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Heartwood assessment of natural *Santalum album* populations for agroforestry development in Sri Lanka

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Abstract Sandalwood (*Santalum album*) is developing as an important agroforestry crop in Sri Lanka. The value of *S. album* depends upon the oil content in the heartwood and its composition with reference to sesquiterpene alcohols *cis*-α-santalol and *cis*-β-santalol. According to the popular belief in Sri Lanka, certain *S. album* trees do not produce oil even after maturity. Therefore the present study was conducted to identify the presence and the variation of essential oil, its composition and the variation of growth parameters of nine distinctive *S. album* populations growing under different agroecological zones in Sri Lanka. According to the results, heartwood content, oil content and its constituents varied within and between the populations. It was interesting to observe that *cis*-α-santalol and *cis*-β-santalol were not detected in certain *S. album* trees though the oil contents of those trees were higher than the average. Heartwood content of the trees did not show a correlation with oil content, dbh and height. However, the oil content was significantly correlated with tree dbh and height. Majority of the trees (62 %) had heartwood essential oil in compliance to ISO standards; only a 31 % of the total sampled trees had the essential oil content above 2 % (w/w). Dry mountainous Badulla district had the highest percentage of trees complying the ISO standards. These findings are vital for identifying suitable sources for agroforestry propagation of *S. album*.

Keywords Sandalwood · Santalum album · Sri Lanka · Alpha and beta santalol

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Introduction

Indian sandalwood (*Santalum album*) is found in the Southern states of India, Sri Lanka and Nusa Tengara province of Indonesian archipelago. Family Santalaceae includes 28 taxa of trees and shrubs, which are distributed from India to Pacific Islands including the Australian continent. Several species generally known as sandalwood, yield fragrant wood and oil rendering them extremely valuable in relation to other woody species (Barrett and Fox 1995; Fox 2000). Essential oil distilled from the wood is highly valued in the perfume industry for both its aroma and fixative properties. Traditionally sandalwood is used in medicine, making religious or ornamental items and to burn as incense (Fox 2000). *S. album* has been harvested from the wild of Sri Lanka over hundreds of years for traditional Ayurvedic medicines used in dermatological and

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pediatric formulations (Ayurveda Pharmacopoeia 1980).

Santalum album is found in many different climatic and geographical regions of Sri Lanka as shown in Fig. 1; however, it is more common in the dry mountainous regions of central Sri Lanka (Subasinghe et al. 2013a; Tennakoon et al. 2000). Rural communities in the Badulla district of Sri Lanka are depending upon sandalwood as a source of income for many generations. Personal communication were held with

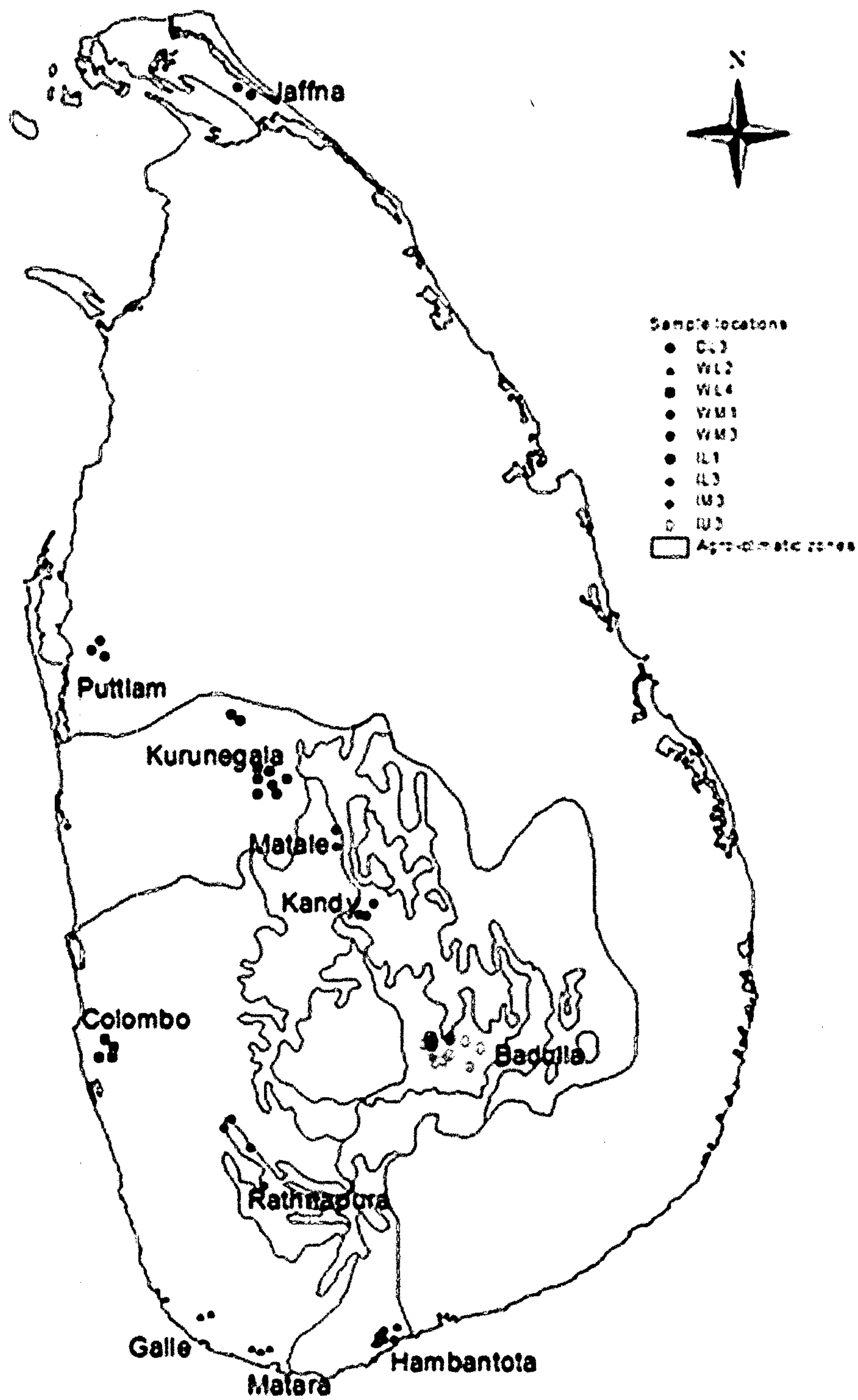

Fig. 1 Map of Sri Lanka illustrating the sample locations with demarcation of agro-ecological regions

the memebers of the communities in the year 2010–2012 to gain more understanding and their experiences and belives are given here. It is the long held belief among the villagers who harvest sandalwood that certain trees do not contain the valuable heartwood, thus called as "Wild Sandalwood". Upon investigation, it was found that the villagers can only identify a suitable tree only by checking the heartwood as the trees show no morphological difference. Badulla district is a dry mountainous region which is known for high quality and high abundance of sandalwood. This region has low temperatures at night although the day time temperature is comparatively high; the folk belief is such climatic conditions favour the essential oil content sandalwood trees.

Value of a sandalwood tree depends on three important characters; the volume of heartwood, the concentration of the heartwood oil and its quality (Brand et al. 2007; Doran et al. 2005; Hettiarachchi, 2012). Studies conducted in Australia and India have reported a larger variation in the essential oil composition and heartwood formation (Brand et al. 2012; Arun Kumar et al. 2014).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 sandalwood aroma (Adams et al. 1975). According to the literature, combination of the above two compounds accounts for 90 % of the total volatile material obtained from the wood (ISO 2002). Minor constituents of *S. album* heartwood oil includes *epi*-β-santalol, *trans*-α-bergamotol, *epi*-β-bisabalol, γ-curcumen-12-ol, β-curcumen-12-ol, *cis*-lanceol, *cis*-nuciferol, *trans,trans*-farnesol and sesquiterpene hydrocarbons such as α and β-santalenes, bergamotenes, α, β and γ-curcumenes and β-bisabolene (Adams et al. 1975; Anonis 1998; Baldovini et al. 2011; Verghese et al. 1990). However, the international standards refer only to four of the major compounds, but the standards were set based on the presence of *cis*-α-santalol and *cis*-β-santalol.

An agroforestry industry based on *S. album* was established in Sri Lanka within this decade; particularly with private sector plantations companies investing on large scale plantations. There is a growing trend among the villagers and small land owners to plant *S. album* in home gardens as timber crop (Subasinghe 2013).

It is important to scientifically investigate the presence of non-productive natural *S. album* populations in Sri Lanka, which could have provided the preparatory material for plantations. Consideration this factor our study was aimed to find the heartwood variation among the *S. album* populations distributed in Sri Lanka. The objective of this study is to identify the variation of heartwood content, oil contents and oil constituents of *S. album* trees sampled from different agro-ecological zones of Sri Lanka. These findings will be matched with physical measurements of the tree and topographical and climatic conditions of the location.

Materials and methods

Plant material and geographical data

Total of 84 trees were selected for the present study from nine distinctive sandalwood populations belonged to nine different agro-ecological zones (Fig. 1) covering all the natural sandalwood growing areas of Sri Lanka. The abbreviations for each agro-ecological region are denoted by a 3-character code. The first two upper case letters of the code denote three major climatic zones, i.e., W wet; I intermediate; D dry and three elevation categories, i.e., L low; M mid; U up respectively. The number placed after above two letters denotes the degree of wetness of the model rainfall probability regime on the scale of 1–5 (Panabokke 1996). Table 1 illustrates the rainfall and geographical variations of the selected agro-ecological regions for this present study.

Field analysis methods and regulatory requirements

Santalum album has been listed as a protected species in Sri Lanka (Fauna and Flora Protection Ordinance 2009) and therefore obtaining samples from the trees growing inside the government forests or similar lands was difficult. In addition, *S. album* trees of suitable sizes were not present in such lands due to high rate of poaching occurred for a long time. Therefore the trees naturally growing in home gardens located closer to forests or forest-like lands were used for sample collection for the present study. It was not possible to determine the age of the trees due to lack of available records. *S. album* does not produce clear growth rings and therefore ring analysis was also not

Table 1 Climatic and geographical details of the selected populations in different agro-ecological regions

Zone	Location	Annual rainfall (mm)	Terrain	Altitude (m)	No of trees sampled
DL3	Jaffna	>650	Flat	10–25	2
	Puttlam			10–25	3
WL2	Galle	>1900	Rolling, undulating	25–50	2
	Matara			25–50	3
WL4	Colombo	>1525	Undulating, flat	15–25	4
WM1	Rathnapura	>3125	Steep, hilly, rolling	425–450	4
WM3	Kandy	>1400	Steep, hilly, rolling	575–600	3
IL1	Kurunegala	>1015	Undulating, flat	150–175	11
IL3	Hambantota	>900	Undulating	15–55	13
IM3	Matale	>900	Steep, hilly, rolling	350–400	4
IU3	Badulla	>1150	Steep, hilly, rolling	900–1100	35

applicable for the age determination. Diameter at breast height (dbh) and total height of sampled trees were measured using diameter tapes and clinometers. A core sample was extracted from each sampled tree from bark-to-bark using a Haglöf increment borer. In order to obtain the maximum amount of heartwood, core samples were taken at 30 cm height from the ground level. Each tree location was recorded using a high quality GPS data receiver (Garmin Montana 650 series).

Hydro-distillation of essential oil

Dried core samples were pulverized using a bench-top grinder to particle size below 2 mm. A representative sample of 5 g was pulverized core sample was introduced to 100 mL round bottom flask with 75 mL of distilled water in an electric heating mantel. An insulated Clevenger's apparatus with a Liebig type condenser was attached and distill for 9 h (Subasinghe et al. 2013). Content at the collection arm was washed with three aliquots of 10 mL *n*-hexane and dehydrated using anhydrous sodium sulfate. Solvent was evaporated under a stream of nitrogen gas. Obtained essential oil was weighed and stored under inert headspace away from light till analysis.

Gas chromatography–mass spectroscopy analysis

Oil samples were dissolved in *n*-hexane to produce a 1 % w/v (1 mg/mL) solution and 1 μL of this solution was injected into a Gas Chromatogram (GC2010, Shimadzu Scientific, Japan) equipped with a flame ionisation detector. 95 % phenyl siloxane coated capillary column (AT-5, Alltech, USA) was used. The injector was kept at 220 °C and oven was programmed for 1 °C min^{-1} gradient from 120 °C to 180 °C. Data were processed by Labsolutions© software (Shimadzu Scientific, Japan). Compounds were identified and quantified using retention indices against internal standard l-menthol (Hettiarachchi et al. 2012; Subasinghe et al. 2013). Method limit of detection was 2.4 ng/mL and the LOD was calculated to be 48 ng/mL per each reported compound. Compounds were verified using the above column in a gas chromatogram with a mass selective detector (GC-6980 and MS-5971, Agilent Technologies, USA). Oven program and injector parameters remained the same, whereas the injector volume was reduced to 0.1 μL and the mass spectra interface was kept at 220 °C and signals were measured at m/z in 40 ms intervals using scan mode. Compounds were identified by comparing the mass fragmentation patterns with published data and online database library (NIST 5, NIST, USA; Wiley 275, John Wiley and Sons Inc, USA).

Statistical analysis

Descriptive statistics and bar charts were used to compare the differences of tested variables between different locations and trees. In addition, correlations were studied among the growth parameters, oil content and the tested constituents. Minitab® software version 14.1 was used for above analysis (Minitab Inc., USA).

Results

Distribution of dbh, height and heartwood

Colombo (WL4) and Rathnapura (WM1) respectively had the highest dbh values among the nine populations used in this study. However, populations sampled in Rathnapura and Colombo had the highest average total height respectively. Trees sampled in the populations of IM3 and WL2 had the highest heartwood contents. Although Badulla region of IU3 agro-ecological zone contain the largest number of sandalwood trees, the average dbh, height and heartwood contents were lower than that of the most of the other populations (Fig. 2). However, the trees of larger sizes, i.e., dbh > 25 cm could not be obtained for sample collection even in Badulla district due to high level of poaching. Kandy sandalwood population (WM3) had the lowest average dbh while the sandalwood populations of Hambantota (IL3) and Kurunegala (IL1) had the lowest average heights (Fig. 2).

Essential oil content

The average variation of sandalwood oil contents extracted from the sampled trees of the selected populations from the nine agro-ecological zones is given in Fig. 3. Out of 35 trees sampled in of Badulla population (IU3 region), 21 trees had oil above 2 % w/w. The highest oil content (4.58 % w/w) was recorded from the Badulla population among all the sampled trees in this study. Supporting the folk belief, trees of this region yielded the average highest oil content (2.05 % w/w). Kurunegala (IL1) population has recorded an average oil content of 1.0 % w/w. None of the other populations sampled had average oil contents higher than 1.0 %.

Chemical composition of essential oil

Variations of *cis*-α-santalol and *cis*-β-santalol of sandalwood oil extracted are illustrated in Fig. 4. According to the ISO standards (ISO 2002), *cis*-α-santalol and *cis*-β-santalol levels of *S. album* heartwood oil should be >41 % and >16 % respectively. The populations sampled in DL3, WL2, WL4, WM1 and WM3 had the average *cis*-α-santalol level above 41 % and all populations other than IL3 and IM3 regions had the average *cis*-β-santalol level higher than 16 % (Fig. 4) irrespective of the varying oil contents (Fig. 3) and tree growth parameters (Fig. 2).

Correlation between tested parameters

The analysis revealed that the oil content was significantly correlated with both tree diameter ($r = 0.323$; $p = 0.030$) and height ($r = 0.325$; $p = 0.031$). *Cis*-α-santalol was significantly correlated with the oil content ($p = 0.372$; $r = 0.000$) and *cis*-β-santalol ($r = 0.832$;

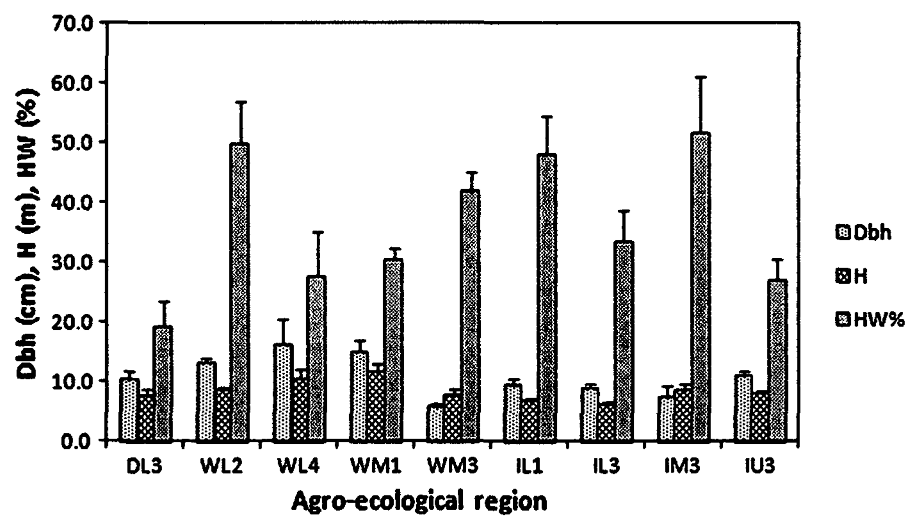

Fig. 2 Variation of dbh, height and heartwood percentage (%w/w) of S. album trees in the selected nine populations from different agro-ecological zones of Sri Lanka. Average percentage value given with ± standard error

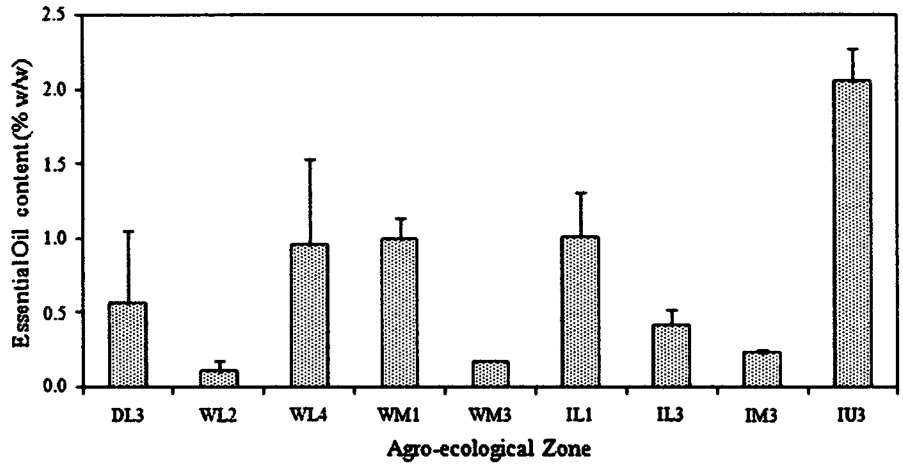

Fig. 3 Variation of heartwood oil contents (% w/w) of *S. album* trees from the selected populations of Sri Lanka. Average percentage value given with ± standard error

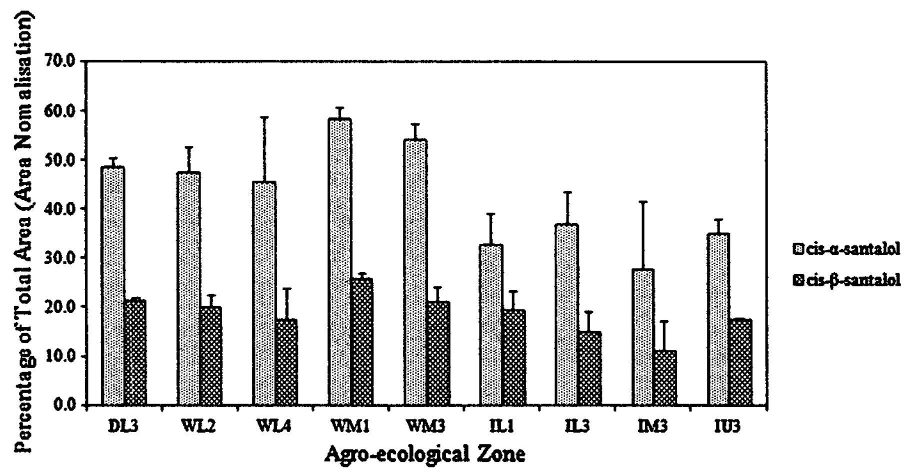

Fig. 4 Variation of mean *cis-α-santalol* and *cis-β-santalol* levels of the heartwood essential oil of *S. album* from the selected populations in Sri Lanka. Mean area percentage given with ± standard error

$p = 0.000$). While *cis*-β-santalol was significantly correlated with the oil content ($r = 0.247$; $p = 0.027$). Correlations between the rest of the tested variables were not siginificant.

Discussion

Present study used core samples for the analysis of heartwood and its constituents of *S. album* growing in the selected population in Sri Lanka due to the legal and ecological constrains of destructive sampling. Similar methods were used on *S. austrocaldonicum* heartwood analysis reported from Vanuatu (Page et al. 2006, 2010; Bottin et al. 2007). This study indicated no correlation between the heartwood content and the dbh. Conversely to the findings of this study Brand et al. have reported a correlation between dbh and heartwood content, in their studies into 16 and 14 year old *S. album* from a plantation in Western Australia (Brand et al. 2012, 2006). This study has also reported a considerable proportion of trees which have not laid heartwood at the age of 14 and 16. A comprehensive study on plantation and wild *S. album* trees by Arun Kumar et al. has reported a larger variation in heartwood content, which included several specimens without heartwood (Arun Kumar et al. 2014).

The average heartwood oil content of *S. album* from Western Australian plantation was 5 % w/w compared to a low 2 % w/w in the current study (Brand et al. 2012). Reports on wild and home garden *S. album* from different locations of Southern India have also shown a variation in oil content (Jain et al. 2003a, b) According to the studies conducted in Vanuatu have found a significant variation of heartwood essential oil concentration within and between populations with a mean value of 2.2 % across all trees sampled (Page et al. 2010).

Six trees of Badulla population of IU3 had very low contents of *cis*-α-santalol and *cis*-β-santalol levels although the oil contents of those trees were higher than 2.50 %w/w (Figs. 3, 4). One tree of Colombo population (WL4) and one tree of Kurunegala population (IL1) showed a low santalol and high oil content. Tree numbered as KG06 of IL1 region had a very low amount of *cis*-α-santalol while its oil content was 2.40 % w/w. Tree age of sandalwood can be considered as a critical factor for the quality of oil constituents (Brand and Pronk 2011; Haffner 1993). This could be the reason of having oil constituents different from the expected values in Hambantota district. It was important to notice the detection of zero or very low levels of both *cis*-α-santalol and *cis*-β-santalol of a few trees where the oil contents were comparatively much higher. This phenomenon was not previously reported nor explained in the literature.

Conclusion

Current study has emphasised on the variation of *S. album* heartwood and its essential oil composition in nine distinct geo-ecological populations in Sri Lanka. The highest essential oil content observed in this study was 4.58 %w/w and only 26 trees out of 84 had essential oil content above 2 %w/w. Current findings are in support of the folk belief, as nine trees had negligible levels of *cis*-α-santalol and *cis*-β-santalol although the oil contents of those trees were above 2 %w/w. Heartwood content did not show significant correlations with dbh, height, oil content or any the constituents. The heartwood essential oil characters of 62 % tree out of the total samples complied with the ISO standard for Indian sandalwood. Dry mountainous Badulla area (IU3) had the highest percentage of trees complying the ISO standards. However, the main concern would be the trees morphologically identified as *S. album* yet contain no santalol type sesquiterpene alcohols. It is necessary to conduct continuous research into sandalwood population in Sri Lanka preferably with parallel analysis on genetic differences. Findings of this data are vital for the emerging sandalwood plantations in Sri Lanka to identify the suitable mother trees for silviculture or tissue culture propagation.

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