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ORIGINAL PAPER

Essential oil content and composition of Indian sandalwood (*Santalum album*) in Sri Lanka

Upul Subasinghe • Manuri Gamage • D.S. Hettiarachchi

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Abstract: *Santalum album* (Indian Sandalwood) is found in the mountainous regions of the intermediate zone of Sri Lanka. Few studies have been conducted on sandalwood ecology in this region, and ours is the first recorded study of essential oil content and chemical composition of heartwood. We harvested two trees with State permission and took cross-sections for analysis. We demonstrated a difference in the heartwood formation and oil yield of the trees. The composition of the oil was found to be consistent between trees and along the trunk of the tree. Main aromatic compounds were santalols and other compounds are recorded in lesser quantities. Results of this study comply with the other published work on sandalwood elsewhere. This initial study on *S. album* in Sri Lanka provided promising results for the future of sandalwood agro-forestry.

Key words: essential oil; Sri Lanka; Sandalwood; Santalum album; santalol

Introduction

Indian Sandalwood (*Santalum album* L.) is found in the tropical mountainous regions of Southern India, Sri Lanka and several islands of the Indonesian archipelago. Sandalwood has many uses in modern and traditional markets. Essential oil distilled from the wood is highly valued in the perfume industry for both its aroma and fixative properties. Traditional markets require carving logs and also powders to prepare incense known as agarbatti (Fox 2000). Although naturally grown *S. album* has been recorded in Sri Lanka, a sandalwood industry has never developed here (Tennakoon et al. 2000). Sandalwood has been harvested from the wild over generations for traditional Ayurvedic medicines used to treat skin conditions and in paediatric formulations (Department of Ayurveda 1980)

The value of a sandalwood tree depends on three important characters (i) the volume of heartwood; (ii) the concentration and (iii) quality of its heartwood oil (Doran et al. 2005; Brand et al 2007). The quality of sandalwood oil depends primarily on the concentration of two major sesquiterpene alcohols (*cis*-α-santalol and *cis*-β-santalol), which produce the pleasant characteristic aroma. According to many standard documents (ISO 3518:2002; Howes et al 2004), the combination of these two compounds (known as total santalol content) accounts for 90% of the total volatile material from the wood. *S. album* heartwood is known to have the highest concentration of oil and the highest proportion of santalols (Verghese et al. 1990; Baldovini et al. 2011). Sandalwood oil content increases with the age of the tree as more heartwood is formed over the time. Biosynthesis of the sandalwood sesquiterpenes depends upon a group of enzymes known as sesquiterpene synthase, which are specific for each type of sesquiterpene. Although the enzymes are regulated genetically, biosynthesis of sesquiterpenes might also be affected by other environmental factors (Jones et al. 2008; Jones et al. 2011).

Global sandalwood resources are diminishing and the demand is increasing (Gillieson et al. 2008). *S. album* populations in Sri Lanka are rapidly decreasing due to habitat destruction, over exploitation and complex silviculture associated with the root hemi-parasitic nature of sandalwood (Tennakoon et al. 2000). For this reason *S. album* was listed as a protected plant (Flora and Fauna Protection Ordinance Act 2009) and its harvest and transport are strictly prohibited in Sri Lanka.

Government of Sri Lanka invited the private sector to invest in forest plantation establishment and management with the intention of increasing national tree cover (Forest Sector Master Plan 1995). Since 2000, a few private sector companies have started forest plantation establishment using broad-leaf mahog-

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Upul Subasinghe • Manuri Gamage
Department of Forestry and Environmental Sciences, Sri Jayewardenepura University, Nugegoda 10250, Sri Lanka.

D.S. Hettiarachchi (✉)
Wescorp Group, Caning Vale, 6155, Western Australia.
Email: danny@wescorp.com.au

Corresponding editor: Hu Yanbo

any (*Swietenia macrophylla*) and teak (*Tectona grandis*). After 2005, they started sandalwood plantation and, at present, approximately 200 ha have been planted to sandalwood and the area of plantation is increasing annually. Globally, sandalwood has become a valuable timber crop. Tropical regions of Western Australia have started large scale plantations of *S. album*, while arid parts of the same state have planted *S. spicatum* plantations (McKinnell 2008). Pacific islands like Vanuautu, Fiji and New Caledonia have started plantations and conducted valuable research on quality and propagation of sandalwood (Page et al. 2010a; Page et al. 2010b).

To date, limited research has been published on *S. album* in Sri Lanka, and therefore our understanding of its heartwood qualities is limited. The intention of our study was to determine the quality of sandalwood grown in Sri Lanka. We discuss the heartwood and oil content of the two naturally grown trees and also their chemical composition. Our findings are compared with previously published work on *S. album* and other sandalwood species.

Materials and methods

Whole tree harvest and destructive sampling was considered the best option for analyzing oil content and oil constituents. To determine heartwood oil variation, two trees (2.5 m apart) were uprooted from a home garden at Welimada (80°54′31 E and 06°53′52 N), 138 km from Colombo. Sandalwood is more common in home gardens, natural forests and reservations in the Welimada region when compared with other parts of the country. In home gardens, the trees were growing 2.5 m apart. Planting records were not available, but the owner estimated the age of the trees to be 15 years. Permission from the Forest Department of Sri Lanka and the Divisional Secretariat of Badulla Division was obtained for harvesting and transporting for research purposes.

Heartwood content

Two trees (tree no.1 with 20.8 cm in DBH and 10.0 m in height, tree no. 2 with 24.5 cm in DBH and 8.5 m in height) were uprooted and marked at ground level and 0.5 m intervals to their crowns (Table 1). Roots were marked at 0.15 m and 0.30 m below ground level. Cross sections were then obtained at all marked points (10 cm in thickness). Heartwood content was measured as a percent of cross-sectional area by marking the area of densely coloured portion. Heartwood was then separated from sapwood for oil analysis.

Hydro-distillation

Heartwood samples were pulverized to less than 10 mesh size and a 20-g subsample was taken from each. Samples were immersed in deionised water overnight and distilled using a Dean-Stalk apparatus for 9 h according to the methods of Hettiarachchi (2008). Collected volatile material was dehydrated, weighed and then stored away from sunlight with inert headspace.

Gas chromatographic analysis

We dissolved oil samples in n-hexane to produce a 1% w/v solution and injected 1 μL of this solution into a Gas Chromatogram (GC2010, Shimadzu Scientific, Japan) equipped with a flame ionisation detector. The column used was 95% phenyl siloxane coated capillary (AT-5, Alltech, USA). The injector was kept at 220°C and the oven had a 1°C per min gradient temperature from 120°C to 180°C. Data were processed by Labsolutions© software (Shimadzu Scientific, Japan). Identification of specific compounds within the oil was verified by using mass spectroscopic data and Kovat's indices (Hettiarachchi et al. 2010).

Results

Heartwood content

Heartwood formation was recorded in both trees, but its presence at sampling points varied between trees. In Tree No.1 (Table 1), heartwood was found at sampling points from 15 cm below to 150 cm above ground, but not beyond these sampling points. Heartwood formation was identified in Tree Two (Table 2) at all sampling points from 30 cm below to 300 cm above ground level.

Essential oil content

Percent oil yield from Tree One (Table 1) was greater than from Tree Two (Table 2) with the highest concentration (6.36% *w/w*) found in the roots (15 cm below ground) of Tree One. The oil concentration in Tree One decreased from the roots to the trunk, with a 2.44% reduction from the lowest (-15 cm) to highest (+150 cm) sampling point. In contrast, oil concentration in Tree Two remained relatively consistent through the heartwood, however the highest oil content was found at ground level (3.35% w/w).

Chemical composition

Total santalols in the roots of Tree One (Table 1) was highest at 56.38%. Tree Two (Table 2) showed highest santalol content of 47.73% at ground level. Limited variation in santalol content was recorded between the two trees. *Cis*-α-santalol was the major sesquiterpene alcohol, followed by *cis*-β-santalol. Variation along the main stem was proportional between the α-santalol, β-santalol and β-curcumen-12-ol. Other compounds recorded were *cis*-nuciferol and α-bisabalol. Composition of these compounds did not change with increasing height of sampling points (Tables 1 and 2). Other identified sesquiterpene alcohols were the *cis*-bergamatol, *epi*-β-santalol, γ-curcumen-12-ol and *cis*-lanceol. *Trans*-farnesol content was below the limit of quantification. Other sesquiterpene hydrocarbons were not recorded in this study.

Statistical analysis (correlation) conducted for the constituents

Correlation tests were conducted separately for the two trees to

identify relationships between tested constituents of total santalol. Seventy-two relationships were tested for each tree. Six of 72 tested correlations between constituents proved significant for Tree 1 while 25 of 72 proved significant for Tree 2.

For Tree One: cis-α-santalol concentration was significantly correlated with epi-α-bisabalol (0.981) and cis-β-santalol (0.973); cis-β-santalol was significantly correlated with epi-α-bisabalol (0.977) and cis-nuciferol (0.886); *epi*-β-santalol was significantly correlated with *cis*-lanceol (0.961) and *cis*-nuciferol was significantly correlated with β-curcumen-12-ol.

Table 1. Heartwood and oil content with chemical composition of Tree 1

Height from ground (mm)	Heartwood (% Area)	Oil yield (%w/w)	Chemical composition									
			cis-α-trans-bergamatol	*cis-α-santalol*	*epi-α-bisabalol*	*epi-β-santalol*	*cis-β-santalol*	*E,E-farnesol*	*cis-nuciferol*	*γ-curcumen-12-ol*	*β-curcumen-12-ol*	*cis - lanceol*
-150	71	6.36	0.37	39	1.98	3.37	17.38	ND	1.35	1.97	6.36	1.99
0	86.5	4.81	0.37	37.36	1.94	2.72	16.65	ND	1.43	1.82	7.14	0.65
500	64.2	4.35	0.35	31.36	1.51	2.55	14.27	ND	1.25	1.99	5.92	0.79
1000	58.6	4.59	0.42	37.2	1.81	2.69	16.36	ND	1.4	1.78	7.18	0.75
1500	43.7	3.92	0.33	31.88	1.5	2.57	13.43	ND	1.08	1.91	3.95	0.68

Table 2. **Heartwood and oil content with chemical composition of Tree 2**

Height from ground (mm)	Heartwood (% Area)	Oil yield (% w/w)	Chemical composition									
			cis-α-trans-bergamatol	*cis-α-santalol*	*epi-α-bisabalol*	*epi-β-santalol*	*cis-β-santalol*	*E,E-farnesol*	*cis-nuciferol*	*γ-curcumen-12-ol*	*β-curcumen-12-ol*	*cis - lanceol*
-300	57.4	2.09	0.15	20.62	0.47	1.68	6.35	ND	0.75	1.26	9.71	0.48
-150	63.7	3.35	0.36	30.83	1.19	2.3	12.37	ND	0.87	1.46	4.59	0.71
0	81	3.07	0.39	33.36	1.31	2.49	14.37	ND	1.01	1.94	3.3	0.53
500	71.2	2.67	0.34	31.67	1.44	2.36	14.5	ND	1.02	1.68	2.35	0.72
1000	57.8	1.46	0.26	17.84	0.53	1.5	7.5	ND	0.7	1.19	8.47	0.47
1500	45.2	2.39	0.22	22.27	0.67	1.78	10.36	ND	0.81	1.32	8.27	0.52
2000	43.6	2.93	1.82	32.66	1.45	2.49	13.83	ND	0.95	2.06	2.91	0.61
2500	41.8	2.99	1.83	33.01	1.61	2.49	14.72	ND	0.94	2.08	2.46	0.62
3000	41.4	2.28	0.38	30.5	1.32	2.16	13.07	ND	0.84	2.15	3.2	0.68

For Tree Two: cis-α-santalol was significantly correlated with epi-α-bisabalol (0.964), epi-β-santalol (0.990), cis-β-santalol (0.952), *cis*-nuciferol (0.901), γ-curcumen-12-ol (0.849), β-curcumen-12-ol (-0.953) and cis-lanceol (0.720). In addition to the significant correlation with cis-α-santalol, epi-α-bisabalol also showed significant correlations with epi-β-santalol (0.954), cis-β-santalol (0.964), *cis*-nuciferol (0.861), γ-curcumen-12-ol (0.849), β-curcumen-12-ol (-0.988) and cis-lanceol (0.742). Epi-β-santalol was also significantly correlated with cis-β-santalol (0.942), *cis*-nuciferol (0.922), γ-curcumen-12 ol (0.819) and β-curcumen-12-ol (-0.933). Cis-β-santalol was significantly correlated with *cis*-nuciferol (0.909), γ-curcumen-12-ol (0.827), β-curcumen-12-ol (-0.964) and cis-lanceol (0.706). *Cis*-nuciferol was positively correlated with γ-curcumen-12-ol (0.682) and β-curcumen-12 ol (-0.864). β-curcumen-12-ol was also significantly correlated with cis-lanceol (-0.760).

Discussion

Substantial variation in heartwood oil concentration and composition was found between these two trees. The results are consistent with the variation found for geographically proximate *S. austrocaledonicum* trees in Vanuatu (Page et al. 2010a). Similarly the oil yields reported for *S. spicatum* in Western Australia varied from tree to tree even though the trees were found within a 50-m radius of each other. Similar trends in oil yield were observed along the tree stem: the amount of oil extracted from the wood above ground level decreased upwards along the stem. The results of the present study are therefore similar to the findings for *S. spicatum* (Moretta et al. 2001). Our two trees had higher heartwood content but oil yields were similar to 14 year-old *S. album* trees from North Western Australia (Brand et al. 2006).

Sesquiterpene compounds were similar to those reported for *S. album*. However, composition is different from mature *S. album* specimens (Howes et al. 2004). Cultivated *S. album* trees from various geographic regions of Western Australia produce oil at quality levels that match or exceed published standards (Brand et al. 2006; Brand et al. 2007; ISO 3518:2002).

Both trees included in this study had lesser santalol content than expected for *S. album*, similar to reports for *S. austrocaledonicum* (Page et al. 2006). However, another study conducted on cultivated 10 year-old Indian sandalwood plantation in North Western Australia reported far lesser santalol content (Jones et al. 2007). We found santalol content to be higher in the roots and the butts of the tree, and declining with height above ground. This gradual decline was less than reported in similar studies of Western Australian Sandalwood (*Santalum spicatum*) (Moretta et al: Piggott et al. 1997) which reported an 80% decline of santalols within the first meter above ground level. The percentage composition differences of compounds in our two trees were not that considerable (Table 1 and 2). Significant negative correlations were not observed for Tree 1 but for Tree 2, β-curcumen-12-ol was significantly negatively correlated with other constitu-

ents. The only significant correlation was of β-curcumen-12-ol with *cis*-nuciferol and was positive for Tree 1. Other sesquiterpene alcohols such as α-bisabalol and *cis*-nuciferol did not increase longitudinally along the main stem, as reported for *S. spicatum*. A recent study identified the biosynthetic pathways for sandalwood sesquiterpene synthesis and a genetic factor governing it (Jones et al. 2006; Jones et al. 2008). These studies showed that sesquiterpenes are synthesised by an enzyme which is genetically regulated. As the santalols are the dominant sesquiterpene type in these trees, other pathways appear to be less prominent. This is a promising finding for this population as they will be rich in santalol.

While this study of heartwood oil chemistry is the first to describe the oil quality of natural sandalwood in Sri Lanka, more widespread sampling is required to confirm its occurrence through the mountainous regions. Such a study would be of particular benefit in identifying and selecting those trees with high oil concentration and santalol content for use in commercial agroforestry. As has been demonstrated in Vanuatu (Page et al. 2010b), sandalwood can become an important cash crop for smallholder farmers in Sri Lanka. Sandalwood with improved oil quality would have a strong demand and attract premium prices in global markets and this would benefit the Sri Lankan domestic economy.

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