Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1028 (2004) 307–312
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Evaluation of the quality of sandalwood essential oils by gas chromatography–mass spectrometry

Melanie-Jayne R. Howes, Monique S.J. Simmonds*, Geoffrey C. Kite

Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK

Received 7 October 2003; accepted 12 November 2003

Abstract

Trade and historic oils from 'sandalwoods', labelled as *Amyris balsamifera*, *Eremophila mitchelli*, *Fusanus acuminatus* (= *Santalum acuminatum*), *Santalum album*, *S. austrocaledonicum*, *S. latifolium*, *S. spicatum* and *S. yasi*, were assessed using gas chromatography–mass spectrometry (GC–MS). Using GC–MS, none of the oils assessed complied with the internationally recognised standard of a 90% santalol content, and only about half of the trade sandalwood oils met with recent International Organisation for Standardisation standards. The majority of trade oils, reportedly from *S. album*, contained approximately 50–70% santalols (*Z*-α and *Z*-β). Thus, the internationally recognised specification (90% santalols) for *S. album* requires re-evaluation by more efficient analysis methods. In view of the issues associated with the quality of sandalwood oils being traded, specifications of ≥43% *Z*-α-santalol and ≥18% *Z*-β-santalol for *S. album* oil estimated by GC–MS are suggested. GC–MS are recommended as it assists with authentication and quality control issues associated with sandalwood oils.

Keywords: Essential oils; *Santalum* spp.; *Amyris balsamifera*; *Eremophila mitchelli*; *Fusanus acuminatus*; Sandalwood; Santalols; Volatile organic compounds

1. Introduction

Sandalwood oil is widely used in the cosmetic, perfumery and aromatherapy industries. The most renowned sandalwood oil is distilled from the sandalwood tree *Santalum album* L. (Santalaceae) and the oil is the subject of international concern regarding its sustainability and quality. There has been a serious decline in the population of *S. album* in India [1,2] due to complex cultivation requirements and continuous harvesting (particularly from smuggling), combined with limited regeneration. As a result the quality of sandalwood oil entering the trade may be compromised, not only due to the declining numbers of trees with developed heartwood (trees of at least 20–25 years old), but also due to adulteration to compensate for the restricted, and thus increasingly expensive, Indian *S. album* oil. In view of the current issues associated with sandalwood, an investigation was conducted to develop an appropriate method to assess the quality of sandalwood essential oils being traded in the UK.

Numerous quality control issues are associated with sandalwood. At present, no standard method is available to determine adulteration of sandalwood oil, or to aid the identification of the species from which the oil was obtained. This is a particular problem as the classification of sandalwood is complex; over 100 taxon names have been published in the genus *Santalum*, probably representing about 25 species, also species in other families (e.g. *Amyris balsamifera* L., Rutaceae) are traded as 'sandalwood' [3]. In addition, *S. album* wood may be substituted with other species [2] or the oil may be adulterated with synthetic or semi-synthetic substitutes such as Sandalore® [3–5]. Substitution and/or (semi) synthetic additives would influence the chemical composition and physical properties of the oil; these factors may affect oil quality and the allergenic potential. Reported non-synthetic adulterants include castor oil, cedarwood oil and oils from 'sandalwood' species other than *S. album* [3,6].

It is recommended that the essential oil from *S. album* should not contain less than 90% w/w of (free) alcohols, calculated as santalols [6–11]. The methods described to assess the santalol content of sandalwood essential oil generally lack specificity and accuracy. For example, the acetylation methods [9,10,12,13] are not specific for the santalols, which

* Corresponding author. Tel.: +44-208-332-5328; fax: +44-208-332-5340.
E-mail address: m.simmonds@rbgkew.org.uk (M.S.J. Simmonds).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.11.093

have been associated with the quality of sandalwood, and do not distinguish among the santalol isomers. Thus, adulteration of sandalwood may be undetected via these methods if some other hydroxylated compounds are amongst the adulterants. It has been suggested that sandalwood oil should be evaluated using gas chromatography (GC) by quantitation of the santalol content, with a proposed range of 40–55% for α-santalol and 17–27% for *Z*-β-santalol [14]. More recently the ISO (2002) has included GC analysis of *S. album* oil and this specifies similar proportions of *Z*-α- and *Z*-β-santalol, 41–55% and 16–24%, respectively [13]. However, these reports do not discuss potential variation in *S. album* oil composition, depending on origin or age, nor do they address the detection of adulterants or the evaluation of other sandalwood oils. These factors are of particular concern if we are to be able to both evaluate the quality and trace the source of the sandalwood being traded. The aim of this study was to develop and evaluate gas chromatography–mass spectrometry (GC–MS) analysis as a means to assess the quality of sandalwood, to distinguish between the different species traded as sandalwood, and to detect adulterants.

2. Experimental

2.1. Samples

Thirty-eight trade samples of sandalwood essential oils were analysed, including 31 oils claimed to be from *Santalum album* L. from India (voucher numbers: BI 10093-6, BI 10106, BI 10132, BI 10168, BI 10172-3, BI 10179-80, BI 10183, BI 10186, BI 10190, BI 10201-2, BI 10206-7, BI 10212, BI 10270-72, BI 10275-76, BI 10279, BI 10287-89, BI 10307, BI 10579) and Indonesia (voucher number: BI 10210), as well as oils from *Amyris balsamifera* L. from India (voucher number: BI 10282), *Eremophila mitchelli* Benth. from Australia (voucher number: BI 10283), *S. austrocaledonicum* Vieill. from New Caledonia (voucher numbers: BI 10277, BI 10280) and *S. spicatum* (R.Br.) A.DC. from Australia (voucher numbers: BI 10211, BI 10281, BI 10580). These samples were obtained from 25 UK companies trading in sandalwood.

Sixteen historical sandalwood oils were obtained from the Royal Pharmaceutical Society of Great Britain (RPSGB) collection (Royal Botanic Gardens, Kew, UK), some dated between 1880 and 1891. Ten of these samples were labelled as *S. album* from Brazil (voucher number: BI 10218), from India (voucher numbers: BI 10221-25, BI 10227-29) and from Madagascar (voucher number: BI 10226). One of these samples was labelled as *Santalum yasi* from Fiji (voucher number: BI 10220). Two samples were labelled as *Eucarya spicata* and *Fusanus spicatus* (voucher numbers: BI 10214, BI 10216, respectively) from Australia. *Eucarya spicata* (R.Br.) Sprague & Summerh. and *Fusanus spicatus* R.Br. are synonyms of *Santalum spicatum* (R.Br.) A.DC. One sample was labelled as *Fusanus acuminatus* (voucher number: BI 10215) from Australia. *Fusanus acuminatus* R.Br. is a synonym of *Santalum acuminatum* (R.Br.) A.DC. One sample was labelled as *Santalum latifolium* (voucher number: BI 10219) and another was an unknown species of sandalwood (voucher number: BI 10217), both labelled as originating from Australia. The sample labelled as *S. latifolium* was dated 1891. Apparently, the only legitimate publication of this name was by Meurisse in 1892 [15]; *S. latifolium* Meurisse is synonymised with *S. paniculatum* Hook. & Arn. [16].

2.2. Chemicals

Solvents (diethyl ether: stabilised with copper gauze) were obtained from Fisher, UK; santalols (mixture of α and β) were obtained from Aldrich, UK.

2.3. GC–MS parameters

The GC–MS system was a Perkin-Elmer AutoSystem XL GC coupled to a Perkin-Elmer TurboMass MS (quadrupole). Chromatography was performed on either a 30 m × 0.25 mm i.d. × 0.25 μm ZB-WAX column (Phenomenex, UK) using an oven program of 40–200 °C at 2 °C/min, or a 30 m × 0.25 mm i.d. × 0.25 μm DB-5MS column (J. & W. Scientific, USA) using an oven program of 40–220 °C at 3 °C/min. In both cases the carrier gas was helium at a flow rate of 1 ml/min and 1 μl injections (split 1:10) at 220 °C were made by an autosampler. Detection was by flame-ionisation detection (FID: 250 °C, 45 ml/min H_2, 450 ml/min air) or MS. The MS was fitted with an EI source operated at 70 eV with a source temperature of 180 °C, and mass spectra were recorded in the range *m/z* 38–300 at 1 scan/0.75 s. The software was Turbomass, version 4.1.1.

Oils were diluted to 1.0% (v/v) with diethyl ether prior to analysis. Retention indicies were calculated against an *n*-alkane series (C10–C44, Supelco, UK) and compounds were identified using authentic standards or by comparing retention indices and/or mass spectra with published data [17–19].

3. Results and discussion

Two GC column phases were evaluated for sandalwood oil analysis. The santalols showed superior chromatographic peak shape on the polar ZB-WAX column, compared to the non-polar DB-5MS column, allowing baseline resolution of the santalols to be obtained using a shallow temperature gradient. Lengthy chromatography of the santalols produced unacceptable peak trailing on the DB-5MS column and so a steeper temperature gradient was necessary; baseline resolution of the santalols was not achieved (Fig. 1). Thus, the ZB-WAX column was deemed more appropriate for the analysis of sandalwood oils. To check the linearity of the MS detector response, oils were analysed at 0.1, 0.2, 1.0, 2.0 and

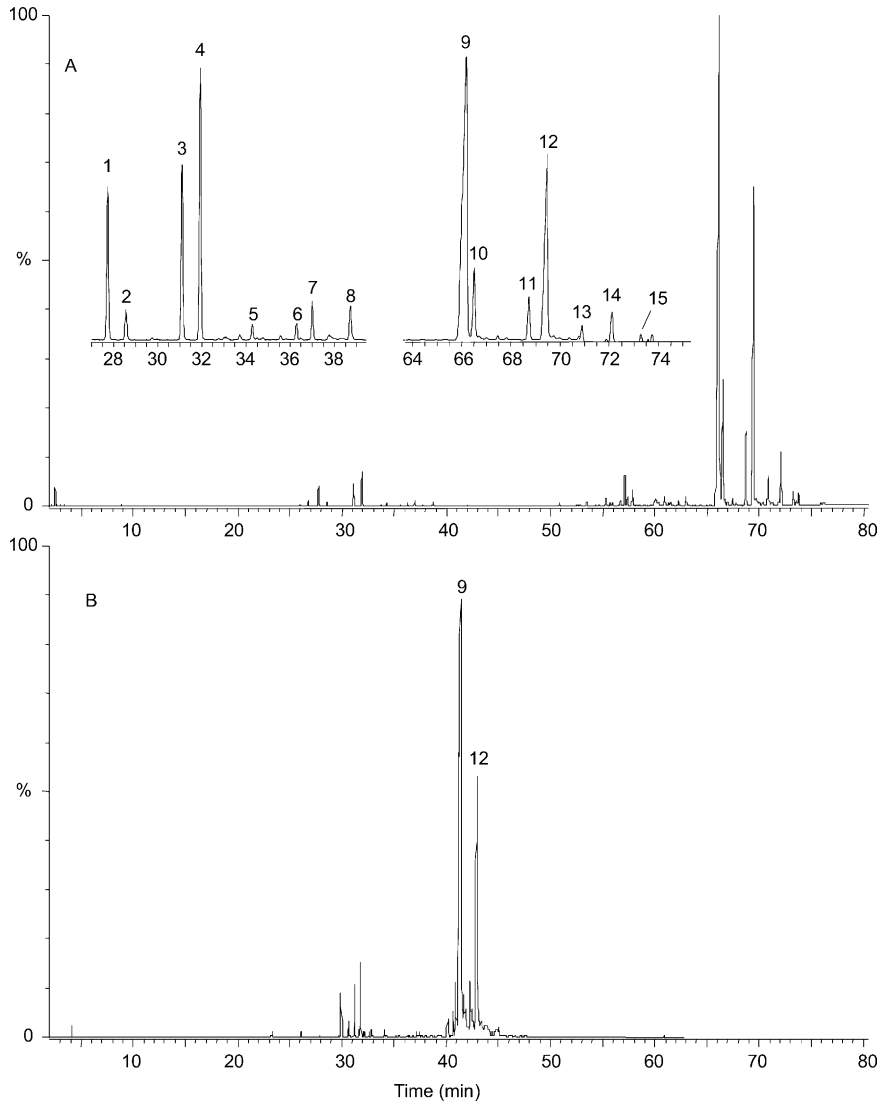

Fig. 1. GC–MS total ion chromatograms of a trade *Santalum album* essential oil using a 30 m × 0.25 mm i.d. × 0.25 μm ZB-WAX column (A) and a 30 m × 0.25 mm i.d. × 0.25 μm DB-5MS column (B). Peak identification: 1, α-santalene; 2, α-bergamotene; 3, *epi*-β-santalene; 4, β-santalene; 5, γ-curcumene; 6, β-bisabolene; 7, β-curcumene; 8, α-curcumene; 9, *Z*-α-santalol; 10, *Z*-α-*trans*-bergamotol (α-bergamotenol); 11, *Z*-*epi*-β-santalol; 12, *Z*-β-santalol; 13, *E*-β-santalol; 14, *Z*-lanceol; 15, *Z*-nuciferol.

10.0% (v/v); the standard errors for the percentage composition (obtained by integration of total ion chromatograms) of *Z*-α- and *Z*-β-santalol ranged from 0.17 to 0.39 and 0.15 to 0.29, respectively. For oils analysed at 1.0% (v/v) ($n = 6$ for each oil analysed) the standard errors for the percentage composition of *Z*-α- and *Z*-β-santalol ranged from 0.09 to 0.13 and 0.05 to 0.15, respectively.

Detection by FID and MS was also assessed specifically to compare the percentage composition estimates of the santalols in the essential oil obtained by integration of FID and total ion chromatograms. Estimates of santalol content (*Z*-α- and *Z*-β-santalol) differed by an average of 2.5% between the two detection methods. GC coupled with FID is included in official Pharmacopoeial methods for the analysis of a variety of essential oils [20–22]. However, the similar santalol composition estimates obtained by MS detection show that this is also appropriate and has the advantage of assisting with the identification of adulterants and oil substitution by comparison of mass spectra with published data [17–19].

The percentage composition of santalols (*Z*-α and *Z*-β) in the essential oils from 31 trade samples reported, or assumed by traders, to be *S. album* oil, are given in Fig. 2. When estimated by GC–MS, none of the samples met with the specified 90% santalol content for *S. album* and only approximately half (17 oils) met the ISO (2002) specifications for both *Z*-α- and *Z*-β-santalol; 18 oils met the ISO (2002) specification for *Z*-α-santalol, and 24 trade oils met the ISO (2002) specification for *Z*-β-santalol. Some of the oils contained only slightly lower levels of *Z*-α- and *Z*-β-santalol and may have met the ISO (2002) specifications with integration of FID chromatograms. Some sandalwood oils being traded contained relatively low levels of the *Z*-α and *Z*-β-santalols

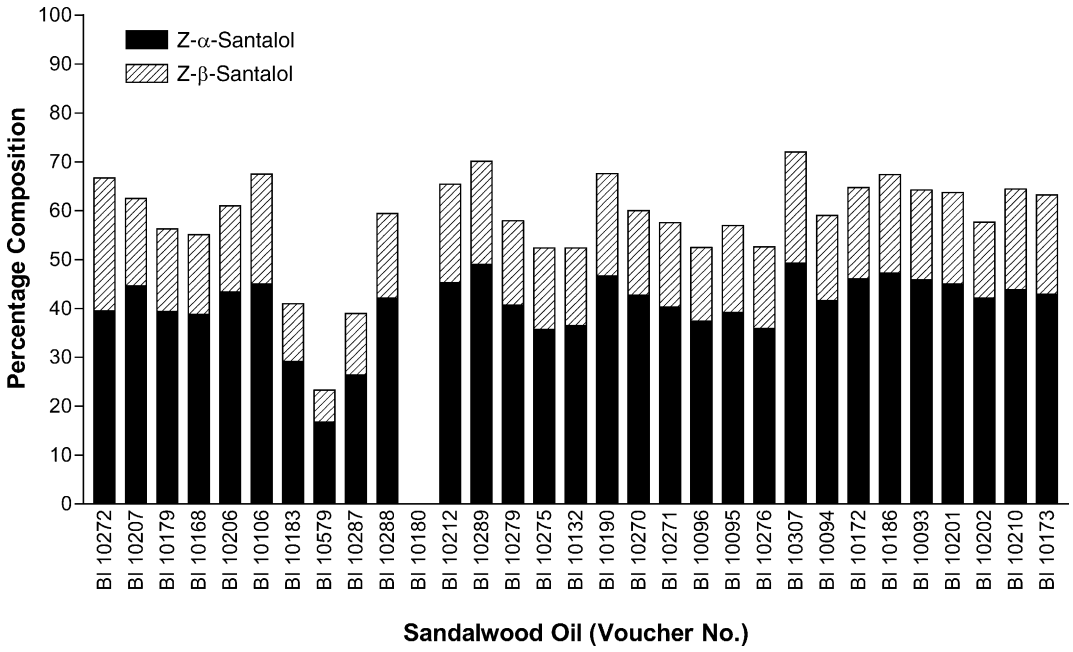

Fig. 2. Percentage composition of *Z*-α- and *Z*-β-santalols in trade sandalwood essential oils (claimed to be from *Santalum album*); results determined using GC–MS.

(four samples were composed of <50% total santalols), including one sample (BI 10180) in which no santalols were detected (Fig. 2). Other santalols detected in the '*S. album*' trade samples were *E*-β-santalol and *Z-epi*-β-santalol; excluding BI 10180, the percentage composition detected in these oils was 0.3–1.8 and 1.4–5.4%, respectively. Peak identification typical for *S. album* oil is shown in the GC–MS chromatogram in Fig. 1.

Of the 10 historical (RPSGB collection, Kew) sandalwood oils reported to be *S. album*, 8 met the ISO (2002) specification for *Z*-α-santalol, but only 5 samples met the specification for *Z*-β-santalol and only 4 samples met specifications for both *Z*-α- and *Z*-β-santalol. The results also showed that the historical samples recorded as *S. album* were similar in chemical composition to the current trade samples of *S. album*, except for one historical sample (BI 10226) which was composed of <2% santalols (Fig. 3). Thus, issues such as correct taxonomy and adulteration of sandalwood may also have been problematic in the past.

Two trade samples (BI 10280, BI 10277 (Fig. 3)) of *S. austrocaledonicum* oil were assessed; both met the ISO (2002) specifications for *S. album*. Thus, *S. austrocaledonicum* (and also *S. album* from Indonesia (BI 10210)) may be an alternative source for 'sandalwood' oil, given the similar chemical composition to the (east) Indian *S. album* oils. However, further analyses of verified plant material and monitoring of the sustainability of these species would be necessary to support their use as alternatives to Indian *S. album*. Three oils, BI 10211, BI 10281 (Fig. 3) and BI 10580, from another source of 'sandalwood', *S. spicatum*, did not meet the specifications (ISO, 2002) for *S. album* and were composed of 17.2–18.7 and 6.6–7.3% of *Z*-α- and *Z*-β-santalol, respectively. Thus, Australian *S. spicatum* does not appear to be an appropriate substitute for *S. album*. One oil traded as 'sandalwood' (BI 10579) was composed of 16.7% and 6.6% of *Z*-α- and *Z*-β-santalol, respectively, a chemical profile more typical of *S. spicatum* than *S. album*. In addition, santalols could not be detected in a trade 'sandalwood' oil (BI 10283) from Australian *Eremophila mitchelli* (Myoporaceae). Thus, there appears to be some confusion in the trade in the classification and chemical differences between 'sandalwoods' obtained from different sources. Five historical sandalwood oils (RPSGB collection, Kew) originating from Australia were also subjected to analysis. Of the two historical samples labelled as *Eucarya spicata* and *Fusanus spicatus* (BI 10214, BI 10216, respectively), neither met the ISO (2002) specifications for *S. album* oil, and showed santalol levels (17.0%, 23.4% *Z*-α-santalol and 5.5%, 9.1% *Z*-β-santalol) consistent with those detected in the trade *S. spicatum* oils.

No santalols were detected in the historical sample labelled as *F. acuminatus* oil (BI 10215); the sample labelled as *S. latifolium* oil (BI 10219) was composed of 33.6% and 9.8% of *Z*-α- and *Z*-β-santalol, respectively; a historical sample labelled as *S. yasi* oil (BI 10220) contained 36.9% *Z*-α-santalol and 26.5% *Z*-β-santalol. These species are not major sources of sandalwood oils currently being traded, and their chemical profiles indicate that they would not be appropriate alternatives to *S. album* oil.

Amyris balsamifera, west Indian sandalwood, has been used to adulterate *S. album* oil, and may be used as a less expensive substitute in some cosmetic or perfumery products. A trade sample of *A. balsamifera* (BI 10282) was also subjected to analysis but no santalols were detected in this oil (Fig. 3). Another trade 'sandalwood' oil (BI 10180) showed

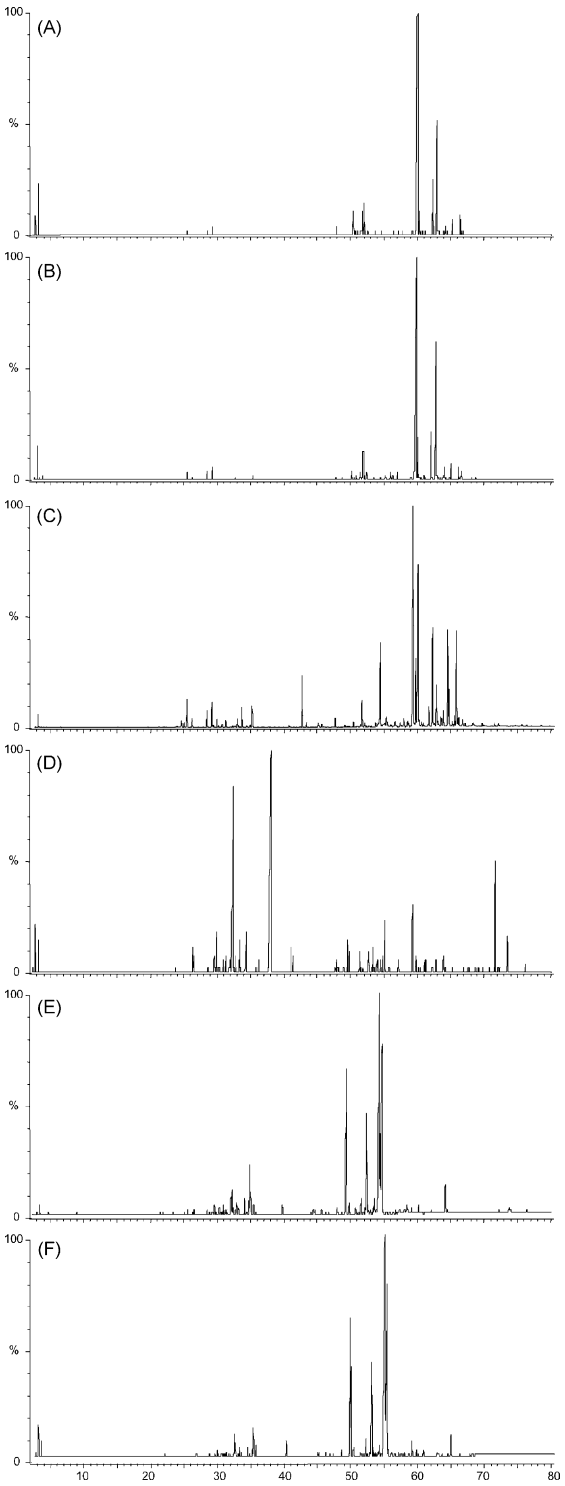

Fig. 3. GC–MS total ion chromatograms of essential oils reported to be from (A) *Santalum album* (historical sample, Bombay (1880), BI 10221), (B) *Santalum austrocaledonicum* (trade sample, BI 10277), (C) *Santalum spicatum* (trade sample, BI 10281), (D) *Santalum album* (historical sample, Madagascar, BI 10226), (E) *Amyris balsamifera* (trade sample, BI 10282), (F) *Santalum album* (trade sample, BI 10180).

a similar profile of essential oil components to the *A. balsamifera* oil with no santalols being detected in this sample (Fig. 3). The major components detected in the analyses of these oils using the ZB-WAX column included elemol (8.7, 10.9%), valerianol (25.0, 26.0%), 10-*epi*-γ-eudesmol (6.8, 7.3%), γ-eudesmol (6.6, 7.8%) and β-eudesmol co-eluting with 7-*epi*-α-eudesmol (18.4, 18.9%). Thus, it is apparent that some 'sandalwood' oils being used in the trade may not be from *S. album*, or they may be of inferior quality, or they may have been substituted with oils from other species, which have different chemical compositions.

Two commercial products (foambaths) reported to contain 'sandalwood' were evaluated for their santalol content. Santalols were not detected in either product but a sandalwood substitute, Sandalore® (secondary alcohols semi-synthetically derived from campholenic aldehyde), was detected in both products.

This study has shown that the chemical profile obtained using GC–MS analysis, and in particular the santalol levels, is valuable in assisting with the species identification and the detection of adulteration of sandalwood oils. The study also highlights that historical samples may not only offer some insight into the species and chemistry of oils used in the past, but may also assist with current methods for authentication. It is also apparent that even though the historical sandalwood oils have been stored for decades, their chemical composition did not appear to be significantly affected.

4. Conclusion

It is evident that sandalwood oil composition may vary depending on its geographical and taxonomic origin, which may reflect current international demand and declining resources. It should also be noted that santalol composition can vary depending on the method of oil extraction [23].

From the present study, it is apparent that generally accepted specifications (90% santalol content) and analysis methods require re-evaluation. Thus, it is suggested that quality control and authentication procedures for sandalwood oils should not rely on old methods of analysis (calculation of the total santalol/alcohol content) and that GC–MS is recommended.

In conclusion, a specification of ≥43% *Z*-α-santalol and ≥18% *Z*-β-santalol by GC–MS for *S. album* oil is suggested, based on *S. album* samples analysed in this study. These suggested specifications support those described by the ISO (2002) for GC-FID analysis of *S. album*; in addition, GC–MS has the advantage of assisting with the detection and identification of adulterants. 'Sandalwood' oils with santalol levels below these specifications may be of inferior quality due to extraction from undeveloped heartwood, adulteration (e.g. with synthetic or semi-synthetic substitutes) or substitution with oils from other species. GC–MS profiles may provide information regarding the species of origin; for example, preliminary investigations in this study suggest that

oils comprised of 16–22% and 6–8% *Z*-α- and *Z*-β-santalol, respectively, are likely to have originated from *S. spicatum* and not *S. album*.

References

[1] J.E.D. Fox, Biologist 47 (2000) 31.
[2] A.M. Radomiljac, H.S. Ananthapadmanabho, R.M. Welbourn, K. Satyanarayana Rao (Eds.), Sandal and Its Products, ACIAR Proceedings No. 84. Australian Centre for International Agricultural Research, Canberra, 1998.
[3] D.P. Anonis, Perfumer Flavorist 23 (1998) 19.
[4] P. Kraft, J.A. Bajgrowicz, C. Denis, G. Fráter, Angew. Chem. Int. Ed. 39 (2000) 2981.
[5] R.E. Naipawer, in: B.M. Lawrence, B.D. Mookherjee, B.J. Willis (Eds.), Flavors and Fragrances: A World Perspective, Proceedings of the 10th International Conference of Essential Oils, Fragrances and Flavors. Elsevier, Amsterdam, 1988, p. 805.
[6] US Dispensatory, 25th ed., Lippincott, Philadelphia, 1955, p. 1836.
[7] British Pharmaceutical Codex, The Pharmaceutical Society, London, 1949, p. 612.
[8] E. Dahlian, Hartoyo, Buletin Penelitian Hasil Hutan. 15 (1998) 385.
[9] Food Chemicals Codex, third ed., National Academy Press, Washington, DC, 1981, p. 268.
[10] International Organisation for Standardisation, first ed., ISO 3518 (1979).
[11] Martindale, 26th ed., Pharmaceutical Press, London, 1972, p. 1250.
[12] International Organisation for Standardisation, ISO 3793 (1976).
[13] International Organisation for Standardisation, second ed., ISO 3518 (2002).
[14] J. Verghese, T.P. Sunny, K.V. Balakrishnan, Flavour Fragrance J. 5 (1990) 223.
[15] M.G. Meurisse, Bull. Soc. Linn. Paris ii (1892) 1026.
[16] W.L. Wagner, D.R. Herbst, S.H. Sohmer (Eds.), Manual of the Flowering Plants of Hawaii, University of Hawaii Press/Bishop Museum Press, Honolulu, 1999.
[17] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy, Allured Publishing Corporation, Illinois, USA, 2001.
[18] P. Ausloos, C. Clifton, S.G. Lias, A. Shamim, S. Stein, NIST/EPA/NIH Mass Spectral Database (v. 4.0), US Department of Commerce, Gaitherburg, USA, 1992.
[19] N.W. Davies, J. Chromatogr. 503 (1990) 1.
[20] British Pharmacopoeia, The Stationery Office, London, 1999.
[21] European Pharmacopoeia, third ed., Council of Europe, Strasbourg, France, 2001.
[22] United States Pharmacopoeia, US Pharmacopoeial Convention, Rockville, USA, 2000.
[23] M.J. Piggott, E.L. Ghisalberti, R.D. Trengove, Flavour Fragrance J. 12 (1997) 43.