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Research Article

Gas Chromatography-Sass Spectrometry (GC-MS) Profiling of Heartwood Oil Composition from 15 Years Old Sandalwood Trees

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ABSTRACT

One of the most important commercially valued products of sandalwood is its aromatic essential oil obtained from the heartwood. The heartwood oil commercially known as East Indian Sandalwood oil is being extensively used in diversified industries like - perfumery, cosmetics, pharmaceutical and aromatherapy. The heartwood oil content and variation in its composition among the similar aged trees grown in homogenous conditions were studied. Fifteen year old trees growing at Institute of Wood Science and Technology campus were considered and core samples were collected from five trees having different girth (37.5, 42, 50.8, 53.5 and 56cm). Oil content extracted from these cores varied from 11 to 43 mg/g of heartwood. Total 35 volatile metabolites were detected and quantified in term of percentage peak area (% A) by using gas chromatography-mass spectrometry (GC-MS) method. A chemically diversified alkanes, sesquiterpenoids, sesquiterpene, fatty acids, and alcohols, were observed. The major constituents were α-santalol (33.55-35.32%), β-santalol (17.16-18.96%), epi-β-santalol (2.23-3.51%), epi-β-santalene (0.80-1.69%), α-santalene (0.56-1.6%), β-santalene (1.12-2.35%), and α-bergamotol (4.03-7.77%).

Key words: *Santalum album* L., Sandalwood oil, Girth, GC-MS, Metabolite

INTRODUCTION

Santalum album L. (Family Santalaceae) commonly known as East Indian sandalwood tree, it is one of the most valued for heartwood and the essential oil[1]. The heartwood oil is being extensively used in diversified industries like - perfumery, cosmetics, pharmaceutical and aromatherapy[2,3,4,5]. The main sesquiterpene alcohols of heartwood oil are α-santalol (1), β-santalol (2)[6] (Verghese et al., 1990), α-bergamotol (3), epi-cis-α-santalol (4), cis-lanceol (5) and α-bisabolol (6)[7] (Fig. 1). The hydrocarbons, α-santalene (7), β-santalene (8), α-bergamotene (9), epi-β-santalene (10), α-curcumene (11), and β-bisabolene (12) are also present in the oil[7,8,9] (Fig. 1). Scanty of literature is available on total volatile constituents of this tropical essential oil-yielding tree. Although the content and composition of oil from the central and transition zones of the Sandalwood disc[10], the GC-MS profiles of steam distilled volatiles[7], preliminary analysis of growth and oil composition[11] and solvent extractable volatile profiling[12] from heartwood of East Indian sandalwood tree are the few studies in this direction. Moreover the biosynthesis of sandalwood oil sesquiterpenes along with heartwood content of wood core and its composition[13], separation of α- and β-santalenes and (Z)-α- and (Z)-β-santalols, (Z)-α-trans-bergamotol and (Z)-lanceol and hydroxylation and epoxidation on (Z)-α-santalol were already reported[14].

[Fig. 1. The main volatile metabolites (1-12) reported from heartwood oil]

Although extensive studies has been carried out for variation in oil content, biosynthesis, separation of main constituent and its composition. But it has been found that in most of the studies the tree age and the growing conditions are not similar while they play an important role in its oil quality and quantity. To date the metabolic variations in the heartwood oil of similar aged trees grown in homogenous conditions have not been studied. Therefore, it is imperative to understand the variation in Sandalwood oil composition among the similar aged trees grown in homogenous conditions. Variations in the phytochemical composition of trees having different girth with similar aged trees grown in homogenous conditions may have an effect on the quality of heartwood oil, which could lead to inconsistency in medicinal and aromatherapy properties. The objective of the present study was to detect and quantification of the volatile metabolites of heartwood oil and their variation among five different girth trees of same age grown in uniform condition using GC-MS analysis.

MATERIALS AND METHODS

General experimental methods: The instrument Buchi Rota Vapor used for evaporation of excess amount solvents from samples. The resulting extracts were analyzed by GC-MS (GC: Agilent 7890 A MS: MS detector 5975 C).

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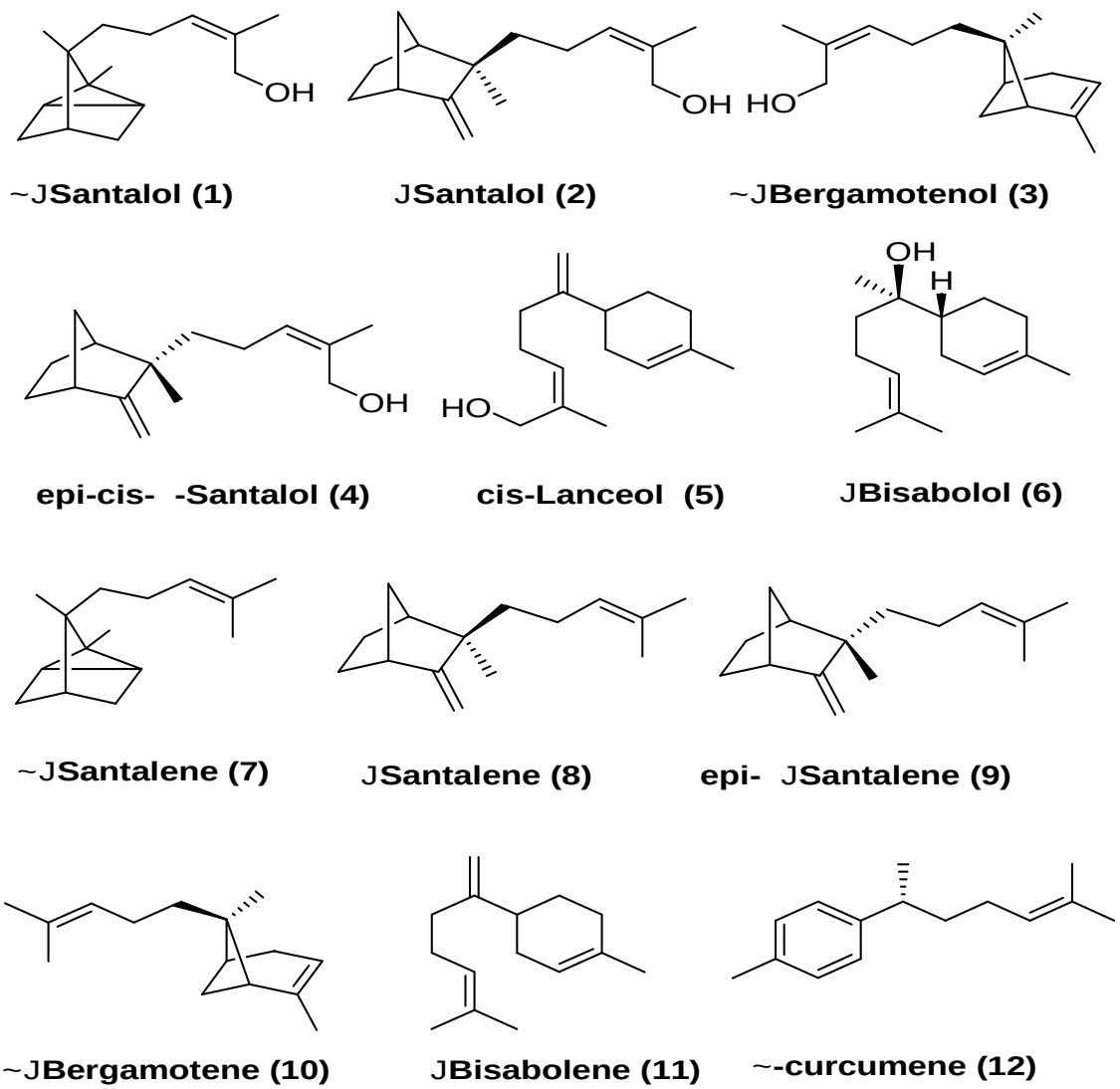

Fig. 1. The main volatile metabolites (1-12) reported from heartwood oil

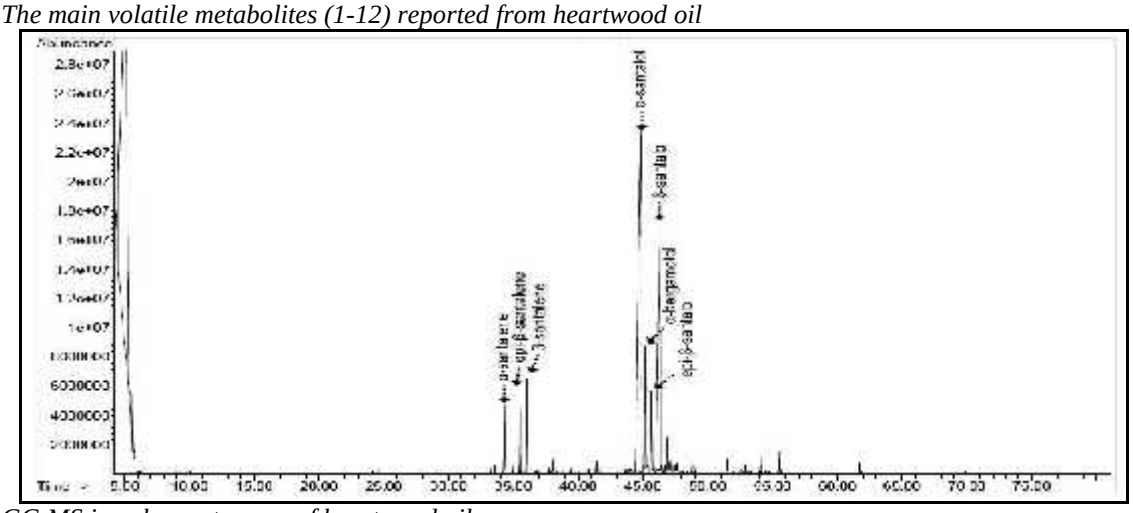

Fig. 2. GC-MS ion chromatogram of heartwood oil

Plant materials and chemicals: Five core samples were collected from sandalwood trees by boring at 30 cm height from the ground. The core samples were separated from sapwood and bark by chopping. It was pulverized into chips/powders form and air dried for 24 h, prior to solvent extraction. The HPLC grade solvents (n-hexane, chloroform, diethyl ether) used for extracting metabolites were purchased from Merck Specialties Pvt. Ltd. and HiMediaLaboratories Pvt. Ltd. Mumbai. Samples were collected from 15-year-old sandalwood trees growing in the campus of Institute of Wood Science and Technology, Bangalore, India.

Extraction of metabolites**:** The chip samples of heartwood were extracted using n-hexane, chloroform and diethyl

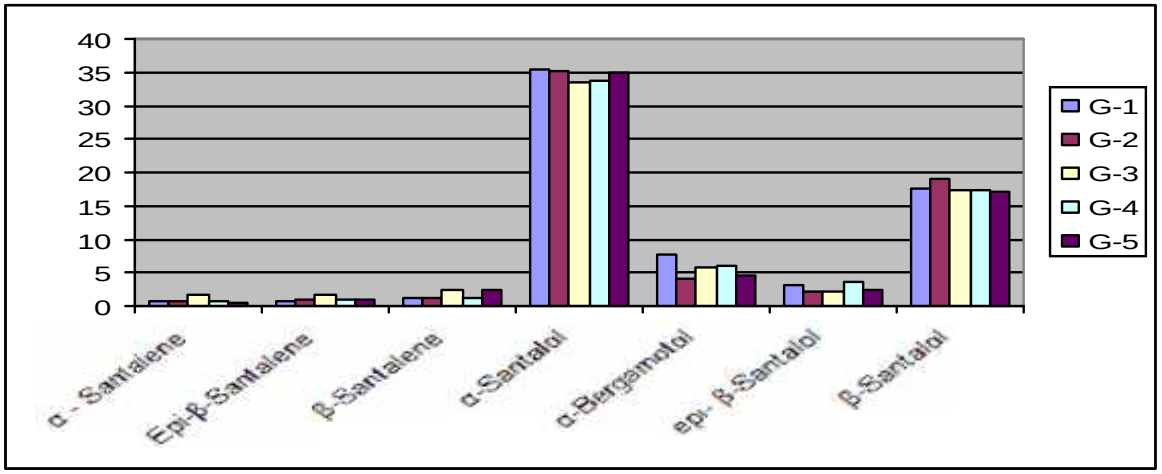

Fig. 3. Major volatile metabolites variation trends in heartwood oil

Table 1: Heartwood oil content of five trees having different girth

Sample code	Girth size (at 30 cm height from ground)	Extractive content in mg/1g of heartwood
G-1	37.5	11
G-2	42.0	15
G-3	50.8	18
G-4	53.5	25
G-5	56.0	43

ether (eluotropic series method). Initially 1g of sample was extracted with hexane (20 mL, 12h) at room temperature. The solvent portion was collected by filtration using Whatman filter paper and this procedure was repeated until the hexane layer became almost colorless. Then extraction continued with chloroform and diethyl ether successively by same procedure. Later all the extracts were dried over Na_2SO_4, pooled and were concentrated under reduced pressure using rotovapor at 40 °C till obtain oily fractions. The resulting oily masses was stored at 4 °C and subjected to GC-MS analysis.

GC-MS analysis: The resulting oily masses were analyzed by GC-MS, equipped with a 30 m (l) 0.25 mm (i.d.), 0.25 mm film thickness, nonpolar TR-5MS fused silica 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. The initial oven temperature was set to 40 °C for 1 min. The GC oven temperature program was as follows: 40 °C-220 °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; 3 scans/s). Maximum ionization time was 25 ms. A solvent delay time of 5 min (setoff) was used to avoid overloading the mass spectrometer with chloroform. The resulting GC-MS profile was analyzed using National Institute of Standards and Technology (NIST, Washington DC, USA) and Dr. Duke's Phytochemical and Ethnobotanical Database (http://www.ars-grin.gov/duke/). Quantity of metabolite was consider by percentage peak area appeared at the total ion chromatogram in GC-MS analysis.

RESULTS AND DISCUSSION

The core samples of heartwood from different girth trees 37.5, 42, 50.8, 53.5 and 56 cm were extracted with n-hexane, chloroform and diethyl ether. The pooled and concentrated volatile fractions were analyzed using GC-MS. The extracted oil content varied from 11 to 43 mg per gram of heartwood (Table 1). Total 35 volatile metabolites were detected by GC-MS (Table 2). These non-polar metabolites were n-alkanes, sesquiterpene, sesquiterpenoids, fatty acids, alcohols and other hydrocarbons.

[Table 1 Heartwood oil content of five trees having different girth]

The quantity of metabolites varied among all the samples from different girth size trees and the metabolites which were detected as concentration percentage peak area ≥0.50 were considered as major metabolites (Fig. 2, Table 3). The results showed that the extract from heartwood of *S. album* had a high concentration of α-santalol and β-santalol. Both α-santalol and β-santalol are responsible for most of the biological activities such as anticancer and tumor inhibitory properties[15] anti-inflammatory effect[16], antiviral properties[17], antimicrobial[18], neuroleptic properties and chemo-preventive effects[19,20,21,22,23]. Clinical trials have identified that α-santalol and β-santalol have a sedative effect and the oil has also been reported to significantly decrease the incidence of papillomas[24]. The concentration of α-santalol ranged from 33.55-35.32%, the lowest being in G-3 and highest in G-1. The concentration of β-santalol ranged

Table 2 Mass fragmentation of GC-MS identified metabolites

No.	tR (min)	Metabolites	Molecular formula	Mol. Wt.	Mass fragmentation (mass m/z values)
1.	24.10	Dodecene	$C_{12}H_{24}$	168	168(M^+), 140, 125, 83, 55, 41
2.	24.50	Dodecane	$C_{12}H_{26}$	170	170(M^+), 141, 127, 85, 57, 43
3.	33.21	Tetradecene	$C_{14}H_{28}$	196	196(M^+), 125, 111, 97, 83, 69, 41
4.	33.57	Tetradecane	$C_{14}H_{30}$	198	198(M^+), 99, 85, 71, 57, 43
5.	34.33	α-Santalene	$C_{15}H_{24}$	204	204(M^+), 189, 161, 107, 55, 41
6.	34.89	Trans-α-Begamotene	$C_{15}H_{24}$	204	204(M^+), 161, 107, 77, 55, 41
7.	35.22	β-Sesquiphellandrene	$C_{15}H_{24}$	204	204(M^+), 161, 133, 93, 69, 41
8.	35.52	Epi-β-Santalene	$C_{15}H_{24}$	204	204(M^+), 122, 94, 79, 41
9.	35.74	Cis-β-Farnesene	$C_{15}H_{24}$	204	204(M^+), 161, 133, 105, 79, 41
10.	36.04	β- Santalene	$C_{15}H_{24}$	204	204(M^+), 161, 122, 94, 79, 55, 41
11.	36.75	α –Funebrene	$C_{15}H_{24}$	204	204 (M^+), 147, 119, 93, 69, 41
12.	36.89	α-Curcumene	$C_{15}H_{22}$	202	202(M^+), 145, 132, 119, 105, 41
13.	36.99	β-Farnesene	$C_{15}H_{24}$	204	204(M^+), 161, 120, 79, 55, 41
14.	37.74	Pentadecane	$C_{15}H_{32}$	212	212(M^+), 113, 99, 71, 57, 43
15.	41.40	Hexadecene	$C_{16}H_{32}$	224	224(M^+), 196, 125, 83, 69, 41
16.	41.70	Hexadecane	$C_{16}H_{34}$	226	226(M^+), 85, 71, 57, 43
17.	44.86	α-Santalol	$C_{15}H_{24}O$	220	220(M^+), 121, 107, 93, 79, 41
18.	45.19	α-Bergamotol	$C_{15}H_{24}O$	220	220(M^+), 187, 145, 119, 77, 43
19.	45.68	epi-β-Santalol	$C_{15}H_{24}O$	220	220(M^+), 134, 107, 94, 79, 55, 41
20.	46.32	β-Santalol	$C_{15}H_{24}O$	220	220(M^+), 122, 94, 79, 55, 41
21.	47.41	Di-Epi-α-Cedrene	$C_{15}H_{24}$	204	204(M^+), 187, 132, 119, 77, 55, 41
22.	47.63	Cis, Lanceol	$C_{15}H_{24}O$	220	204(M^+), 187, 159, 134, 93, 79, 43
23.	48.39	Bicyclogermacrene	$C_{15}H_{24}$	204	204(M^+), 170, 133, 121, 93, 67, 43
24.	48.81	Octadecene	$C_{18}H_{36}$	252	252(M^+), 224, 125, 83, 41
25.	49.05	Octadecane	$C_{18}H_{38}$	254	254(M^+), 113, 99, 85, 71, 43
26.	53.34	α-Bisabolene Epoxide	$C_{15}H_{24}O$	220	220(M^+), 205, 151, 121, 93, 81, 43
27.	53.72	β-Caryphyllene Oxide	$C_{15}H_{24}O$	220	220(M^+), 205, 161, 135, 95, 67, 43
28.	55.52	Cycloeicosane	$C_{20}H_{40}$	280	280(M^+), 139, 97, 83, 69, 55, 41
29.	55.72	Eicosane	$C_{20}H_{42}$	282	282(M^+), 113, 99, 85, 71, 57, 43
30.	60.12	Vaccenic Acid	$C_{18}H_{34}O_2$	282	282(M^+), 149, 120, 111, 93, 71, 57, 32
31.	60.85	Octadeconoic Acid	$C_{18}H_{36}O_2$	284	284(M^+), 121, 105, 97, 83, 76, 55, 41
32.	61.68	Heneicosanol	$C_{21}H_{44}O$	312	312(M^+), 308, 111, 97, 83, 69, 55, 43
33.	61.86	Docosane	$C_{22}H_{46}$	310	310(M^+), 127, 99, 85, 57, 43
34.	69.84	Docosene	$C_{22}H_{44}$	308	308(M^+), 139, 125, 97, 83, 57, 43
35.	70.14	Tetracosane	$C_{24}H_{50}$	338	338(M^+), 225, 127, 85, 57, 43

m/z = Mass/charge ratio, M^+ = molecular ion peak.

from 17.16-18.96%, the lowest being in G-5 and highest in G-1.

Fig. 2. GC-MS ion chromatogram of heartwood oil

Biological activities such as DNA polymerase (*pol*) inhibitory, cancer cell (human colon carcinoma, HCT116) growth inhibitory, antiallergic, and anti-hexosaminidase release activity in rat have been credited to the santalene type sesquiterpenoids, i.e., α - and β - santalenes and santalols[25]. Santalenes (α-santalene and β-santalene) has been reported as the products of α /or β-santalene synthase mediated enzymatic reactions[26] and an important ingredient of scent[1]. The α-santalene and β-santalene were detected in all the five samples with varying quantities ranged 0.56-1.6%, and 1.12-2.35% respectively. The epi- β-santalene was found to be active against *S. typhimurium*[18] and its content found in ranged 0.80-1.69%.

Table 2 Mass fragmentation of GC-MS identified metabolites]

[Table 3 Qualitative and quantitative variability in volatile metabolites of heartwood oil using GC-MS method]

The fulfillment with legal authenticity requirements, identification and quantification of α-bergamotol also have been included into the list of compounds regulated by Australian Standard (AS2112-2003), International Organization for Standardization ISO 3518:2002 and European Union[27]. In oil extract, the content of α-bergamotol ranged from 4.03-7.77% while the epi-β-santalol with the concentration ranged from 2.23-3.51%.

Further the trend in variation of major metabolites concentration among the different girth trees was analyzed (Fig. 3). There was no particular relationship observed between the girth size, concentration and

Table 3 Qualitative and quantitative variability in volatile metabolites of heartwood oil using GC-MS method

tR (min)	Metabolites	Percentage peak area (%A)				
		G-1	G-2	G-3	G-4	G-5
24.10	Dodecene	0.03 ± 0.01	0.03 ± 0.02	0.04 ± 0.02	0.03 ± 0.01	0.02 ± 0.01
24.50	Dodecane	0.04 ± 0.01	0.05 ± 0.02	0.05 ± 0.01	0.04 ± 0.02	0.03 ± 0.01
33.21	Tetradecene	0.07 ± 0.02	0.07 ± 0.02	0.12 ± 0.02	0.07 ± 0.01	0.07 ± 0.01
33.57	Tetradecane	0.14 ± 0.02	0.09 ± 0.01	0.16 ± 0.03	0.07 ± 0.01	0.07 ± 0.02
34.33	α - Santalene	0.72 ± 0.02	0.80 ± 0.05	1.6 ± 0.01	0.84 ± 0.01	0.56 ± 0.02
34.89	Trans-α-Begamotene	ND	0.13 ± 0.02	ND	0.17 ± 0.01	0.14 ± 0.01
35.22	β-Sesquiphellandrene	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.02	0.01 ± 0.01
35.52	Epi-β-Santalene	0.80 ± 0.01	0.87 ± 0.03	1.69 ± 0.01	0.89 ± 0.01	1.01 ± 0.01
35.74	Cis-β-Farnesene	0.02 ± 0.01	0.02 ± 0.02	0.06 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
36.04	β-Santalene	1.12 ± 0.01	1.19 ± 0.02	2.35 ± 0.02	1.23 ± 0.02	2.33 ± 0.01
36.75	α –Funebrene	0.03 ± 0.01	0.05 ± 0.02	0.04±0.00	0.05 ± 0.01	0.03 ± 0.00
36.89	α-Curcumene	0.08 ± 0.00	0.06 ± 0.02	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01
36.99	β-Farnesene	0.02 ± 0.00	0.02 ± 0.01	0.05 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
37.74	Pentadecane	0.03 ± 0.02	0.07 ± 0.03	0.04 ± 0.01	0.01 ± 0.00	0.04 ± 0.01
41.40	Hexadecene	0.17 ± 0.02	0.13 ± 0.02	0.17 ± 0.02	0.11 ± 0.01	0.19 ± 0.01
41.70	Hexadecane	0.09 ± 0.01	0.08 ± 0.02	0.12 ± 0.02	0.06 ± 0.01	0.07 ± 0.01
44.86	α-Santalol	35.32 ± 0.06	35.25 ±0.05	33.55 ± 0.02	33.84 ± 0.03	35.06 ± 0.02
45.19	α-Bergamotol	7.77 ± 0.01	4.03 ± 0.06	5.83 ± 0.02	6.12 ± 0.03	4.50 ± 0.01
45.68	epi- β-Santalol	3.18 ± 0.02	2.25 ± 0.03	2.23 ± 0.01	3.51 ± 0.04	2.33 ± 0.02
46.32	β-Santalol	17.63 ± 0.02	18.96 ± 0.05	17.42 ± 0.02	17.27 ± 0.25	17.16 ± 0.05
47.41	Di-Epi-α-Cedrene	0.75 ± 0.02	0.10 ± 0.01	0.75 ± 0.02	0.15 ± 0.02	0.44 ± 0.01
47.63	Cis, Lanceol	ND	0.15 ± 0.02	ND	1.75 ± 0.01	1.57 ± 0.02
48.39	Bicyclogermacrene	0.01 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.00
48.81	Octadecene	0.35 ± 0.01	0.22 ± 0.01	0.35 ± 0.01	ND	ND
49.05	Octadecane	0.23 ± 0.01	0.08 ± 0.01	0.23 ± 0.02	0.11 ± 0.02	ND
53.34	α-Bisabolene Epoxide	0.45 ± 0.02	0.03 ± 0.03	ND	0.15 ± 0.1	0.45 ± 0.01
53.72	β-Caryphyllene Oxide	ND	0.05 ± 0.03	0.05 ± 0.01	0.18 ± 0.01	0.22 ± 0.01
55.52	Cycloeicosane	ND	0.31 ± 0.03	ND	0.17 ± 0.02	0.22 ± 0.02
55.72	Eicosane	0.35 ± 0.01	0.06 ± 0.04	0.23 ± 0.01	ND	0.37 ± 0.01
60.12	Vaccenic Acid	0.13 ± 0.01	ND	0.23 ± 0.02	0.12 ± 0.01	0.25 ± 0.02
60.85	Octadeconoic Acid	0.25 ± 0.02	ND	0.16 ± 0.01	0.10 ± 0.01	0.34 ± 0.02
61.68	Heneicosanol	0.04 ± 0.02	0.13 ± 0.03	0.11 ± 0.01	0.02 ± 0.01	0.13 ± 0.02
61.86	Docosane	0.04 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.04 ± 0.02	0.06 ± 0.01
69.84	Docosene	0.12 ± 0.02	0.05 ± 0.01	0.12 ± 0.02	0.08 ± 0.01	0.17 ± 0.02
70.14	Tetracosane	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.00	ND	ND

(ND = not detected; ± = SD of percentage peak area)

composition of metabolites. The seven major metabolites (with concentration ≥ 0.50 mg/g) were present in all five samples with little variation in concentration of α-santalol, β-santalol, and epi-β-santalol while α-santalene, β-santalene, epi-β-santalene, α-bergamotol were detected with high range deviation.

[Fig. 3. Major volatile metabolites variation trends in heartwood oil]

CONCLUSION

In conclusion, metabolite profiling of the heartwood oil has provided a vast array of metabolites which included n-alkanes, sesquiterpene, sesquiterpenoids, fatty acids, alcohols and other hydrocarbons. Thirty five volatile metabolites from the heartwood of 15 year old Sandalwood tree were detected by GC-MS method. The extracted oil yield from heartwood of different girth size trees were varies as well as the compositions of metabolites were differing. The major metabolites were present in all the samples with little variation in concentration of α-santalol, β-santalol, and epi-β-santalol while α-santalene, β-santalene, epi-β-santalene, α-bergamotol were detected with high range deviation.

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