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Note

Thin-layer chromatographic characterization of essential oils

DANIELA HEIMLER* and VERIANO VIDRICH
Dipartimento di Scienza del Suolo e Nutrizione della Pianta, Universita degli Studi di Firenze, Piazzale delle Cascine, 28-Firenze (Italy)
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The use of thin-layer chromatography (TLC) for the analysis of essential oils has been very limited in the last 10 years[1,2], gas chromatography (GC) on packed and capillary columns and the head-space technique being the chromatographic methods most widely used[3,4]. The interpretation of GC data is quite difficult and, when possible, mass spectrometry is employed[3].

The characterization of these compounds is of importance in industry (production and quality control) and in physiologic and genetic studies of the plants from which they are extracted[5].

This paper deals with a rapid method which can differentiate and characterize the essential oils of different plants (pine and juniper), different species (*Pinus halepensis, Pinus cembra, Hybridus P. halepensis* × *P. brutia*) and different parts of the same plants (needles and small branches in the case of pine and needles and fruits in the case of *Juniperus phoenicia*).

EXPERIMENTAL

The chromatographic determinations were carried out on silica layers 60 F_{254} (Merck). There are no substantial differences between the data on these layers and those on high-performance ones, there being no problems of sensitivity or of elongated spots. The spots were visualized by spraying first with 5% sulphuric acid solution in ethanol and then with 10% vanillin solution in methanol[1]. The plates were then heated at 100°C for 10 min. The migration distance was 10 cm. Fresh standard solutions were prepared by dissolving the pure compounds (Aldrich, Fluka, Roth) or the essential oils in methanol. The amount deposited on the layer was between 0.1 and 2 μg in the case of the reference compounds and between 10 and 30 μg in the case of essential oils. All the measurements were carried out at 20°C.

The quantitations were effected with a Shimadzu LS 200 densitometer. The measurements were carried out at a wavelength of 540 nm, which gave the best results both in terms of the sensitivity and baseline constancy; in this way the separation of spots which differ very little in their R_F values is possible. Most spots were visualized even by fluorimetric measurements using an excitation beam wavelength of 365 nm and an emission filter of 400 nm. In this case, however, the densitometric peaks were less sharp and the baseline quite high and therefore the interpretation is rather dif-

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ficult. The layers were scanned with the densitometer 30 min after the end of heating: the intensity of the spots decreased considerably in the first 20 min, but after this time the colour intensity was constant for about 1 h.

The essential oils were obtained by steam distillation of minced needles small branches or fruits of *Pinus halepensis, Pinus cembra, Hybridus* (*P. halepensis* × *P. brutia*) and *Juniperus phoenicia.*

RESULTS AND DISCUSSION

Table I lists some constituents of essential oils, the colours observed upon spraying with the vanillin solution and the R_F values on silica gel layers in four eluents chosen among those which gave the best results[1,2]. Among the reference compounds studied were the most common consistuents of pine and juniper essential oils. It should be noted that, with the vanillin solution, only those compounds with a certain degree of double bond conjugation can be detected. The compounds are listed according to their increasing R_F values in chloroform–toluol (75:25) mixture. From the data, it is seen that quite good separations can be obtained even with a single eluent and that the identification of the spots may be improved by the use of two plates in two different eluents. For instance, with toluol–ethyl acetate (90:10),

TABLE I

R_F VALUES OF SOME COMPONENTS OF ESSENTIAL OILS ON SILICA GEL LAYERS AND COLOURS OF THE SPOTS WHEN SPRAYED WITH VANILLIN SOLUTION

Eluents: (a) chloroform–toluol (75:25); (b) toluol–ethyl acetate (90:10); (c) two developments in *n*-hexane–ethyl acetate (90:10); (d) first development in dichloromethane followed by a second one in *n*-hexane–ethyl acetate (90:10).

Compound	*Colour*	*Eluent*			
		a	*b*	*c*	*d*
Nerol	Blue	0.20	0.30	0.30	0.42
α-Terpineol	Blue	0.22	0.27	0.32	0.32
D-citronellol	Red	0.23	0.29	0.32	0.40
Borneol	Red	0.25	0.35	0.35	0.43
Geraniol	Red	0.26	0.27	0.30	0.38
Myrtenol	Violet	0.28	0.35	0.39	0.32
Terpinen-4-ol	Blue	0.31	0.43	0.47	0.51
Linalool	Blue	0.34	0.43	0.47	0.46
α-Phellandrene	Blue	0.35	0.59	0.37	0.32
Carvacrol	Pink	0.40	0.56	0.65	0.57
Eugenol	Yellow	0.42	0.55	0.68	0.44
Thymol	Red	0.43	0.62	0.70	0.55
1,8-Cineole	Blue	0.47	0.54	0.68	0.65
α-Terpinene	Brown	0.50	0.58	0.70	0.64
Carvone	Pink	0.50	0.61	0.65	0.73
Isobornyl acetate	Violet	0.67	0.66	0.82	0.85
Geranyl acetate	Blue	0.68	0.78	0.83	0.85
Methylchavicol	Pink	0.85	0.95	0.90	0.98
β-Caryopillene	Violet	0.97	0.96	0.98	0.98
β-Myrcene	Violet	0.98	0.97	0.95	0.98

linalool and α-phellandrene can be separated (R_F = 0.43 and 0.59, respectively), while this separation is not possible in chloroform–toluol (eluent a). In the latter eluent, terpinen-4-ol and linalool (R_F = 0.31 and 0.34) can be separated, in contrast to their behaviour in eluent b. The reference compounds listed in Table I can be detected if present at the level of 1 or 2 μg at most. The retention of the compounds is correlated to the polarity of their molecules. The higher the polarity of the compounds, the more they are retained by the silica gel layers. Terpenes with an alcoholic group are the most strongly retained, then the phenylpropane derivatives, the acetates and finally compounds such as β-myrcene and β-caryophillene which migrate with the solvent front (see Table I).

In Fig. 1 are shown the chromatograms of different essential oils obtained by steam distillation. Very good separations are achieved and some components can be identified (see Fig. 1). The data are in good agreement with these obtained by GC[6,7].

As regards the quantitation of the different components, some critical factors must be considered, that is the temperature and the time during which the layers are heated, and the time after which the densitometric measurements are made. Because of the influence of these parameters, besides those which are peculiar to TLC quantitations[8], a standard of known concentration must always be deposited on the layer.

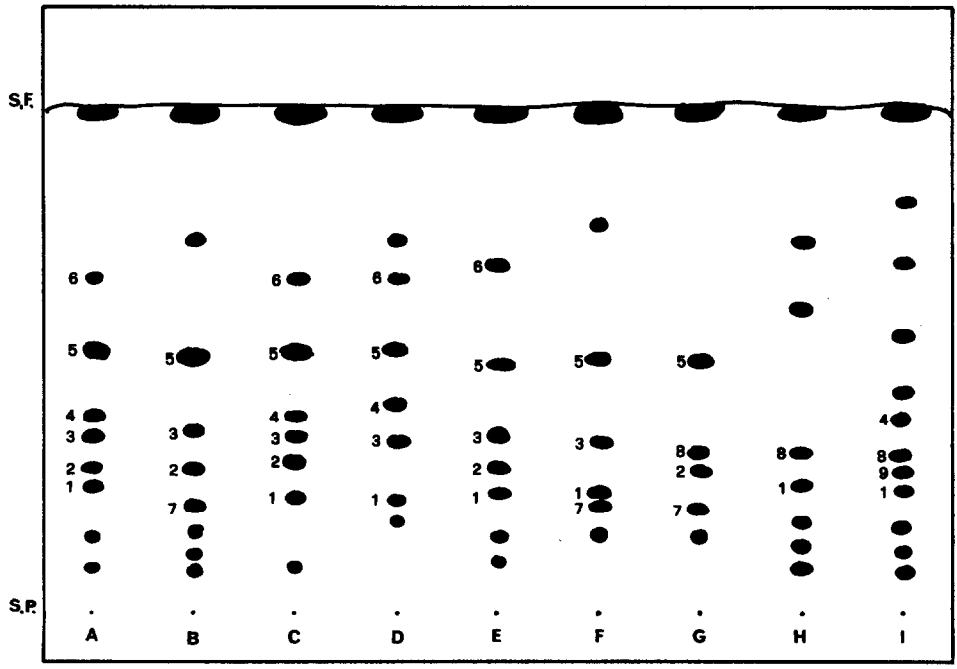

Fig. 1. Thin-layer chromatograms of essential oils. Eluent: chloroform–toluol (75:25). A = *Hybridus* (needles); B = *Hybridus* (branches); C = *Pinus halepensis* (needles); D = *Pinus halepensis* (branches); E = *Pinus cembra* (needles); F = *Pinus cembra* (branches); G = *Pinus cembra* (commercial oil); H = *Juniperus phoenicia* (fruits); I = *Juniperus phoenicia* (needles). 1 = D-Citronellol; 2 = myrtenol; 3 = linalool; 4 = carvacrol; 5 = 1,8-cineole; 6 = geranyl acetate; 7 = α-terpineol; 8 = terpinen-4-ol; 9 = geraniol.

TABLE II

RATIOS OF INTEGRATION UNITS FOR THE DIFFERENT COMPONENTS OF PINE ESSENTIAL OILS TO THOSE OF LINALOOL (SEE FIG. 2)

The ratios are the means for eight determinations. S.D. = Standard deviation.

Component	*Ratio*	*S.D.*	*Component*	*Ratio*	*S.D.*
P. halepensis (needles)			*P. halepensis (branches)*		
1	1.78	0.05	1	1.05	0.18
2	4.46	0.33	3	1	
3	1		4	0.90	0.14
4	3.51	0.33	5	6.68	1.61
5	10.38	1.47	6	0.61	0.02
6	0.84	0.06			
P. cembra (needles)			*P. cembra (branches)*		
1	2.89	0.08	1	1.08	0.16
2	2.88	0.12	3	1	
3	1		5	1.69	0.13
5	0.37	0.01	7	0.78	0.10
6	0.18	0.02			
Hybridus (needles)			*Hybridus (branches)*		
1	3.98	0.66	2	1.13	0.12
2	3.67	0.71	3	1	
3	1		5	10.22	1.33
4	2.40	0.52	7	1.95	0.07
5	16.14	1.67			
6	0.66	0.07			

Notwithstanding such difficulties, quite reproducible data are obtained; however the whole procedure is time-consuming, since fresh solutions of all the reference compounds must always be used.

In order to effect a quality control or to identify the origin of an essential oil (for instance from small branches, needles or from different species), the quantitative data may be used as ratios with respect to a given component which must always be present so that the above-mentioned critical factors can be neglected. In the case of the pine essential oils, we calculated the ratios of the integration units for the different components identified relative to those of linalool. We chose linalool as the reference since it is present in all samples, can easily be identified and its concentration is relatively low (its amount changes from 0.5 to 1.5% in the different essential oils studied). Table II lists some ratios for the compounds identified; the values are the averages from eight determinations; the standard deviations are also listed.

In Fig. 2 are reported maps of the different oils. The results are very interesting, since significant differences among them can be seen. These maps can also be used for the study of the essential oil composition as a function of the genetic origins of the plants[9,10].

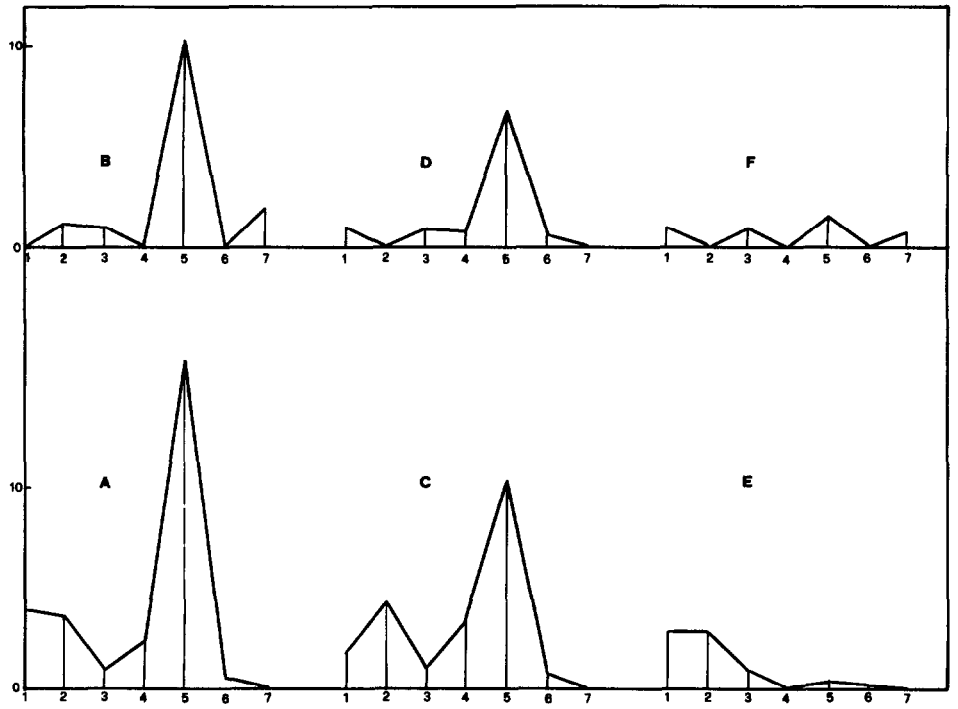

Fig. 2. Maps of essential oils. A = *Hybridus* (needles); B = *Hybridus* (branches); C = *Pinus halepensis* (needles); D = *Pinus halepensis* (branches); E = *Pinus cembra* (needles); F = *Pinus cembra* (branches). *X* axis, numbers as in Fig. 1; *Y* axis, ratios of integration units (see text).

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