Comparison of Oil Recovered from Tea Tree Leaf by Ethanol Extraction and Steam Distillation

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Two methods for the determination of oil and oil major components from tea tree (*Melaleuca alternifolia*) leaf are quantitatively compared. A microwave assisted ethanol extraction and a 2-h hydrodistillation technique were used on both dry and fresh leaf from a low and a high oil concentration tree. There was no significant difference between dry and fresh leaf. The distillation technique recovered 88% and 82% of the extractable oil for the low and high concentration material, respectively. For both samples this distilled oil was composed of lower absolute amounts of sesquiterpenoids and marginally lower amounts of monoterpenoids. Extending the distillation to 6 h increased the sesquiterpenoid recovery but this resulted in a reduction in both the absolute and relative amounts of the oxygenated monoterpenoids, terpinen-4-ol and 1,8-cineole.

Australian tea tree oil is a popular medicinal oil obtained from the subepidermal oil glands in leaves of *Melaleuca alternifolia* (Maiden and Betche) Cheel by steam distillation or hydrodistillation (Southwell and Lowe, 1999).

Steam distillation is the technique employed to commercially process tea tree for oil production. Although steam is usually generated from an external boiler, hydrodistillation, where leaf material is immersed in or above boiling water, is also used. Gas chromatography (GC) is used to determine the contribution of individual components in the oil.

In his research on the theories of essential oil distillation, Von Rechenberg (1910) demonstrated the early appearance of oxygenated components with the distillation of oils from intact plant material. This was explained by hydrodiffusion (the diffusion of the aqueous solution through the cell membrane), rather than boiling point and was proposed as the rate-determining step in the distillation. Von Rechenberg (1910) also found that it was not possible to recover 100% of oil from a plant sample by distillation. He concluded that some volatiles were retained by their affinity to nonvolatile lipids. This was confirmed by Koedam et al. (1979) who extended distillations for 24 h but found that some hydrocarbon fractions of the volatile oil were not recovered.

Previous researchers have documented the losses and artifact formations associated with the distillation of essential oils. While Koedam et al. (1980) could not recover all of the oil from cypress leaf (*Abies x arnoldiana* Nitz) with 18 h of distillation, they recovered the remaining oil (sesquiterpenes) by grinding and redistilling the spent leaf. During the distillation of tea tree, the oil extracted changes composition as components are recovered at different rates (Southwell, 1988; Brophy et al., 1989; Johns et al., 1992; Stiff, 1995). Increasing the distillation time increases the proportion of sesquiterpenoids at the expense of the oxygenated monoterpenes. In line with Von Rechenberg's hydrodiffusion

theories, the oxygenated components, particularly terpinen-4-ol and 1,8-cineole, extract faster despite their higher boiling points. Johns suggests that their recovery is mass-transfer film controlled, whereas the components extracted later (monoterpenes and sesquiterpenes) are controlled by diffusion. An increased resistance to diffusion by these components is attributed to the hydrophobic properties of the monoterpenes plus the larger molecule size of the sesquiterpenes (Johns et al., 1992). In addition, the flush leaf precursors, sabinene, *cis*-sabinene hydrate, and *trans*-sabinene hydrate, are thermally transformed to terpinen-4-ol, α-terpinene, and γ-terpinene with distillation (Southwell and Stiff, 1989).

Solvent extraction is an alternative method for removing oil from the leaf. The addition of a known weight of internal standard enables the determination of oil concentration in leaf by GC analysis. Solvent extraction is not reliant on component volatility nor are the labile precursors in flush leaf exposed to prolonged thermal conversions. Polar components of oils are also partially water soluble to various extents and not fully recovered from the distillation process. The solvent extraction technique can be streamlined for multiple samples and sample size can be reduced to less than 1 mg of plant material (Stiff, 1995). Taskinen (1974), however, investigated the alcoholic extraction of sweet marjoram and found that monoterpenoids represented a lower proportion of the total than they did in the steam distilled product. Other authors (Boland et al., 1982; Weston, 1984) have suggested that solvent extraction is preferable to steam or hydrodistillation in providing a better indication of the components present in the plant.

A microwave-assisted dry method for extracting essential oils was described by Craveiro et al. (1989). The oil produced from *Lippia sidoides* was qualitatively similar to the steam distilled oil but significantly different quantitatively. Stiff (1995) and Southwell et al. (1995) have used microwave-assisted solvent extraction for the rapid GC analysis of tea tree leaf samples down to 1 mg. They found that 10 s of microwaving

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Table 1. Mean Yield (mg/g) of Total Oil and Selected Constituents (Parentheses Indicate GC %) for Ethanol Extraction and Steam Distillation from Low and High Oil Leaf

method	leaf-type	monoterpenoids			sesquiterpenoids	total oil
		1,8-cineole	terpinen-4-ol	total		
extraction	low oil	1.2 (2.5)	18 (38)	36 (75)	10 (21)	48
distillation (2 h)	low oil	1.1 (2.6)	16 (38)	34 (81)	6 (14)	42
distillation (6 h)	low oil	0.8 (1.9)	13 (31)	31 (74)	10 (24)	42
extraction	high oil	2.9 (3.8)	31 (40)	59 (81)	16 (20)	77
distillation (2 h)	high oil	2.6 (4.1)	30 (44)	57 (94)	5 (7)	63
distillation (6 h)	high oil	2.2 (3.3)	27 (41)	54 (82)	10 (15)	66

reduced extraction time for tea tree leaf from 30 h to 1 h. They also examined the potential for a microwave pretreatment to alcohol extraction of tea tree and concluded that 30 s of microwaving reduced the required ethanolic extraction time and produced oil most closely reflecting the oil within the leaf. In a second study Baker and Stiff (1995) found that for air-dried tea tree leaf (1 g) the optimum time for microwave initiated ethanolic extraction was 3 days.

Tea tree leaf drying (Murtagh and Curtis, 1991) and maceration (Johns et al., 1992) do not appear to affect the yield or composition of distilled oil. To our knowledge similar studies on tea tree leaf for solvent extraction have not been reported. This paper validates a solvent extraction method for a tea tree breeding project by comparing quantitative and qualitative results for fresh and dry leaf with conventional hydrodistillation yields and composition data.

MATERIALS AND METHODS

Sample Preparation. The two methods were compared on leaf from two mature tea trees of different oil concentrations. Twigs were sampled to avoid flush leaf from both a low (<50 mg/g) and a high (>60 mg/g) oil concentration tree. These twigs were cut and separated into fine twiglets (<2.5-mm stem diameter), which were then chopped into lengths less than 30 mm, bulked, mixed, and divided into 18 low concentration and 22 high concentration 10-g samples. Eight low concentration and nine high concentration fresh samples (approximately 50% dry matter (DM)) were stored at 5 °C in airtight plastic bags until required for distillation. The remaining 23 samples (10 low and 13 high concentration) were air-dried (approximately 90% DM) for a minimum of 5 days in paper bags prior to extraction. Samples were then distilled or partitioned into leaf and fine stem for solvent extraction of the leaf and mean yields calculated.

Dry Matter and Percentage Leaf Determination–Steam Distillation. At the time of distillation, dry matter was determined by drying replicate 10-g samples of the fresh and air-dried material from both trees in an oven at 60 °C for 2 days. The dried samples were then separated into leaf and stem and redried and the components weighed. The percentage leaf on a dry matter basis was then calculated.

Leaf Separation and Dry Matter Determination–Solvent Extraction. The fresh and air-dried samples were separated into leaf and stem. A 1-g equivalent of dried leaf was placed in a McCartney bottle for extraction and the remaining leaf was dried (60 °C for 2 days) to determine dry matter content.

Steam Distillation. A bank of eight glass microdistillation units was used. Heating was achieved with a LPG manifold and microburners with individual controls. Glassware consisted of 250-mL round-bottom flasks, 200-mm water condensers, and 250-mm calibrated collectors. Tubing between the flask and the condenser was insulated to prevent condensation back into the flask. The 10-g samples were distilled from 60 mL of water at the rate of 2 mL/min with the first condensate appearing 3–5 min after initial heating. Condensate water was cohobated back to the distillation flask and distillation con-

tinued for 2 or 6 h from the time of first condensate appearance. On completion of the distillation, the oil (0.05–0.30 mL) was transferred from the collector to a McCartney bottle by three 1-mL washings with *n*-hexane. To provide an internal standard for the GC analysis, a solution of tridecane in ethanol was quantitatively prepared to give a concentration of approximately 2 mg per gram. The use of this internal standard enabled GC area percentages to be expressed as absolute concentrations of volatiles in the leaf (mg/g). For each 0.1 mL of oil, approximately 8 g of internal standard solution was added accurately by weighing. The final solution containing both hexane/oil mixture and the internal standard was thoroughly shaken and a 2-mL subsample quantitatively analyzed by GC.

Solvent Extraction. Leaf samples (1 g) were extracted in ethanol (12 mL) with internal standard (0.22% tridecane). The capped samples were then microwaved for 25 s and left to stand for 3 days, enabling full oil extraction. Bottles were shaken and 2 mL of solution withdrawn and pipetted into a vial for GC analysis.

Gas Chromatographic Analysis. Oil was analyzed on a Hewlett-Packard 5890A gas chromatograph, with a 3390A integrator, an Alltech AT 35 column (60 m × 0.25-mm i.d.), and a flame ionization detector operating at 300 °C. A 1-μL sample was injected at 200 °C. Hydrogen was used as the carrier gas (40–50 cm³/min) with an oven temperature program of 10 °C/min from 50 °C (1 min) to 250 °C (4 min). Major components were identified by comparing retention times against laboratory supplied Aldrich/Sigma pure components. Monoterpenoid and sesquiterpenoid recoveries were based on a summation of the peak percentages from their respective chromatogram regions, which are clearly separated on the intermediate polarity AT35 stationary phase. Response factors for tea tree oil, five major components, and the tridecane internal standard were calculated by determining the peak area to oil weight ratio (Grob and Kaiser, 1982) over four concentrations and fitting a linear response line. Relative response factors (RRF) for the oil and major components were then calculated against the internal standard and applied to the calculations of final oil and component concentration.

RESULTS AND DISCUSSION

No significant difference in the quantity or quality of oil extracted from fresh (approximately 50% DM) and air-dried leaf (approximately 90% DM) sampled from either low or high oil concentration trees was found. The fresh and air-dried results were averaged and the mean yields for oil, monoterpenoid, sesquiterpenoid, 1,8-cineole, and terpinen-4-ol contents are shown in Table 1.

Ethanol extraction gave 48 and 77 mg of oil/g of leaf for the low and high oil concentration trees, respectively. This was 14 and 22% higher, respectively, than the amounts distilled in 2 h from the same trees. Total amounts of monoterpenes extracted with ethanol were 4–6% higher than those recovered from the 2-h distillation with 36 and 34 mg/g for the low oil concentration tree and 59 and 57 mg/g for the high oil concentration tree, respectively. The sesquiterpenoid recovery was greater by extraction than 2-h distillation. For the low

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oil concentration tree, ethanol extracted 10 mg/g compared to only 6 mg/g recovered from the distillation. For the high oil concentration tree the difference between methods was greater, with 16 mg/g extracted and distillation recovering only 5 mg/g. The higher concentrations obtained by extraction reflected both enhanced levels of those volatiles common to both methods as well as the presence of nonsteam distillable volatiles detected by GC.

In the high oil leaf distillation, a lower sesquiterpenoid region component recovery (5 mg/g or 7%) was balanced by a higher terpinen-4-ol concentration (30 mg/g or 44%). In contrast, leaf extraction gave more sesquiterpenoid region components (16 mg/g or 20%) and an increased terpinen-4-ol concentration (31 mg/g), which contributed a decreased proportion (40%) to the total extract. Hence the enhanced yield from the solvent extraction was reflected in lower proportions of key monoterpenoid constituents.

The difference in the sesquiterpenoid recovery is the main cause of the quantitative difference between the two methods. When distillation time is extended to 6 h, sesquiterpenoid recovery is increased from 6 to 10 mg/g in the low oil concentration leaf type and from 5 to 10 mg/g in the high oil concentration leaf type (Table 1). However, by increasing distillation time, the absolute monoterpenoid recovery is reduced, with 1,8-cineole reduced from 2.6 to 2.2 mg/g and from 1.1 to 0.8 mg/g in the high and low oil leaf types, respectively. Likewise the terpinen-4-ol levels were reduced from 30 to 27 and 16 to 13 mg/g. These reductions are attributed to the dissolution of the more hydrophobic isolates in the increased volumes of condensate.

These findings were consistent with earlier findings on fresh tea tree leaf (Stiff, 1995), where steam and 2.5-h hydrodistillation extracted 80 and 89%, respectively, compared to alcohol extraction. The distilled oil also contained lower levels of sesquiterpenoids with a higher terpinen-4-ol and marginally higher 1,8-cineole level than the extracted oil. This reduced sesquiterpenoid recovery with distillation and the differences between the 2- and 6-h distillation recoveries from our study (Table 1) emphasize that the oil derived from distillation is dependent on the distillation time and conditions.

Both techniques are appropriate for tea tree analysis. Steam distillation delivers an oil equivalent to the commercial product. Solvent extraction delivers oil most closely matching the in-situ leaf oil composition. When results are expressed on a percentage basis, components such as terpinen-4-ol and 1,8-cineole may vary depending on the technique used and, in particular, the total amount of sesquiterpenoids included in the recovered oil. In projects such as the tea tree breeding project where large numbers of samples are analyzed, the solvent extraction method is expedient. However, routine "spot" comparison between the two procedures is a necessary component of method validation. Also, it is important that the differences outlined above between the oil resulting from solvent extraction and oil resulting from the generally accepted industry production technique of steam distillation are understood.

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