



Research article

Variability in yield and composition of oil from Indian Sandalwood (*Santalum album* L.) trees grown in homogeneous conditions

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Abstract: The study evaluated the variability in yield and composition of oil from the heartwood of Indian Sandalwood (*Santalum album*) trees grown in homogeneous condition. Trees grown at Institute of Wood Science and Technology campus, Bengaluru were considered in this study. Wood samples were collected from trees having different girth size (47.1, 53.4, 61.2, 69.1, 72.2, 74, 75.4, 81.6 and 82.4 cm). About 500 g of heartwood powder per sample was extracted by hydro-distillation method using Clevenger's apparatus. Oil yield extracted from these samples varied from 1.6–3.6 % of heartwood. Chemical profiling of the oil samples was carried out by gas chromatography-mass spectrometry (GC-MS-QP-2010 Ultra Auto Sampler). A chemically diversified alkanes, sesquiterpenoids, sesquiterpene, fatty acids, and alcohols, were detected. The major constituents were α -santalol (41.7–53.67 %), β -santalol (18.2–27.9 %), epi- β -santalol (2.7–7.18 %), β -santalene (1.39–5.30 %), α -santalene (0.4–4.87 %), and α -bergamotol (3.1–9.3 %). In this study, it was concluded that the oil yield and its composition varies among the trees with different girth. But no particular trends were observed between the girth size and oil yield.

Keywords: *Santalum album* L. - Indian Sandalwood oil - Composition - Girth - GC-MS.

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INTRODUCTION

Santalum album L. is a small to medium-sized, evergreen semi parasitic tree of family Santalaceae (Fox 2000, Chaudhary *et al.* 2016). *S. album* commonly known as Indian Sandalwood is one of the oldest and precious sources of natural fragrance with immense medicinal and commercial significance (Burdock & Carabin 2008, Hansda 2009, Bajpai *et al.* 2016). Sandalwood has been esteemed all over the world for its peculiar, sweet, long lasting and medicinally valued fragrant oil. Indian Sandalwood oil derived from heartwood of the tree have been used in various traditional systems of medicine, like Ayurveda, Siddha and Unani medicine in the treatment and prevention of wide range of ailments (Sensarma 1989). The value of Indian Sandalwood tree mainly depends on the volume of its heartwood and the quantity and quality of the heartwood oil. The quality of SW oil mainly because of the concentration of oxygenated sesquiterpenes *i.e.* α -santalol and β -santalol, due to which it has a pleasant characteristic aroma. Earlier reported main sesquiterpene and sesquiterpene alcohols of Indian Sandalwood (*Santalum album* L.) oil were α -santalol (1), β -santalol (2), α -bergamotol (3), epi-cis- β -santalol (4), cis-lanceol (5) and α -bisabolol (6) (Fig. 1). The hydrocarbons, α -santalene (7), β -santalene (8), epi- β -santalene (9), α -bergamotene (10), β -bisabolene (11) and α -curcumene (12) were also present in the oil (Fig. 1) (Verghese *et al.* 1990).

Various studies have been carried out for documenting variation in oil content and its composition. The study conducted on heartwood oil yield in *Santalum yasi* Seem and *S. album* by Doran *et al.* (2005) and Xiaojin *et al.* (2011). The variation of oil content in 6 year old *S. album* was estimated from 0.64% to 1.78%. The studies carried out in Sri Lanka by Subasinghe *et al.* (2013) on *S. album* showed a higher variation of oil content and they found high oil content, *i.e.* up to 6.36%. Further, the research was conducted on the estimation of alpha and beta santalol levels in *S. album*, *Santalum spicatum*, *S. yasi*, *Santalum austrocaledonicum* by some other

researcher as well (Brand *et al.* 2006, 2007, Xiaojin *et al.* 2011). Moreover, the content and composition of oil from the central and transition zones of the Sandalwood disc (Shankaranarayana *et al.* 1998), analysis of growth and oil composition (Xiaojin *et al.* 2011) and solvent extractable volatile profiling (Misra *et al.* 2013) from heartwood of East Indian sandalwood tree are the few studies in this direction.

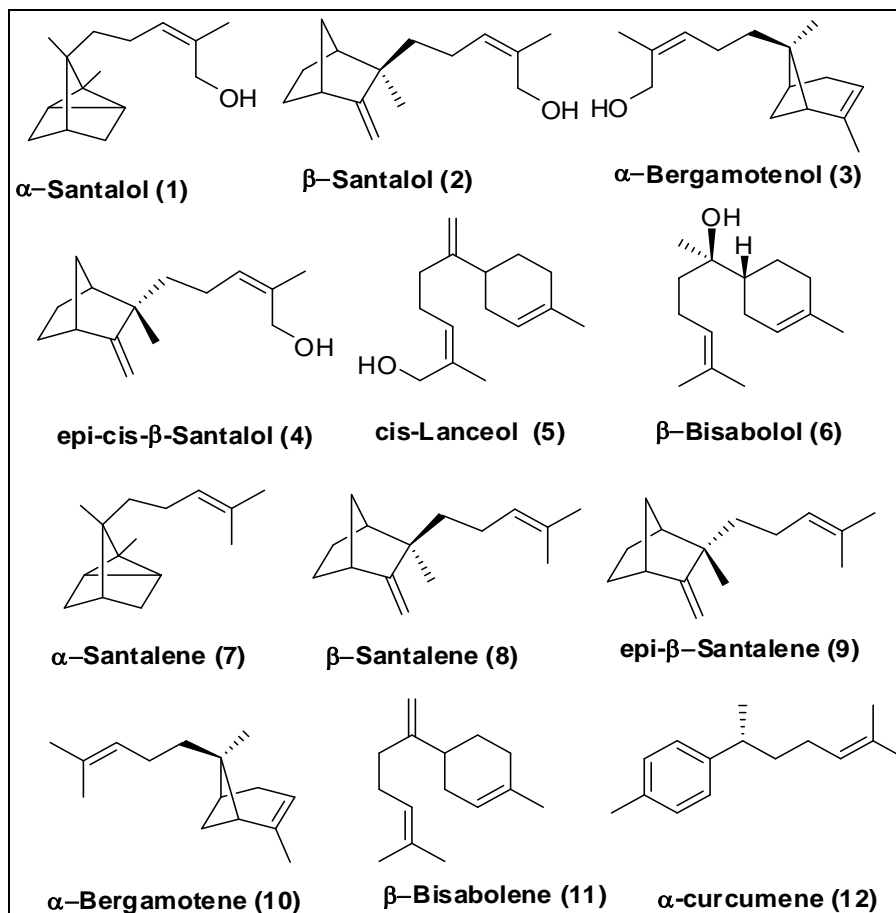


Figure 1. The volatile compounds reported from Indian sandalwood oil.

It has been found that in most of these studies the growing conditions or environments were not similar. Moreover, the quantities of the heartwood for extracting oil were not sufficient, which also plays an important role in estimating oil yield and its composition. Variations in the composition of oil from trees grown in homogenous condition may have an effect on the quality of SW oil, which could lead to inconsistency in medicinal and aromatherapy properties. In this connection, it is imperative to understand and estimate the yield and chemical composition of Sandalwood oil from mature Sandalwood tree grown in homogenous conditions. Therefore, the aim of this study was to analyze the variation in yield and composition of oil from Sandalwood trees grown in uniform condition.

MATERIALS AND METHODS

General experimental methods

Heartwoods were collected from Sandalwood trees grown in Institute of Wood Science and Technology campus. SW oil was extracted by hydro-distillation method using Cleavenger's apparatus. Refractive index was measured by Abbe 5 Refractometer. Relative density was measured gravimetric method using glass specific gravity bottle. The instrument Buchi Rota Vapor used for evaporation of excess amount solvents. The resulting extracted oils were analyzed by GC-MS (GCMS-QP 2010 Plus, Shimadzu make).

Plant materials and chemicals

SW samples were collected from different girth size sandalwood trees growing in the campus of Institute of Wood Science and Technology, Bengaluru, India. Collected wood samples were processed for separation and chipping of heartwood by chopping method. Heartwood chips were pulverized into powders and air dried for 24 h, prior to hydrodistillation. The LR and HPLC grade solvents (n-hexane, chloroform, diethyl ether etc) were purchased from Merck Specialties Pvt. Ltd. and HiMediaLaboratories Pvt. Ltd. Distilled water was used during the experiment.

Extraction of SW oil

Total 500 g of heartwood powder was hydro distilled for 14–15 hr by using Clevenger's apparatus (Hettiarachchi 2008). The hydrodistilled oil was separated from water by separating funnel using organic solvent (petroleum ether). Collected oil and solvent mixture was dried over Na_2SO_4 and concentrated under reduced pressure using rotovapor at 35–40 °C to get oily fractions. The resulting oily mass was subjected to GC-MS analysis (Bisht & Hemanthraj 2014).

Physicochemical analysis

Refractive index was recorded by Abbe 5 Refractometer at room temperature. Relative density was calculated by the ratio of the mass of a given volume of the oil to the mass of an equal volume of distilled water at room temperature.

The relative density was calculated by the following equation:

$$\text{Relative Density} = \frac{m_2 - m_0}{m_1 - m_0}$$

Where,

- m_0 was the mass in grams of the empty specific gravity bottle,
- m_1 the mass in grams of the specific gravity bottle filled with water,
- m_2 is the mass, in grams, of the specific gravity bottle filled with the essential oil.

Equal volumes of the essential oil and water were weighed successively in a glass specific gravity bottle at room temperature.

GC-MS analysis

The resulting oily masses were analyzed by GC-MS, equipped with RTX-Wax capillary column, connected to anion trap quadrupole (ITQ) mass selective detector, with a unit mass resolution. The split was 1:90, with helium as the carrier gas at a flow rate of 1 ml/min, while the damping gas flow was 0.3 ml min⁻¹. The GC oven temperature program was as follows: 40°C to 300°C, by ramping at 3°C, and held at 220°C for 20 min. The injector temperature was maintained at 220°C and the transfer line was held at 220°C. The detection was performed by a Thermo ITQ 900 mass spectrometer in the EI mode (ionization energy of 70 eV, ion source temperature of 180°C, emission current of 220 mA). The acquisition was made in full scanning mode (mass range 50–900 m z⁻¹; 3 scans s⁻¹). Maximum ionization time was 25 ms. A solvent delay time of 5 min (setoff) was used to avoid overloading the mass spectrometer with solvent. The resulting GC-MS profile was analyzed using National Institute of Standards and Technology (NIST-2011, Washington DC, USA) and Dr. Duke's Phytochemical and Ethnobotanical Database (<http://www.ars-grin.gov/duke/>). Qualitative analysis of metabolite was done using its percentage peak area appeared at the total ion chromatogram in GC-MS analysis (Bisht & Hemanthraj 2014).

RESULTS AND DISCUSSION

Wood samples were collected from trees having different girth size *i.e.*, 47.1, 53.4, 61.2, 69.1, 72.2, 74, 75.4, 81.6 and 82.4 cm. Oil yield from these samples varied from 1.6 to 3.6 % of heartwood (Table 1). Highest % yield of oil (3.6%) was observed in tree with girth 72.2 cm while the lowest % oil yield *i.e.* 1.7 and 1.6% in tree with girth size 69.1 cm and 72.2 respectively. The values of physical parameters such as relative density and specific gravity of the oils were observed within the limit of high-quality SW oil (ISO 2002). The colour were observed as colourless to pale yellow and refractive index (1.501–1.5025) of the oil were almost consistent with the all samples while specific gravity was varies from 0.9435-0.974 (Table 1).

Table 1. Oil yield and physical parameter of extracted SW oil. [\pm standard error]

Sample	Girth size (cm)	Oil yield (%)	Oil Colour	Refractive index (RI)	Specific gravity
T1	47.1	2.5 \pm 0.1	Colourless	1.5010	0.960 (\pm 0.01)
T2	53.4	2.0 \pm 0.1	Pale yellow	1.5015	0.964 (\pm 0.02)
T3	61.2	2.2 \pm 0.2	Pale yellow	1.5020	0.963 (\pm 0.01)
T4	69.1	1.7 \pm 0.2	Pale yellow	1.5025	0.957 (\pm 0.03)
T5	72.2	3.6 \pm 0.1	Pale yellow	1.5010	0.962 (\pm 0.02)
T6	72.2	1.6 \pm 0.1	Colourless	1.5020	0.942 (\pm 0.02)
T7	74.0	2.0 \pm 0.2	Colourless	1.5020	0.9435 (\pm 0.01)
T8	75.4	2.0 \pm 0.2	Pale yellow	1.5020	0.974 (\pm 0.03)
T9	81.6	2.6 \pm 0.1	Colourless	1.5010	0.953 (\pm 0.01)
T10	82.4	3.2 \pm 0.2	Pale yellow	1.5025	0.963 (\pm 0.01)

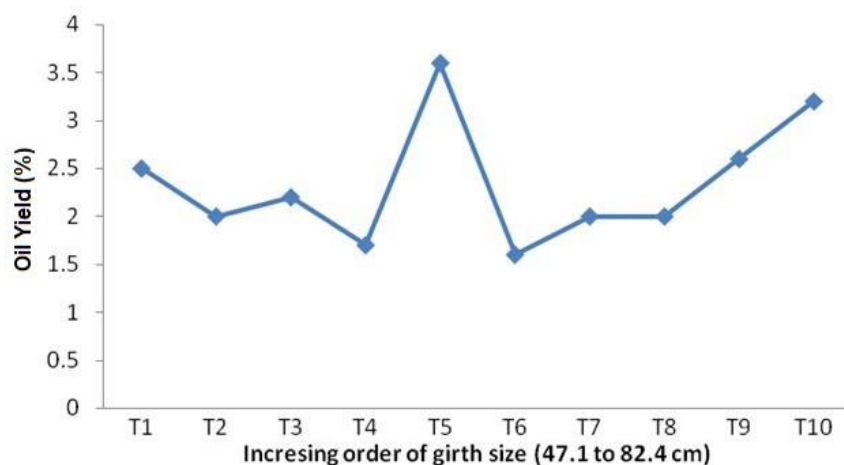


Figure 1. Variation trend of % SW oil yield in different girth size SW trees.

Total 35 volatile metabolites were detected with significant area percentage in total ion chromatogram (Fig. 2). Diversified phytochemicals such as alkanes, sesquiterpenoids, sesquiterpene, fatty acids, and alcohols were observed in extracted Sandalwood oil. The major constituents were α -santalol (41.7–53.67 %), β -santalol (18.2–27.9 %), epi- β -santalol (2.7–7.18 %), β -santalene (1.39–5.30 %), α -santalene (0.4–4.87 %), and α -bergamotol (3.1–9.3 %) (Table 2 & 3).

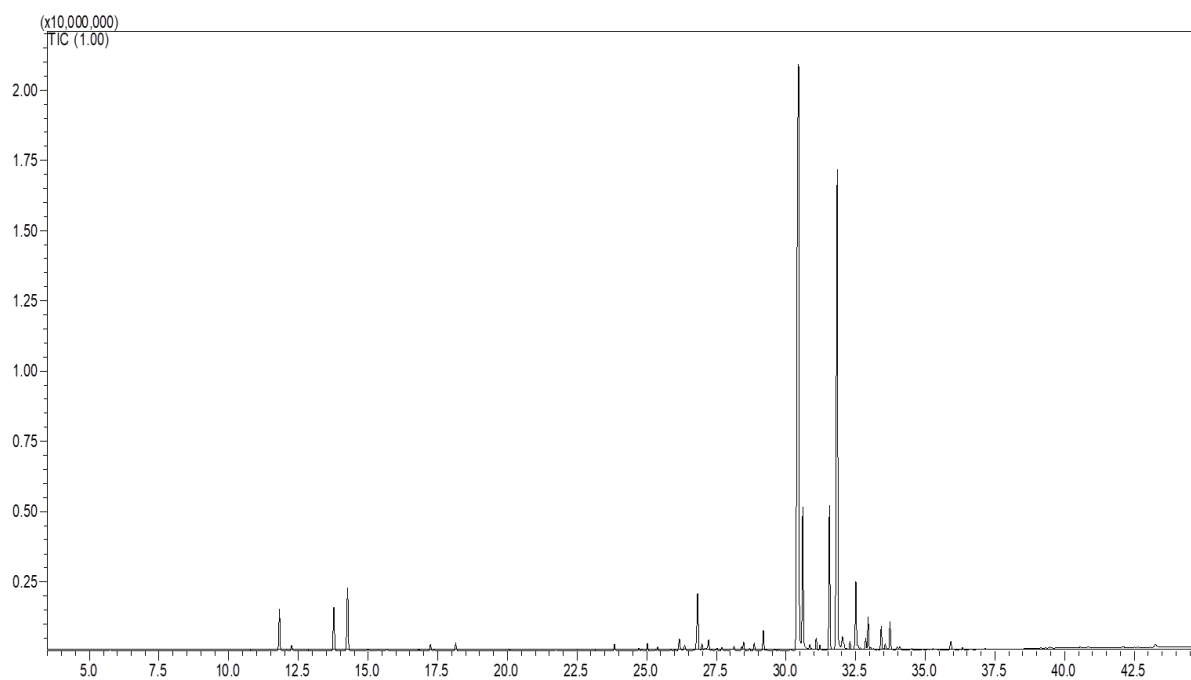


Figure 2. GC-MS Chromatogram of Heartwood Oil.

Table 2. Mass fragmentation of GC-MS identified metabolites.

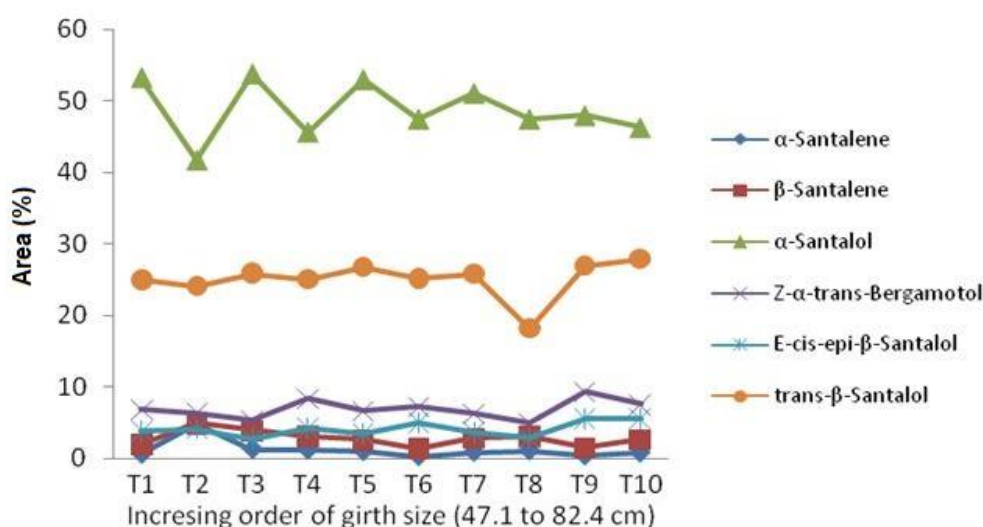
tR (Min)	Metabolites	Molecular formula	Mol. Wt	Mass fragmentation (mass m/z^{-1} values)
11.81	α -Santalene	$C_{15}H_{24}$	204	204(M+), 189, 161, 121, 107, 69, 41
14.26	β -Santalene	$C_{15}H_{24}$	204	204(M+), 122, 94, 79, 67, 55, 41
30.44	α -Santalol	$C_{15}H_{24}O$	220	220 (M+), 187, 121, 107, 93, 79, 41
30.6	Z- α -trans-Bergamotol	$C_{15}H_{24}O$	220	220(M+), 132, 119, 107, 93, 79, 41
31.55	E-cis-epi- β -Santalol	$C_{15}H_{24}O$	220	220(M+), 122, 107, 94, 79, 67, 41
32.50	trans- β -Santalol	$C_{15}H_{24}O$	220	220 (M+), 189, 122, 94, 79, 67, 41

The quantity of metabolites varied among all the samples and the metabolites which were detected in area percentage ≥ 0.50 of total ion chromatogram considered as major metabolites (Fig. 2; Table 2). The results showed that the extracted oils contain high concentration of α -santalol and β -santalol at retention time (tR) 30.44 min and 32.5 min respectively. The concentration of α -santalol varied from 41.7–53.67 %, the lowest being in T-2 and highest in T-3. The content of β -santalol ranged from 18.2–27.9 %, the lowest being in T-8 and highest in T-10.

Table 3. Variation of metabolites (area %) in extracted SW oil.

Metabolites	Area %									
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
α -Santalene	0.6	4.8	1.3	1.2	1.0	0.3	0.9	1.1	0.4	0.8
β -Santalene	2	4.9	3.9	3.1	2.6	1.4	2.9	3.1	1.5	2.7
α -Santalol	53.2	41.7	53.7	45.6	52.9	47.4	51.0	47.4	47.9	46.2
Z- α -trans-Bergamotol	6.8	6.3	5.2	8.4	6.6	7.2	6.2	4.9	9.3	7.5
E-cis-epi- β -Santalol	3.8	4.1	2.7	4.2	3.4	4.9	3.7	2.8	5.5	5.5
trans- β -Santalol	25.0	24.1	25.9	25.1	26.8	25.2	25.8	18.2	27.0	27.9

The six metabolites were present in significant amount in all samples with little variation in concentration of α -santalol and β -santalol. While α -santalene, β -santalene, epi- β -santalene, α -bergamotol and epi- β -santalol were detected with high deviation. Further the trend in variation of major metabolites among the different girth trees was analyzed. In general the relative variation of the α -santalol and β -santalol among the all samples was almost same (Fig. 3). Although there was no particular relationship observed between the girth sizes and oil yield of Sandalwood trees grown in the same location. There may be a strong influence of genetic characteristics to the quantity and quality of sandalwood oil variations of *S. album* trees of similar size growing under similar soil and climatic conditions.

**Figure 3.** Variation trend of SW oil composition in different girth SW trees.

CONCLUSION

Metabolite profiling of the heartwood oil by using GC-MS techniques has provided a vast array of metabolites, which included n-alkanes, sesquiterpene, sesquiterpenoids, fatty acids, alcohols and other hydrocarbons. Total % oil yield from trees having different girth size (47.1–82.4 cm) were observed from 1.6–3.6 % of heartwood. Although, there were no particular trends observed between the girth sizes and % oil yield. But in general, almost similar type trends in the relative ratio of α -santalol and β -santalol content were observed. The physical parameters such as relative density and specific gravity of the oils were observed within the limit of high quality SW oil. These variations can be suspected that the genetic factors may play a significant role in oil formation. The unknown causes of the variation of sandalwood oil compounds show the importance of conducting further research on sandalwood oil formation with consideration of the external and internal parameters.

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