

# Sandalwood Research Newsletter

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## EDITOR'S NOTE

The quality and value of sandalwood oil is typically determined by the level of  $\alpha$ - and  $\beta$ -santalol. There are various methods of extraction currently used such as solvent extraction, super critical fluid extraction, water distillation and steam distillation. Making direct comparisons between studies using different means of oil extraction can be problematic given the variable results between them. Since steam distillation is the primary method used by industry using bulk wood samples, there is a need for a standardised 'desk top' method that gives equivalent results. In this issue Hettiarachichi compares the relative merits of two widely used methods of extraction and proposes a standardised method that may be developed for use across industry to allow for more accurate comparison between studies.

With interest in improving product quality across all sandalwood markets there is also a need for rapid determination of  $\alpha$ - and  $\beta$ -santalol in both harvested logs and standing trees. This will assist sandalwood merchants in stratifying their harvest into those suitable for oil extraction and powdering for agarbatti. There is also a need for determining the levels of santalol in living trees that may contribute to future improvement programmes and cultivar development. To meet this challenge Wedding *et al.* has developed a rapid method using Near Infra-Red Spectroscopy to assess oil quality in heartwood across a large number of samples. There are many potential applications of this technology for both sandalwood science and industry alike.

Developing a sound understanding of the breeding systems for sandalwood often involves meticulous work with controlled pollination and isolation of very small and fragile flowers. Given that only a small percentage of flowers pollinated this way are useful in studies of breeding systems Shepherd has developed a laboratory-based protocol that is less painstaking to undertake, which could have implications for reproductive biologists working with sandalwood.

Tony Page

## Volatile oil content determination in the Australian sandalwood industry: Towards a standardised method

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### Abstract

Quality assurance of Western Australian Sandalwood has been identified as being important for industry. The main parameters of sandalwood timber quality are yield and quality of its oil. Sandalwood oil already has an Australian standard, but there is no specified method to determine yield. A hydro-distillation method was developed along with a simple solvent extraction method to represent the currently contemporary methods. Despite the significant time and resource consumption of hydro-distillation it was found to be a superior method of analysis since it more accurately reflects the results obtained during industrial processing. More economical and rapid methods are demanded by different sectors involved in sandalwood industry. This article is an early step in seeking the most appropriate method suited for volatile oil determination.

### Introduction

Sandalwood plays an important role in the Western Australian economy as an export commodity and involves many organisations for its large scale processing. Western Australian Sandalwood (*Santalum spicatum*) is placed fifth in the quality scale (Erligmann 2001) relating to its relatively lower percentage of santalol when compared with its Indian and Pacific cousins. While different species have different chemical composition quality also varies within species which may be influenced by factors

such as tree age, soil, climatic conditions, host vegetation, pests or other idiosyncratic causes.

Most of the sandalwood harvested in Western Australia is distilled for its oil since it has a very high perfumery value. The balance of the annual harvest is used in the incense industry and for certain other cultural uses in different parts of the world. Australian (AS 2112:2003) and international (ISO 3518:2002) standards have been drawn for the quality parameters of the oil with reference to santalol levels and physical parameters. Gas

chromatography is the preferred method of analysis to determine the  $\alpha$ - and  $\beta$ -santalol and the other sesquiterpene concentrations (Howes *et al.* 2004).

The two primary parameters determining the quality of timber will be the yield of volatile oil and the santalol content of the obtained oil. While a standard exists for oil quality, a standard method for the determination of volatile oil yield accepted by the Sandalwood industry in Australia is still required.

To study the volatile oil content of plant material there are few methods

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published in 'standards' documents. The ISO method (ISO 6571: 2008) of determining volatile content of spices and condiments shows much similarity with other widely accepted methods stated on British Pharmacopeia (Appendix XI E 2008) and AOAC international (962.17-2006). All these methods follow a similar procedure of hydro distillation with minor variations. Other methods which are commonly used to isolate the volatile oil content are solvent extraction, microwave extraction, super critical fluid extraction, dense liquid carbon dioxide extraction, solid phase micro extraction and headspace gas analysis (Min (2007); Lucchesi *et al.* 2004; Moretta *et al.* 2001).

The current study has focused on contemporary analytical methods used in the Western Australian sandalwood industry, namely hydro distillation and solvent extraction (Brand *et al.* 1999). Optimal experimental conditions and possible errors of both these methods are discussed as an initial step to develop a standard method for the industry.

## Methods

### Plant material

A root sample of *Santalum spicatum* was selected out of 54 specimens kept at Wescorp Sandalwood. Particle size was reduced with a hammer mill and separated into two samples (i) as coarse (420-4760 $\mu$ m / 4-40 mesh size) and (ii) fine (<420 $\mu$ m / 40 mesh).

### Hydro-distillation

Samples of 50g and 25g were each placed in a 1000mL round bottom flask, 600mL distilled water was added to each, and kept on an electric heating mantel set to boil (Isopad LG2ER, Tyco Thermal controls GmbH). Dean-Stalk arm (12.5mL / 0.1 mL, LAB GLASS) was fixed to the flask with a Liebig condenser (Figure 1). Temperature in the condenser was monitored by a thermocouple (HI935005, K type, Hanna) (within bottom 10cm) and water flow was controlled to maintain <80°C. Distillation operated for 18 hours, oil was collected in the Dean-Stalk arm and volume was recorded every 3hrs. Samples of same timber with different weights 15g, 25g, 40g, 50g, 75g and 100g were distilled for 9hrs to obtain their percentage yields.

Particle size	Time of Extraction	% Extract in wood	% Volatiles in wood	% Volatiles in extract	% Non-volatiles in extract
> 40 mesh	1 hr.	7.24	3.83	53	47
> 40 mesh	2 hrs.	8.08	3.39	42	58
> 40 mesh	4 hrs.	7.02	2.93	41.5	58.5
> 40 mesh	8 hrs.	7.21	2.96	41	59
> 40 mesh	24 hrs.	7.31	2.92	40	60
4 – 40 mesh	1 hr.	5.58	1.89	34	66
4 – 40 mesh	2 hrs.	5.56	1.97	35.5	64.5
4 – 40 mesh	4 hrs.	5.63	2.47	44	56
4 – 40 mesh	8 hrs.	5.80	2.38	41	59
4 – 40 mesh	24 hrs.	5.77	2.36	41	59

**Table 1.** Variations in solvent extraction yield with change to time and particle size

### Oil recovery

A similar distillation unit was employed as mentioned above, sample of wood was replaced by a Sandalwood oil samples (oil of *S.austrocaledonicum* standardised for total santalols of 33.87%). Distillation was carried out for 18 hrs, volume of oil collected in the Dean-Stalk arm was noted every three hours. This was employed to determine the recovery of volatile oil.



**Figure 1.** Dean-Stalk apparatus used for hydro-distillation of the sandalwood oil from milled heartwood.

### Solvent extraction

Five sub-samples (1g) were taken from each of the coarse and fine samples and placed in a sintered glass vial and 10mL of *n*-hexane (HPLC grade, Lab Scan, Ireland) was added and shaken intermittently. Solvent was withdrawn from one sample from each particle size after 1, 2, 4, 8 and 24 hours. Extract was filtered through a Whatman filter paper and collected to a pre-weighed vial. Hexane was evaporated under air flow for 24 hours and observed for constant weight. Dry extract obtained was weighed and dissolved in 2mL of hexane, 100 $\mu$ L was further diluted to 1000 $\mu$ L with hexane. Final concentration of the sample was measured by gas chromatography using a calibration curve.

### Gas chromatography

Shimadzu GC2010 Ver.2 instrument (Shimadzu scientific, Japan) attached to a Shimadzu AOC-20i auto sampler was used. Column used was Rtx-WAX (Restek, PA, USA) 60m X 0.25mm with 1 $\mu$ m film thickness. 1 $\mu$ L of the sample was injected as a split injection with a ratio of 10. The injector port and flame ionisation detector was kept a constant temperature of 220°C. Column was held at 100°C for 5 min and increased by 3°C/min until it attained 220°C where it maintained for 25 min. The carrier gas was helium (ultra high purity grade, BOC gas, Australia) with a linear velocity of 3mL/min.

Parameter	Hydro distillation	Solvent extraction
Sample size	40g or above	~1g
Electricity consumption	significant	nil
Water consumption	significant	nil
Time	9 hrs	4 hrs
Solvent consumption	nil	modest
Non-volatile composition	nil	~50%
Auxiliary methods	nil	separation of volatiles
Actual interpretation	appropriate	non appropriate
General experimental error	very modest	significant
Human error	modest	significant
Standard methods	many	nil
Effect to oil quality	very modest	quite significant

**Table 2.** Comparison between the hydro-distillation and solvent extraction over 12 different parameters.

### Calibration Curve

Oil obtained from hydro distillation was prepared in dilution sequence from 0.5% to 10% in 0.5% increments. Sample was analysed by gas chromatography to produce a calibration curve considering  $\alpha$ -santalol as the internal standard. This calibration curve was used to find the concentration of volatile composition of the solvent extracts. Auto generated calibration curve (GC Lab Solutions, Shimadzu Scientific, Japan) was manually verified using Microsoft Excel<sup>®</sup>.

## Results

### Distillation time

An progressive increase in oil yield was observed in all timber and oil distillation experiments from 3 to 9 hours. No increase in oil yield was measured after distillation from 9 to 18 hours (Table 1). These results were reproduced in a second replicated experiment. Using

the described method it is therefore proposed that hydro-distillation for 9hrs is sufficient to separate the volatile oils from timber. All the experiments hereafter were performed only for 9hrs. Shorter distillation time is beneficial to the industry, as it not only consumes less resources but it also manages to provide a result within a quicker time frame.

### Sample weight and particle size for distillation

Different sample weights of the same timber were distilled for 9 hours and the oil yield estimate was highest in the 15g followed by the 25g sample. All three oil yield estimates for the 40, 50 and 75g samples were the same (Figure 2). The 100g samples and samples of fine particle size (<420  $\mu$ m) samples exhibited irregular particle movement and colliding, hence the experiments were terminated to avoid any accident. With the above

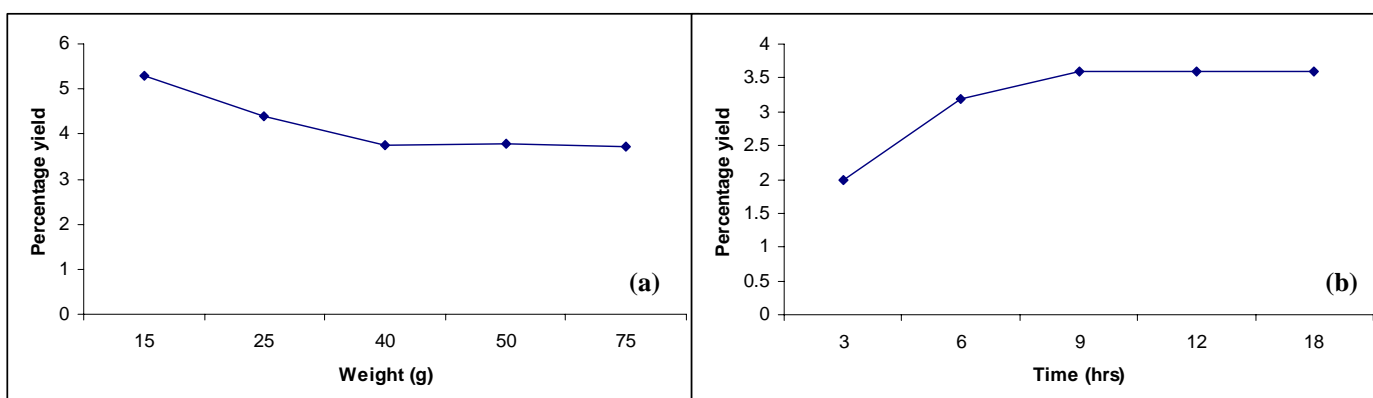
results 50g coarse particle sample was identified as an appropriate standard for a 1000mL capacity distillation vessel to perform hydro distillation.

### Solvent extraction

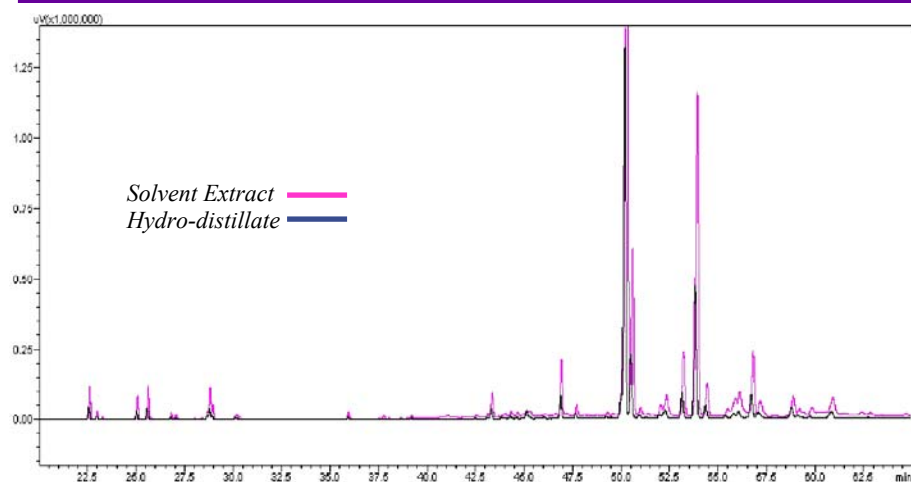
Solvent extraction with hexane has shown greater variation with the time and particle size (Table 1). Solvent extracts consist of both volatile oil and non-volatile hydrocarbon soluble compounds such as waxes and lignans. The non-volatile component of solvent extracts can comprise more than 50% of the extract, which contrasts with the hydro distillation method where very little non-volatile components. Calculated percentage yield for solvent extraction varies nearly 2% v/w, which is inadequate for estimating yield of an industrial sample. Solvent extraction estimate is closest to steam distillation is when a coarse sample of 4760-420 $\mu$ m (4-40 mesh) used and extracted with hexane for 4 hrs in a solvent to sample ratio of 1:10. Future research can find a better suitable particle size, solvent, ratio and an extraction time.

### Gas chromatography

An increase in the oil percentage was observed with solvent extraction compared with hydro distillation, since more compounds were extracted thus reducing the percentage of total santalol content. Chromatogram of the solvent extract showed higher concentration of non-polar minor compounds due to higher extraction capacity and is substantially different the chromatogram of the hydro distilled sample (Figure 3). The misinterpretation of quality using solvent extraction is disadvantageous to the industry. These results support the findings of Piggott *et al.* (1997) which revealed that steam distillation yields fewer



**Figure 2.** Hydro-distillation volatile oil yield with (a) increasing time and (b) sample weight



**Figure 3** Gas chromatogram of the extracts obtained from the two methods.

extracts but consists of a higher percentage (>90%) of volatile oils compared with solvent extraction. Sesquiterpene analysis of the above study revealed that the percentages of major compounds in solvent extracts are nearly half of those found in the distilled oil.

#### Discussion

Hydro distillation is a time and resource consuming method, it is also necessary to have a much larger sample for analysis than solvent extraction. For more accurate measurement of oil yield a v/w% can be replaced by w/w% where the yielded oil will be dried and weighed. The ISO method can also be practiced followed by drying and evaporation of solvent used in oil recovery. Possible experimental error or the likelihood of human error is very high in solvent extraction method when compared to hydro distillation, a minor fault can cause a significant difference as many factors will affect the final results such as temperature, sample size, solvent ratio and frequency of particle movement where as in hydro distillation there is a broader window for experimental error (Table 2).

In this study total volatile composition of the solvent extract was determined using a calibration curve, in a routine experiment an external standard can be used instead of the internal standard method used in this study, which again is subject to human error. A more accurate method is needed to separate the volatile from the non-volatile compounds in an extract, such as headspace analysis, liquid chromatography or partial distillation analysis. These methods can verify the

volatile composition accurately, but a possible drawback will be the cost effectiveness of the instrument.

#### Conclusion

Steam distillation is the most widely accepted commercial method for sandalwood oil extraction. Any 'bench top' analysis should therefore closely match the commercial yield rather than reporting total volatiles or extractable matter. Analysis of smaller samples from living trees provided by the plantation and forestry sector cannot practically use steam distillation method. Researchers and process operators need to continue to develop cost effective and rapid new methods for utilising small test samples to accurately estimate commercial yield and quality. Even though such a method may be developed in future, proper quality management must be enforced to minimise the experimental error. Until a generally accepted method is developed for sandalwood industry the findings of this study suggest the continued use of the standard method of hydro distillation. This conclusion can be justified, as the hydro distillation is the closest bench top method to the industrial process and less affected by experimental error when compared to solvent extraction. Hydro distillation has already been used in different standard documents in the pharmaceutical, flavour and fragrance industries, and is inarguably the most accepted method despite its high consumption of resources and time.

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