aroma profile. Odour profile was quantitatively recorded and compared with the gas chromatographic data to analyse and identify the effect of each minor constituent class on the aroma of ISO.

**Conclusion:** Minor constituents contribute to the unique aroma of natural ISO, a unique balance of these constituents is required for specific uses such as fine perfumery and fragrance in cosmetics. Unlike synthetic or biotechnologically developed molecules, natural ISO has the complete odour profile of sandalwood note.

**Keywords**: Santalum album; Indian Sandalwood; Essential oil; olfactory; minor constituents

## Introduction

Indian sandalwood oil is the steam distilled essential oil from the heartwood of *Santalum album* L. (Santalaceae) and has beenused as a perfumery, cosmetic and aromatherapy ingredient for many centuries. *Santalum album* is a root hemi-parasitic tree and grows naturally in southern India, Sri Lanka, Nusa Tenggara province of Indonesia and Timor Leste. It has been historically valued as a perfumery ingredient for its unique sweet, lactonic, woody aroma[1]. The first reference of sandalwood comes in an ancient Sanskrit literature *Yasaka Nirukta* written in 500 BCE, which describes its aroma for ritualistic use. Following this, since then numerous references to the aroma of sandalwood paste, wood carvings and burnt incense were seen in Hindu, Buddhist and Jain scriptures. Several early Indian treatises of perfumery and cosmetics have regularly referred to sandalwood as an essential element in perfumery. Medieval Indian books on perfumery and fashion regularly mentioned sandalwood as a luxury item, thus its value has been identified from an early stage [2]. Around the $10^{th}$ century India first distillation of sandalwood oil was conducted, where it played a crucial role in capturing floral notes and solubilising other solid aromatics to a make unique perfume product, Attar [3].

Traditional European perfumery has always used sandalwood oil as an essential base note and a fixative. Some of the sandalwood oil was distilled in Europe and Northern America, while h the bulk of commercial product was produced at the Mysore state distillery. Modern perfumery has always included sandalwood, in current estimates over 60% of perfumes contain either natural or synthetic sandalwood products [4].

Sandalwood was highly exploited from the wild due to the high demand in perfumery, traditional medicine and incense religious applications around the world. Certain historical state restrictions on sandalwood have also made the situation more difficult to manage, resulting in sandalwood being included on the International Union of Conservation of Nature (IUCN) red list as a vulnerable species. Australian researchers have identified northern tropical Australia as a suitable cultivation region for sustainable sandalwood [5].

Essential oil is found only in the heartwood of the tree, which is formed as the tree matures naturally. Lignification of xylem tissue is Indian sandalwood oil contains more than 125 reoccurring constituents. Their composition changes with different factors such as age of the tree, maturity of heartwood, geographical location, climatic conditions, soil nutrition and host tree nutrition [1].

The unique smell of sandalwood comes from two main sesquiterpene alcohols, namely alpha and beta santalol. In combination these two constituents make upto 80% of the total composition of Indian sandalwood oil. Beta-santalol is the main aroma constituent giving the sweet, lactone woody aroma while alpha-santalol has a lower olfactory intensity with woody aroma [6]. Indian sandalwood oil has been standardised by the International Standard Organisation, Indian Standards and British Pharmacopoeia [7-8]. Together with physical parameters, these standards describe the gas chromatographic profile and quantification of the major compounds. Major constituents of Indian sandalwood include, the santalols and bergamotols which are biosynthesised through the same pathway. Other class sesquiterpene alcohols are regularly detected; although they have a weak odour profile, their composition is important to detect to determine whether the Indian sandalwood has been adulterated with other *Santalum* species[6, 9]. The majority of pure essential oils obtained from Indian sandalwood from different origins shows a similarity when tested on standards. However, the odour profile of Indian sandalwood essential oils distilled from different sources shows a clear difference. Indian sandalwood oil is reported to contain numerous minor constituents of different chemical classes. Sesquiterpene hydrocarbons make up most of the minor constituents, which are unoxidized precursors of the sesquiterpene alcohols. Among other compounds contributing to the odour are the aldehyde, ketones and carboxylic acids from further oxidation of santalols, monoterpenes and wood breakdown products from distillation. Upon high resolution gas chromatographic analysis, a subtle variation of minor components was observed between odour profiles[10].

As the commercial sandalwood plantations commenced in early 1990s in Australia, it opened a sustainable and legal source of sandalwood. However, challenges were faced when distilling heartwood from relatively young Indian sandalwood trees, which require understanding of the subtle variations of odour profile. This current study is aimed at understanding the olfactory profile of minor constituents of Indian sandalwood oil which contribute to the odour profile variation. These findings will enable us to convert the quantitative gas chromatographic data to olfactory understanding of Indian sandalwood oil.

## Methodology

Indian sandalwood oil was sourced from different commercial sources including plantation grown sandalwood from the Kununurra region of Western Australia. Certain distillation trials were conducted on pilot scale at the processing and laboratory facility of Quintis Sandalwood Ltd., Albany, Western Australia.

Fractional distillation was conducted in laboratory scale using a molecular distillation unit comprised of a 30 cm glass bead packed column. A glass round bottom flask was maintained at 110ºC on an electrically heated oil bath, under the vacuum of 200mmHg 20 L/min (Scilogex, USA). Fractions were collected in 30 min intervals into separate inline collectors.

GC analysis was conducted using a Thermo Fisher Scientific Q-Exactive orbitrap mass spectrometry system-Trace 1310 (Scoresby, Victoria, Australia). Chromatographic separation was performed on a DB5 column, 30 m x 0.25 mm x i.d 0.25 µm (Thermo Scientific TG-5MS, Scoresby, Victoria, Australia) using a gradient temperature oven program. The oven was initially set at a temperature of 100 ºC for 1 min and ramped at 2ºC/min up to 200ºC, it was heated to 300°C at a rate of 20°C/min and constantly held for 1 min. The carrier gas was helium at a flow rate of 1.0 mL/min. The injection volume was 0.2 µL and the injector temperature was held constant at 250°C with a split flow set to 10 mL/min. The mass spectrometer was used in the positive electron impact mode at 70 eV. A mass range of 50 to 750 m/z was scanned with a resolution of 60,000 and a AGC target of 1e6. The filament delay was set at 4 mins and the mass transfer line at 280 °C. The software used was Xcalibur©.

Solid phase extraction was performed on a short glass column of dimensions 33 cm × 24 mm × 28 mm (Sigma-Aldridge, USA) filled with silica gel 60 (Supelco, USA). Indian sandalwood oil samples were introduced to the column stabled on silica gel and eluted with n-hexane, dichloromethane, methanol and aqueous acidic solution at pH 3.

Indian sandalwood oil samples and liquid chromatographic fractions were evaluated by a trained olfactory panel lead by a qualified perfumer. Each sample or fractions were given a quantitative measure for olfactory notes.

## Results

GCMS analysis of the samples and fraction have quantified the major and minor constituents. Fractions rich with certain chemical classes were further analysed to determine its identification. Constituents were identified through matching the mass fragmentation patterns previously published.

Santalols comprised of α-santalol, β-santalol, z-α-trans bergamatol, epi-β-santalol. On average Indian sandalwood oil is comprised of over 80% santalols, in which α-santalol makes up between 41-50% and β-santalol 20-24%, on an average range of essential oils tested. Further chromatographic separations managed to isolate fractions rich in α-santalol and β-santalol. The olfactory identification of α-santalol was found to be soft woody, nutty, milky, musky, warm, cedarwood. The intense warm woody, milky, animalic notes that sandalwood is renowned for comes from the β-santalol.

The second most important class of constituents in Indian sandalwood oil are the sesquiterpene hydrocarbons. Some of the key compounds are α-santalene, β-santalene, α-bergamatene, curcumene and α-bisobalene comprising an average of 6% of the total essential oil volume. These compounds in combination showed an odour profile similar to α-santalol which is soft woody, nutty, milky, musky, warm, cedarwood as recorded by the olfactory detection.

Other sesquiterpene alcohols contribute to an average percentage composition of 5% of the total essential oil. Molecules derived from either the bisabalol pathway of single aromatic ring are bisabalol, curcumen-12-ol, lanceol and nuciferol. Farnesol, which is the oxidised precursor of the farnesyl pyrophosphate pathway also remain at less than 1% of the total volume. These molecules exert a low odour contribution which can be easily masked by the major constituents and other minor constituents.

Oxidised sesquiterpene alcohols form their corresponding aldehydes and ketones with an average of 4% of total volume. Constituents are identified as α-trans-bergamotenone, α and β santalals with powerful odours, present at low concentrations. These olfactory properties are important contributors to the milky, nutty, fatty tonalities, much desired for Indian sandalwood oil.

Carboxylic acids which are derivatives of sesquiterpene alcohols makes up less than 1.0% of the Indian sandalwood oil. Carboxylation causes the sesquiterpene alcohol molecule to cleavage, resulting in constituents nor-tricyclo-eka-santalic acid and *epi*-β-nor bicyclo-eka-santalic acid. Samples obtained from wood damaged by fungal infections were identified to contain more of these compounds. While the compounds in trace amounts add to the animalic and musky odour of Indian sandalwood, excessive amounts give a repulsive "smelly sock" odour.

Samples prepared by change to the distillation process such as prolonged distillation and increased steam temperature and pressure have developed a malodour. Phenolic compounds were produced as a result of breakdown of the lignin of the wood. These molecules in excess give a harsh middle smoke note. Regular samples of Indian sandalwood oil have less than 0.05% of the phenolic compounds such as vanillin giving a sweet aroma, eugenol with a medicinal note and allyl syringol producing a smoke note.

The current study has identified two main furan derivates 5-methyl furfural and furfural pyrrole from Indian sandalwood oil. The carbohydrate content of the wood can undergo a cyclic reaction to form furan and its derivatives. These furans and pyrroles are usually found in volumes less than 0.1% in Indian sandalwood oil giving a caramel, gourmand and smoky notes.

The overall odour profile for Indian sandalwood is identified as a top note of weak discrete green terpenic, followed by a weak and discrete heart note of flowers, spice, amber, incense, leather and animalic. The most important base note of Indian sandalwood is identified as persistent and substantive warm woody, nutty and milky that lasts on dry down (Figure 1).

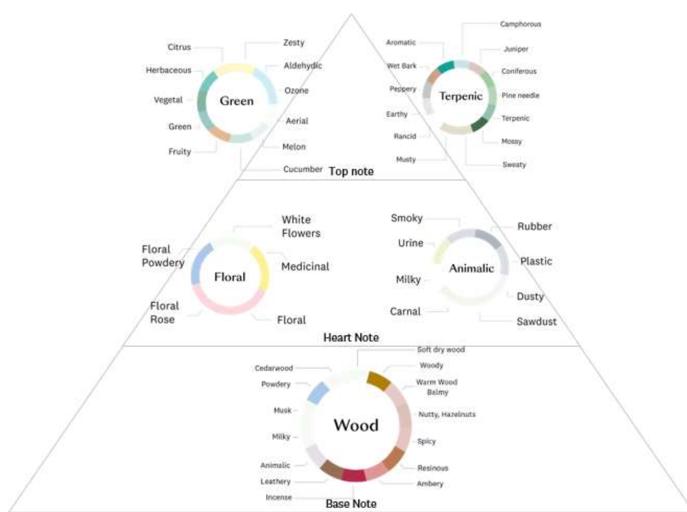


Figure 1: Olfactory description of the top, heart, and base note of Indian sandalwood oil

**Discussion**

The main constituent in Indian sandalwood oil is z-α-santalol. It is generally accepted that while this molecule has the greatest contribution by weight it does not contribute greatly to the odour, having a week woody odour reminiscent of cedarwood and as such is likely the contributor to the slight nutty cedar characteristic of Indian sandalwood oil [6]. Literature generally refers to z-β-santalol as the source of the intense warm, woody, lactonic notes of Indian sandalwood oil [11].

Trans isomers are found in small quantities in Indian sandalwood oil and typically contribute about 2% of the mass of Indian sandalwood oil. There are two further major sesquiterpene alcohol that are present, trans-α-z-bergamotol and epi-β-santalol [10, 12]. These four molecules make up about 80% of the mass of Indian sandalwood oil but an isolate of these four molecules, while having a clear, woody lactonic odour does not have the character of a good Indian sandalwood oil, it lacks the intensity and complexity for which Indian sandalwood oil is renowned. The origin of these minor constituents can be put down to three broad categories. Precursor molecules, which are molecules that have not been fully synthesised by the tree, for example santalenes. Molecules that are produced from other biosynthetic pathways curcumens via monoterpene synthesases and breakdown products, for example aldehydes and ketones [4].

The olefinic constituents are an important contributor to the Indian sandalwood oil odour. They constitute about 6% of the total mass of the oil and contribute a woody, green terpenic note to sandalwood. The contribution of these molecules to the sandalwood odour can best be described when these molecules a removed by vacuum fractionation. The resulting oil lacks intensity and loses the slight green terpenic notes that are typical of sandalwood. Some of the terpenes are normally removed from the oils during distillation by the removal of foreruns during the distillation process [4].

There is not a lot of information available on the effect of other sesquiterpene alcohols, which are considered to have a week woody odour [12] and as such are not considered to contribute much effect to the odour. of Indian sandalwood oil. These molecules are produced by enzymatic system through the monoterpene synthase acting on farnesene pyrophosphate as opposed to santalene synthases [9].

The oxidation of sesquiterpene alcohols with the corresponding aldehyde or ketone produce some important intense odour molecules that contribute to the lactonic milky woody notes of sandalwood [12]. The effect of these molecules on sandalwood oil odour can be ascertained by isolation with Girard reagent, with the resulting isolate being intensely woody. Most of the alcohols have their corresponding aldehyde or ketone present [14].

The breakdown and rearranged products of santalols are also seen in this group of molecules. While the alcohols have a cis (z) configuration it appears that the aldehydes and ketones are produced in the trans (E) configuration [12]. These constituents with a powerful woody odour, may be part of the reason why it is generally considered that the odour of the oil improves with age and that oil distilled from old heartwood is considered superior to oil distilled from the heartwood of young trees [4, 10].

The isolation of carboxylic acid constituents from sandalwood oil yields a sweaty, over ripe fruit odour and thus is a likely contributor to the animalic and sweet notes of sandalwood oil. This group of molecules does not contribute wood notes to sandalwood oil. The formation process of these carboxylic acids involves cleavage of the hydrocarbon chain [14] and the subsequent formation of the nor (C14), eka (C13) or tere (C12) santalic acids. Brunke et al made the observation that these acids were esterified, although the esterified moieties are rarely observed. It is not clear if the carboxylic acid constituents are produced in the live tree or after harvest or by oxidative action on the oil. *Aspergillus niger* has also been also identified to produce teresantalic acid, tere-santalols and photosantalols from a-santalene [15].

Smaller hydrocarbons can be found in Indian sandalwood oil. These can broadly be divided into two groups. One being derived from degradation pathways via a mechanism that has not been determined at this stage, these being santene, albene and dihydroalbene. Santene in particular is important as it has a very powerful leather odour and is readily found in Indian sandalwood. It can be isolated by fractional distillation of the oil or foreruns under vacuum[4, 12].

Also present are monoterpenes such as α-pinene, limonene, ρ-cymene and ρ-cymenene [6]. These constituents are though to be produced by monoterpene synthases in response to mechanical damage to the woody parts of the tree. These molecules have powerful odours and contribute to undesirable odours in Indian sandalwood oil.

During the extraction process of Indian sandalwood oil, a number of constituents can be formed which if allowed to accumulate in the oil can lead to undesirable odours [12]. Phenols produced from the breakdown of lignin these and be highly odoured and contribute harsh and smoky characteristics to the oil. Constituents include p-cresols, guaiacol, eugenol and vanillic and syringic acid [4].

Furfuals can also be produced from cellulose and these then can undergo Mallard type reactions with hydroxy proline which is found in the aromatic heartwood to produce furfural pyrrole [4, 10, 12]. This constituent has a vegital odour which is not considered as favourable for Indian sandalwood oil.

## Conclusion

The importance of minor constituents in the olfactory qualities of Indian sandalwood oil cannot be underestimated. Major constituents compose 80% of the Indian sandalwood oil and contribute to the woody, sweet, lactonic notes unique to sandalwood. However, the complexity of a natural essential oil is lacking with the absence of a mix of the minor constituents. Considering the complex notes of sandalwood's olfactory character, it is crucial to have the nuances from the minor constituents for the balanced odour profile. It is observed that the composition of major constituents listed on standard documents remain unchanged whilst the oils have a significant difference in their odour profile. The current study has established the necessity to detect minor components in odour to identify the olfactory character. It is also found that the source of wood and processing have an effect on the composition of minor constituents which can significantly change the odour profile. The study could also provide information to the forestry and processing scientists to develop sustainable sandalwood source to match the most appealing olfactory profile for natural Indian sandalwood oil. This study stands as a testament that synthetic or biotechnological methods to produce or mimic major constituents of Indian sandalwood would be unable to produce the natural aroma balanced by a myriad of minor constituents produced by the tree and other factors.


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## Conflict of Interest

The authors declare no conflict of interest.

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