

Gas chromatography: an investigative tool in multiple allergies to essential oils

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Essential or fragrant oils are volatile odourous mixtures of organic chemical compounds that are widely used in aromatherapy and in the perfume industry. Because of their frequent use, allergy to essential oils is being increasingly recognized. We report 2 cases of multiple allergies to essential oils in professional aromatherapists. Gas chromatography/mass spectrometry was used to analyse the oils in order to identify a common allergen responsible for the contact dermatitis. In both the cases, α - and β -pinene were found to be the most common constituent in the oils and thus appeared to be key allergens. α -pinene was confirmed as an allergen on repeat patch testing with pure α -pinene in both cases. 12 controls tested were negative for the same. Gas chromatography-mass spectrometry was found to be an extremely useful tool that could be utilized in investigating multiple allergies to essential oils.

Key words: α -pinene; aromatherapy; contact allergy; essential oils; gas chromatography-mass spectrometry (GC-MS); occupational. © Blackwell Munksgaard, 2002.

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Essential or fragrant oils are volatile odourous mixtures of terpenes, aldehydes, alcohols, esters, and ketones. These fragrant oils are widely used in aromatherapy and in the perfume and cosmetics industry. In aromatherapy, essential oils are used in various ways such as massaging, adding to the bath, compresses, inhalers, mouthwashes and in incense lamps. When utilizing these oils for massaging, therapists often have unprotected bare hands and these result in frequent contact with the skin. Because of their constant use, contact allergy to essential oils has been increasingly recognized (1–3). Due to the volatile nature of these oils; airborne allergic contact dermatitis can also occur from their presence in the surrounding air (2). Every oil has many constituents, so that, when contact allergy occurs, identification of the specific sensitizer is often difficult (4).

Gas chromatography (GC) is a technique used for checking the purity or identity of volatile liquid samples. We employed the combined technique of gas chromatography and mass spectrometry (GC-MS) (5) to identify the common constituent among the essential oils that produced contact allergy in 2 patients. α - and β -pinene were found to be common to these oils. We used pure samples of the monoterpenes α - and β -pinene for testing in both our cases.

Case Reports

Case No. 1

A 51-year-old professional aromatherapist presented with suspected allergic contact dermatitis. 2 months previously, she had applied tea tree and lavender oils for an abrasion on her shin following an injury. Within a few days, she developed an eczematous eruption on her legs that soon spread all over her body. She had been working as an aromatherapist for over 2 years and handled a variety of essential oils for massaging (using unprotected hands), in baths, and in incense burners. She gave no history of atopy in herself or in the family. She was patch tested to the standard series, fragrance series and the essential oils that she used in her practice (Fig. 1). In all, 60 such oils were tested.

Case No. 2

A 52-year-old woman was referred with an intermittent patchy eczematous eruption affecting her hands, arms and legs of 2 years' duration. She had practised as an aromatherapist for 9 years, and handled essential oils with ungloved hands for massaging. An allergic contact dermatitis was suspected and she was patch tested to the standard



Fig. 1. Patch tests to essential oils at D 4 in Case No. 1.

series, fragrance series and 22 of the essential oils that she used regularly.

Materials and methods

Patch testing was performed using the standard and fragrance series from Chemotechnique Diagnostics (Milton Keynes, UK). Both patients were also tested to their own essential oils. The oils were diluted in white petrolatum at various concentrations as described in Table 1. The occlusion time was 48 h. The initial reading was half an hour after removal of patches. A second reading was done at day (D) 4. For the purpose of GC-MS analysis,

the Shimadzu GC-17 A (Kyoto, Japan) linked to a QP5000 (Kyoto, Japan) mass spectrometer was used. The GC was fitted with a fused silica DB1 capillary column, 30 m long, 0.25 mm internal diameter, and 0.25 μ m film thickness (J.W. Scientific, Köln, Germany).

The technique briefly was as follows. In each case 50 μ L of oil was diluted with 1000 μ L acetone, 1 μ L of which was injected onto the GC column. In some cases samples of different concentrations were also run. Any standards were purchased from Aldrich Chemical Company (Poole, UK). 50 μ L of standards were diluted in 10,000 μ L acetone. The solution injected onto the column vaporizes and is carried in a stream of carrier gas (helium) on to the chromatography column. As different components of the oil have different volatility and affinities for the non-polar surface of the column; each component moves through the column at a different rate. The components pass directly into the ionization chamber of the mass spectrometer where they are ionized by electron impact, and then analysed according to their mass/charge ratio (m/z). The mass spectrum produced is a plot of m/z against ion abundance. By comparison with the intensity of standard samples it is possible to quantify the percentage of given components in any oil. Each component of oil when input into the mass spectrometer is fragmented and gives a characteristic pattern. The MS detector, in addition to enabling quantification of components, also facili-

Table 1. Allergic PT reactions of both patients to standard battery and fragrance series at D4. Concentrations in percentage v/v in white petrolatum. Response ranges down from + + + (ulcerative/bullous reaction) to no detectable response

Series tested	Substance	Conc. (% in pet)	Case No. 1	Case No. 2
Standard	Colophonium	20	—	+++
	Balsam of Peru	25	—	+
	(Myroxylon Pereirae resin)			
	Wool alcohols (lanolin alcohol)	30	—	++
	Fragrance mix	8	++	++
	Quaternium-15	1	—	++
	Nickel sulphate	5	++	—
	Kathon CG	0.01	++	—
Fragrance	(methylchloroisothiazolinone + methylisothiazolinone)			
	Cinnamic aldehyde (cinnamal)	1	+	—
	Cinnamic alcohol	2	+	—
	Eugenol	2	—	++
	Isoeugenol	2	+	+++
	Geraniol	2	+	++
	Lavender absolute	2	+++	+++
	Cananga oil	2	+	+++
	Rose oil Bulgarian	2	+	+
	Ylang ylang oil	2	++	+++
	Geranium oil	2	++	++
	Jasmine absolute Egyptian	2	—	+

Table 2. PT reactions at D4 and the approximate concentrations of suspected sensitizing agents in the essential oils as indicated by GC-MS. 1, 2 are Cases Nos. 1 and 2. Blank space indicates the test was not done. For patch testing: + denotes palpable erythema, ++ oedematous/vesicular reaction and +++ ulcerative/bullous reaction. For the GC/MS results: –denotes below the limit of detection (<0.01%), + denotes >0.01%, ++ denotes >0.1%, +++ denotes >1%, and ++++ denotes >10%

Essential oils	PT results				GC-MS results							
	at D4 (2%v/v)		%v/v α -pinene		%v/v linalool		%v/v geraniol		%v/v terpineol		%v/v carophyllene	
	1	2	1	2	1	2	1	2	1	2	1	2
Angelica seed	++		+++		++		++		–		++	
Basil	++	+	+++	+	++++	++++	+++	–	–	–	+++	++
Benzoin	+		–		–		–		–		–	
Bergamot	++	++	++	+	++++	++++	++++	+++	–	–	+++	++
Black pepper	++	+++	+++	++++	–	–	–	–	–	–	+++++	++++
Cajuput	++	+++	+++	++	–	–	–	–	++++	+++	+++	++
Cedarwood		+	+	–	–	–	–	–	–	–	++++	+++
Chamomile	++		+	++	–	–	–	–	–	–	–	–
Citronella		+		–		–		++++		–		++
Clary sage	++	++	++	–	++++	+++	++++	+++	++++	++	++++	+++
Coriander seed		+		+++		++++		–		–		–
Cypress	++		++++		+++		–		+++		+++	
Elemi		++		+		–		–		+++		++
Eucalyptus	+		+++		–		–		–		–	
Frankincense		++		++++		++++		–		++		++++
Galbanum	+		+++		–		–		–		–	
Geranium	++	++	++		++++		++++		+++		+++	
Ginger	+		–		+++		+++		+++		++	
Grapefruit	+	+	++	+	–	–	–	–	–	–	+++	–
Hyssop	++		++		–		+++		–		+++	
Juniper	++	+++	++++	++	–	–	–	–	–	++	–	–
Lavender	+++	++	+	+	+++	++++	++++	+++	++	++	+++++	++
Lavandin	++		++		–		–		–		–	
Lemon	+	+++	+++		–		–		–		+++	
Mandarin	++		++		–		–		–		–	
Marjoram	++	+++	++	+	++++	+++	++++	+++	++++	+++	+++	+++
Melissa blend	+		–		+++		++++		–		+++	
Myrrh		++										
Niaouli	++		+++		–		–		++++		++++	
Peppermint		++		+		–		–		–		+++
Petitgrain	+		+		++++		++++	–	++++		+++	
Pine	+		++++		–		–		+++		++++	
Ravensora	++		+++		–		–		++++		+++	
Rosemary	++		++++		++++		–		+++		++++	
Spearmint mentha	+		–		–		–		–		+++	
Sweet orange	+		++		–		–		–		–	
Sweet thyme thyme	+		+++		+++		–		+++		+++	
Tea tree oil	+++		+++		+++		–		++++		+++	
Valerian		+										
Vetiver		++										
Ylang ylang	++	++++	+		++++		–		–		++++	

tates identification of separated components using a library database of stored mass spectra. In practice, without optimizing for particular pairs of analytes, baseline separation was not always achieved. This contributes to the error in estimating percentages of different components, particularly if 2 or more components coelute, as do geraniol and linalyl acetate.

Results

Table 1 shows the patch test (PT) reactions of both patients to the standard and fragrance series. Table

2 shows the positive patch test reactions to the essential oils handled by them at D4. In order to identify a common constituent, all the oils that showed positive reactions on patch testing were subjected to GC-MS analysis. The approximate concentrations of suspected sensitizing agents on GC are also shown in Table 2.

As seen, both the patients had been sensitized to numerous essential oils. It was noted that many of the oils were complex mixtures of compounds, as shown in the mass spectrogram of tea tree oil (Fig. 2). 2 of the compounds common to many oils eluted early at 8.08 min and 9.2 min, respectively,

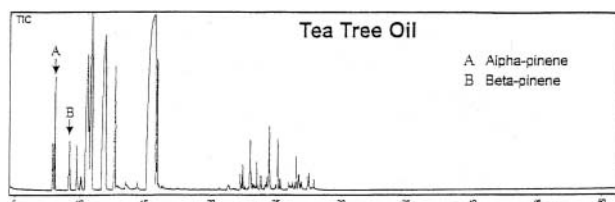


Fig. 2. Mass spectrograph of tea tree oil.

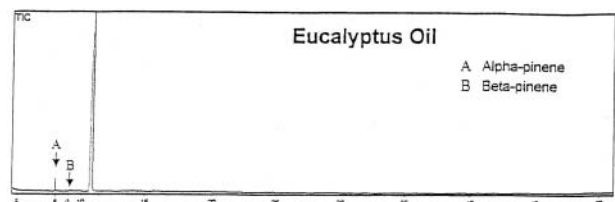


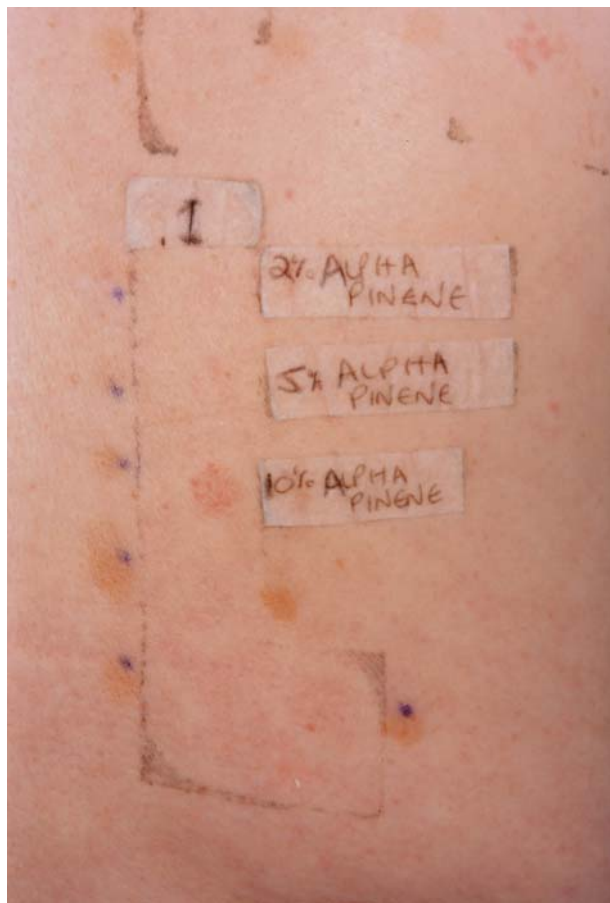
Fig. 3. Mass spectrograph of eucalyptus oil.

on the mass spectrometer. These were compared with the library database and found to be α -pinene and β -pinene, respectively. A standard sample of α -pinene (from Aldrich Chemical Company) was found to elute at 8.08 min and to have the same mass spectrum as the component in the oils. Other analytes were similarly identified.

Eucalyptus, one of the oils which showed a positive reaction in Case No. 1, in contrast to many of the oils, was found to be composed only of eucalyptol together with α - and β -pinene (Fig. 3).

It should be noted that the oils were from different manufacturers and had been open for different periods of time. As there was no specification of oil content, different samples were expected to differ both in concentrations and perhaps even in components.

Pure α -pinene, β -pinene and eucalyptol were obtained through the manufacturer (Sigma-Aldrich Co. Ltd) and further patch tests were carried out at dilutions of 2.0%, 5.0% and 10% in petrolatum (Fig. 4). The results (Table 3) show positive reactions to α -pinene at 5% and 10% concentrations in the 1st case and at 10% in the 2nd. 12 control subjects were negative to all 3 oils. There were some oils that showed positive reactions but had no detectable levels of pinenes at the concentrations analysed. We therefore examined in more detail Case No. 1 oils where the α -pinene content did not relate to the allergic response, and identified 4 additional peaks in the chromatograms that were present in many of these oils, though by no means as uniformly as α -pinene. The components eluted at 12.85 min, 17.52 min, 15.48–15.54 min, and 22.34–22.4 min. The MS identified these, respec-

Fig. 4. Patch tests to 2%, 5%, 10% pet. α -pinene at D 4 in Case No. 1.

tively, as linalool, geraniol and linalyl acetate (depending on the oil), a mixture of terpineol isomers and a mixture of carophylene isomers. GC-MS runs with standard samples of these compounds confirmed the identifications. Linalyl acetate and linalool when patch tested at 5 and 10% concen-

Table 3. Patch test reactions to different concentrations of α - and β -pinene and eucalyptol at D4 in both cases

	Case No. 1	Case No. 2
α -Pinene		
2%	—	—
5%	+	—
10%	++	++
β -Pinene		
2%	—	—
5%	—	—
10%	—	—
Eucalyptol		
2%	—	—
5%	—	—
10%	—	—

tration showed positive results in Case No. 1. Similarly carophylene tested positive in Case No. 2.

Discussion

Multiple sensitivity to essential oils used in cosmetics was first reported by Rudzki et al. in 1976 (6). The oils are obtained by a process of expression or distillation from a single botanical form or species (7). There are 2 main groups: the terpenes (monoterpenes, sesquiterpenes); and the oxygenated compounds (mainly esters, aldehydes, ketones, alcohols, phenols and oxides) (8). The common terpene hydrocarbons include pinene and limonene. Esters include linalyl acetate and geranyl acetate. The constituents of essential oils could vary according to their origin, time of harvest, and manufacturing process. Due to the high volatility of the oils, the quantity of each component in oil may vary with time and use. Allergens still being commonly encountered in oils are pinenes (9, 10), sesquiterpenes and geraniol.

Alpha pinene is a monoterpene and may be obtained from turpentine, an oleoresin that is exuded from many species of pine trees. It is widely distributed, and is one of the commonest constituents of essential oils from leaves, fruits, seeds, barks and woods of many plants (11). We found α -pinene to be the most common constituent in the oils analysed. The fact that it was a key allergen was confirmed on further patch testing with the purified product in both our cases. It thus appears to be a low-level potential sensitizer, being a constituent of nearly all the oils to which the patients reacted. Other components, linalool, linalyl acetate, geraniol, carophylene and terpineol, were also present in subsets of the oils and were probably responsible for part of the allergic responses noted with some of the oils. Linalool and linalyl acetate when patch tested in Case No. 1 and carophylene when tested on patient No. 2 support this hypothesis.

Alpha pinene, being more volatile, elutes early, suggesting that variations in the manufacturing process to remove it early could be used to reduce the amounts of these components and make the oils less allergenic. Meanwhile, since α -pinene is a

potential sensitizer and a common constituent in many essential oils, we suggest that α -pinene be considered in the investigation of fragrant oil sensitivity. Perhaps, it ought to be included in the fragrance series when investigating suspected allergy to essential oils.

We have also shown that gas chromatography-mass spectrometry (GC-MS) is an extremely useful tool that can be utilized in investigating multiple allergy to essential oils. In addition to α -pinene, we have identified a further 5 sensitizing components in the oils tested.

References

1. Selvaag E, Holm J, Thune P. Allergic contact dermatitis in an aromatherapist with multiple sensitisation to essential oils. *Contact Dermatitis* 1995; 33: 354-355.
2. Schaller M, Korting H C. Allergic airborne contact dermatitis from essential oils used in aromatherapy. *Clin Exp Dermatol* 1995; 20: 143-145.
3. Cockayne S E, Gawkrödger D J. Occupational contact dermatitis in an aromatherapist. *Contact Dermatitis* 1997; 37: 306-307.
4. Scheinman P L. Allergic contact dermatitis to fragrance: a review. *Am J Contact Dermatitis* 1996; 7: 65-76.
5. Lewis R, Evans W. *MacMillan Foundations Chemistry*, 1st edn. London: Macmillan Press, 1997: 345-346.
6. Rudzki E, Grzywa G, Bruo W S. Sensitivity to 35 essential oils. *Contact Dermatitis* 1976; 2: 196-200.
7. Price S. *Practical Aromatherapy*, 3rd edn. London: Thorson's, 1994.
8. Lawless J. *The Illustrated Encyclopaedia of Essential Oils*, 1st edn. town?: Element Books, year?: 48-49.
9. Cachao P, Menezes Brandao F et al. Allergy to oil of turpentine in Portugal. *Contact Dermatitis* 1986; 14: 205-208.
10. Lear J T, Heagerty A H et al. Transient re-emergence of oil of turpentine allergy in the pottery industry. *Contact Dermatitis* 1996; 35: 169-172.
11. Read J, Gunstone F D. *A Textbook of Organic Chemistry*. London: G Bell and Sons, 1958: 545-551.

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