Composition of *Eucalyptus camaldulensis* Volatiles Using Direct Thermal Desorption Coupled with Comprehensive Two-Dimensional Gas Chromatography–Time-of-Flight-Mass Spectrometry

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Abstract

The direct qualification and quantitation of the volatile organic components of four *Eucalyptus camaldulensis* fruit samples, obtained from different geographical areas in Turkey, is studied using a direct thermal desorption (DTD) technique coupled with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry. It is found that the *E. camaldulensis* sample from Adrasan gave a slightly higher oil yield (1.18%) than the others. The number of components quantitatively identified from Adrasan, Belek, Kuyucak, and Cesme were 46, 54, 55, and 59, respectively. The main compounds found in the volatile oils were: aromadendrene (6.45–15.02%), eucalyptol (0.17–12.61%), γ-gurjunene (8.40–10.08%), terpinolen (1.98–8.39%), spathulenol (1.42–8.34%), α-pinene (0.85–6.81%), ledene (0.94–6.72%), and longifonene (0.07–6.22%). The composition of the volatiles desorbed from samples from all four different areas varied qualitatively and quantitatively. All identified compounds were quantitated using total ion chromatogram peak areas. DTD is a good method for qualitative and/or quantitative analysis of complex mixtures, and in particular for quantitative analysis of plant samples, which can yield data without the traditional obligation for costly and time consuming extraction techniques.

Introduction

The genus of Eucalyptus includes more than 700 species. *E. camaldulensis* is native to Australia. It has spread worldwide, particularly in Africa (1). Eucalyptus trees are also widespread in all of the Mediterranean basin (2). It is a fast-growing, medium-sized tree, standing up to 40 m tall and up to 0.8 m in diameter. Flowering is from February to April, and fruiting is from May until August.

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There are many reports on the composition of *E. camaldulensis* volatiles, whether obtained from the leaves (1,3,4), flowers (5), fruits (1,6) or the bark (6). The essential oils distilled from *E. camaldulensis* are used in medicine, in perfumes, and as a food flavouring material. The nature and quantities of the oil components are characteristic of the different Eucalyptus species. Among the different *E. camaldulensis* leaves studied in the previous literature, three chemotypes were distinguished: one rich in 1,8-cineole (28–84%); one rich in *p*-cymene (20–30%), and one rich in spathulenol (18%) (1).

The analysis of organic volatiles usually first requires an extraction step from the plant matrix, followed by pre-concentration of analytes, chromatographic separation, and, finally, detection. Solvent extraction (6), distillation (6,7), supercritical fluid extraction (1), superheated water extraction (7), automatic thermal desorption (8) dynamic headspace (9), and solid-phase micro-extraction (9,10) techniques are all used as sample preparation methods for plant and food volatiles before analysis using chromatographic techniques.

The dynamic headspace technique is a popular method for analyzing volatile compounds in food and in plant materials, such as *Luffa acutangula* and *Momordica charantia* (9). As a new analytical approach, direct thermal desorption (DTD) is a comparable alternative to dynamic headspace techniques requiring cryogenic trapping of the liberated analytes but extracting components irrespective of volatility. Chromatographic profiles of plant volatile fractions obtained using steam distillation (7,8) and DTD (7) are similar in terms of components. However, recoveries of both thermally labile and low volatility compounds have been found to be better using DTD. Quantitation of volatiles by DTD is possible (11–13).

Two-dimensional gas chromatography (GC×GC) provides increased resolution and peak capacity over single column methods. The use of a mass spectrometry (MS) system is highly desirable for identification of the numerous separated com-

pounds found during a GC×GC run and provides a third dimension of specificity to the analysis. Dalluge et al. (14) reviewed the use of GC×GC in various samples.

The direct thermal desorption technique used here has been applied previously only in a limited fashion (11), and we believe that there is significant scope for the study of direct thermal desorption (DTD) coupled with online GC×GC separation. The objective of this present study was to find new compounds and improve resolution from the fruit of *E. camaldulensis* using DTD comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry GC×GC–TOF-MS whilst at the same time adding to the types of sample that cryo-cooled DTD has been used for.

Experimental

Materials

Eucalyptus camaldulensis Dehl. fruits were collected in July 2006 from Adrasan and Belek, (close to Antalya on the south coast of Turkey) and also from Kuyucak and Cesme (both nearby Izmir, western Turkey). In all 4 different geographical areas, the fruits were collected from the same tree then stored in airtight containers kept in a cool place until drying. All 4 stations' samples were collected within a week, dried in the sun on sheets of paper, and then stored in airtight containers stored in a refrigerator utill use. The results are the mean of five experiments. Dodecane (as an internal standard) was provided by Aldrich (Gillingham, Dorset, UK). HPLC grade pentane was supplied by Fisher Scientific (Loughborough, UK).

Direct thermal desorption method

E. camaldulensis fruits were cut into two pieces just before the experiment. The glass tube liner of the GC injection port was removed and 3.0 ± 0.1 mg of one of the halves were placed into it (SGE, Ringwood, Australia) using tweezers to ensure that no contamination of the sample occurred. Glass wool was used to hold the sample in place. Dodecane (3.0 µL of a 0.1 mg/mL pentane solution) was spiked directly onto the sample in the GC liner as an internal standard. The GC inlet was held at 40°C, and the liner containing the sample carefully inserted into it. The GC liner was purged for 2 min at ambient temperature using helium to remove water vapour and oxygen. This equilibration period is needed when switching the carrier gas from its normal operation to pass through the sample. The head of the primary column (around 10 cm long) was cryo-cooled using liquid nitrogen. The temperature of the GC inlet was then quickly raised to 150°C and held isothermally for a further 5 min to ensure maximum desorption of all volatile organic materials. After a five-minute desorption, the liquid nitrogen was cut off and the oven programme initiated to begin the GC×GC analysis. Glass wool was replaced after each run.

Chromatographic analysis

The GC×GC–TOF-MS system consisted of an HP 6890 (Agilent Technologies, Palo Alto, CA) GC and a Pegasus III TOF-MS (LECO, St. Joseph, MI). The first column was a non-polar DB5 (5% phenyl–95% methyl polysiloxane, 30 m × 0.32 mm i.d. x 0.25-µm film thickness) and the second column a DB17 (50% methyl–50% phenyl polysiloxane, 1.9 m × 0.10 mm i.d. × 0.10-µm film thickness). Both columns were purchased from J & W Scientific (Folsom, CA). The columns were connected by means of a press-fit-connector. The first dimensional separation was based on separation by volatility in a non-polar column. The second dimensional separation was based on separation by polarity using a polar column. The inclusion of this made this overall a two dimensional chromatogram. The second dimension column was installed in a separate oven, which was maintained in the main GC oven. The separate oven provided a more flexible system because it allows fine-tuning of the retention in the second column by using a higher or lower temperature relative to the first dimension column. In this particular system, the need to use a two-oven set-up was driven by detector stability considerations, requiring accurate and stable control of the second column's temperature. This temperature control of both ovens enabled more rapid and higher resolution separations.

The system does not require any valving or switching facilities. The modulator is the key to the performance of the GC×GC experiment. Cryogenic modulation was performed using a jet-type modulator which was installed at the top of the second dimension column. This consists of two cold and two hot jets, with the nozzles providing the cold jets mounted orthogonal to the hot jets. Nitrogen gas was cooled by heat exchange through copper tubing immersed in liquid nitrogen outside the GC system and delivered through vacuum-insulated tubing to the cold jets to provide two continuous jets of approximately 10 L/min of cold nitrogen gas. The modulation time was 6 s. When the hot downstream pulse was fired the analytes were effectively injected into the second dimension column. Most of the compounds were modulated into two or three second dimension peaks and were seen more than once.

Helium (flow rate 1 mL/min, column head pressure was 100 kPa) was used as a carrier gas; *m/z* ratios between 20–350 amu were collected at 50 Hz. The initial temperature of the first dimension column was 60°C for 30 s and the subsequent temperature programme was a heating rate of °C/min until 280°C was reached and held isothermally for a further 2 min. The initial temperature of the second dimension column was 75°C for 30 s and a 5°C/min heating rate was used until 300°C was reached and held isothermally for further 2 min. Peak identification was made using TOF-MS with electron ionisation. The mass spectrometer used a push plate frequency of 5 kHz, with transient spectra averaging to give unit resolved mass spectra between 45 and 350 amu at a rate of 50 spectra/s.

Peaks in the total ion chromatogram (TIC) profiles for the *E. camaldulensis* fruits were characterized or tentatively identified from their mass spectral data using the NIST and Wiley mass spectrometry libraries. Identifications were confirmed from their chromatographic retention times using linear retention indices (LRI). The LRI of each component was collected from the literature for column DP5. Concentration values, expressed as a percentage, were directly calculated from TIC peak areas. Quantitative values were obtained using dodecane as an internal standard (a 0.1 mg/mL pentane solution was spiked onto the dry samples). They are the means of five experiments.

Table I. Percentage Compositions of Volatile Components of Various *E. camaldulensis* Isolated Using The Direct Thermal Desorption Technique

Compound*	RI†	%‡ Adrasan	Kuyucak	Cesme	Belek
Acetaldehyde	320	0.50 (0.04)	0.21 (0.02)	–	0.06 (0.01)
Pentanal	732	–§	0.06 (0.01)	–	–
Acetic acid	600	–	0.06 (0.01)	–	0.05 (0.01)
Pentanol	759	–	0.31 (0.04)	0.07 (0.01)	–
Hexanol	851	–	0.07 (0.01)	–	–
Hexanal	801	–	–	–	0.05 (0.01)
2-Pentanol	950	–	0.07 (0.01)	–	–
Pentyl acetate	888	–	0.05 (0.01)	0.06 (0.01)	–
2-Heptenal	951	0.05 (0.01)	0.06 (0.01)	–	–
α-Thujene	938	0.78 (0.06)	–	0.14 (0.03)	1.01 (0.13)
α-Pinene	939	0.85 (0.05)	6.81 (0.59)	2.35 (0.31)	2.27 (0.19)
Camphene	953	0.07 (0.1)	0.47 (0.05)	0.26 (0.05)	0.10 (0.02)
Sabinene	972	–	0.06 (0.01)	–	1.13 (0.09)
β-Pinene	976	2.78 (0.28)	0.78 (0.04)	0.34 (0.02)	2.43 (0.18)
Myrcene	992	1.41 (0.16)	2.02 (0.19)	0.84 (0.10)	0.81 (0.11)
Nerol	1233	0.55 (0.05)	–	5.07 (0.63)	1.25 (0.13)
α-Phellandrene	1006	2.62 (0.18)	1.92 (0.22)	2.82 (0.23)	3.89 (0.43)
Limonene	1033	0.45 (0.03)	3.07 (0.27)	3.09 (0.26)	1.75 (0.21)
Terpinyl acetate	1352	–	–	4.54 (0.38)	–
3-Carene	1009	1.94 (0.18)	0.13 (0.02)	1.68 (0.19)	–
p-Cymene	1018	4.43 (0.40)	3.90 (0.42)	3.94 (0.28)	1.14 (0.15)
m-Cymene	1037	0.54 (0.06)	0.98 (0.12)	0.66 (0.09)	–
Carveol	1197	1.53 (0.10)	3.33 (0.24)	0.08 (0.01)	–
Eucalyptol	1030	0.17 (0.02)	12.61 (1.03)	9.97 (1.12)	5.33 (0.46)
Linalool	1100	0.26 (0.02)	–	–	–
Terpinolene	1088	4.26 (0.51)	8.39 (0.63)	5.64 (0.61)	1.98 (0.22)
Solusterol	1103	0.05 (0.01)	3.05 (0.21)	–	–
γ-Terpinene	1074	2.00 (0.16)	2.44 (0.19)	2.72 (0.29)	2.23 (0.15)
Linalool oxide	1212	–	0.07 (0.01)	–	–
Phenylethyl alcohol	1118	–	0.21 (0.03)	0.23 (0.03)	0.11 (0.03)
Fenchyl alcohol	1139	–	0.22 (0.04)	0.08 (0.01)	–
2,4-Hexadienal	910	–	–	0.14 (0.02)	–
Myrtenol	1194	–	0.34 (0.03)	0.05 (0.01)	–
cis-Verbenol	1140	0.41 (0.05)	0.35 (0.04)	0.16 (0.04)	0.13 (0.02)
Nerol oxide	1131	–	0.08 (0.01)	0.08 (0.02)	–
cis-Carveol	1229	–	0.15 (0.02)	1.68 (0.09)	0.96 (0.11)
Borneol	1162	–	0.56 (0.06)	0.25 (0.03)	–
Terpinen-4-ol	1179	1.60 (0.22)	–	1.66 (0.18)	1.53 (0.09)
trans-Carveol	1217	–	–	0.18 (0.03)	–
Cryptone	1188	–	0.81 (0.07)	0.51 (0.04)	0.54 (0.08)
α-Terpineol	1195	0.16 (0.02)	3.13 (0.34)	0.50 (0.07)	0.30 (0.05)
p-Cymenol	1166	–	–	0.38 (0.05)	0.06 (0.01)
Sabina ketone	1156	3.15 (0.28)	–	–	–
Piperitol	1233	–	–	–	0.91 (0.10)
cis-Sabinol	1144	1.96 (0.17)	0.91 (0.10)	0.85 (0.09)	2.02 (0.23)
Isobornyl formate	1245	–	0.15 (0.02)	0.05 (0.01)	
Terpinyl acetate	1352	1.19 (0.08)	0.16 (0.02)	0.09 (0.01)	0.08 (0.01)
Piperitone	1245	0.19 (0.02)	1.47 (0.16)	–	0.14 (0.03)
Cuminic alcohol	1284	1.24 (0.11)	–	–	0.26 (0.04)

* As identified by GC×GC–TOF-MS software; names according to NIST mass spectral library, and by comparing their Kovats retention indices.

† RI = Retention index; Kovats retention indices (column: DB5).

‡ Percentage of each component is calculated as peak area of analyte divided by peak area of total ion chromatogram times 100 (in the case of multiple identification, the areas of the peaks that belong to one analyte were combined to find the total area for this particular analyte). The results are the mean of five experiments and the data mentioned in parentheses are the corresponding standard deviations (±) of the readings.

§ – = Not detected or percentage of the component is lower than 0.05%.

Results and Discussion

The optimization of DTD temperature has been studied previously and 150°C was found to be the best from the 100–250°C range studied. Thermal desorption temperatures in excess of 200°C are not recommended due to an observed production of degradation products within the injection liner (15). A number of species, however, appear only at the higher temperatures of 200°C and 250°C, and are not components of volatile oil, but are browning reaction products (15). In this study, amongst various other compound classes, alkanes, alcohols, aldehydes, ketones, esters, acids, and substituted aromatic compounds were identified by library search (Table I). However, a number of unknown compounds still occurred.

The composition of volatiles obtained from dried *E. camaldulensis* using DTD is presented in Table I. It should be noted that the peak identification of components is based on both library mass spectra and LRI. Identification was based on a mass spectral library search using similarity and reverse factors > 750 and 800, respectively. Lower values than these were counted as unknown and components having these low values were not compared for their LRI. Dalluge et al. (16) and Ozel et al. (15) also used similarity and reverse factors > 750 and 800, respectively.

The yields of the volatile fractions of *E. camaldulensis* samples from Adrasan, Kuyucak, Cesme, and Belek, obtained using DTD, were 1.18%, 1.05%, 0.93%, and 0.89%, respectively. The yields are given as a percentage of the weight of the dried fruit sample used. They are the means of five experiments and the relative standard deviations were 8.12, 14.62, 11.13, and 13.32, respectively. The number of components identified in samples from Adrasan, Belek, Kuyucak, and Cesme were 46, 54, 55, and 59, respectively. Each identified component has also been quantitated using TIC peak areas and quantities are expressed as a percentage of the total volatile. In earlier studies, El-Ghorab et al. (6) found 38 compounds and Tsiri et al. (3) found 52 compounds from fruits of *E. camaldulensis*. Differences in the quality or quantity of the composition of volatiles may be due to collection time, differing chemotypes, drying conditions, mode of distillation, and/or extraction, analyzing technique and geographic or climatic factors.

The nature and quantities of the oil components are known to be characteristic of the different Eucalyptus species and places of origin. In

this study, the four samples were of the same species but were collected from different places. Twenty two of the components identified were common to all four samples. The main components found were aromadendrene (6.45–15.02%), eucalyptol (0.17–12.61%), γ-gurjunene (8.40–10.08 %), terpinolen (1.98–8.39%), spathulenol (1.42–8.34%), α-pinene (0.85–6.81%), ledene (0.94–6.72%), and longifonene (0.07–6.22%). Predominantly these volatiles were rich in sesquiterpenenoids. El-Ghorab et al. (6) found aromadendrene (17.99%), α-pinene (12.68%) drimenol (12.35%), cubenol (9.23%), α-vetivone (8.28%), α-gurjunene (6.65%), and *p*-cymene (6.15%) as the major constituents in the volatiles of *E. camaldulensis* fruits. Tsiri et al. (3) found spathulenol (19.0%), β-pinene (8.8%), and *p*-cymene (4.8%) as the main components of the fruits of *E. camaldulensis*. Table I shows that percentages of myrcene, α-phellandrene, γ-terpinen, *cis*-sabinol, and α-gurjunene exhibit a similar pattern in the 4 different samples. However, percentages of α-pinene, eucalyptol, terpinolen, longifolene, and spathulenol varied dramatically. Tsiri et al. (3) also noted variation in the components of essential oils obtained from fruits of *E. camaldulensis* during the course of one year.

Esteban et al. (8) recently showed that although the chromatographic profiles of plant volatiles obtained by steam distillation and DTD are similar; the recovery of both low volatility and thermally labile compounds were better using the DTD technique. Our previous study also showed a similar trend using the DTD, steam distillation, and superheated water extraction techniques (7). Analyzing plant volatiles usually requires sample preparation steps before chromatography and/or any other analyzing techniques. In this study, Table I shows that DTD may be used to analyse volatiles without any sample preparation procedures (apart from drying the sample).

Table I. (Continued) Percentage Compositions of Volatile Components of Various E. camaldulensis Isolated Using The Direct Thermal Desorption Technique

		%‡			
Compound*	RI†	Adrasan	Kuyucak	Cesme	Belek
6-Camphenol	1110	–§	–	–	0.08 (0.03)
Bornyl acetate	1283	0.07 (0.01)	0.06 (0.01)	0.10 (0.02)	0.05 (0.01)
Thymol	1290	–	0.18 (0.03)	0.52 (0.07)	0.16 (0.02)
Verbenone	1204	0.81 (0.09)	–	0.20 (0.03)	0.30 (0.06)
Carvacrol	1299	–	1.46 (0.20)	0.73 (0.06)	0.92 (0.09)
α-Cubebene	1463	0.05 (0.01)	–	–	1.54 (0.17)
Eugenol	1364	–	–	0.06 (0.01)	–
Limonene oxide	1132	–	0.19 (0.03)	–	–
α-Copaene	1377	–	–	0.15 (0.02)	0.60 (0.08)
Linalyl acetate	1261	–	0.40 (0.04)	–	–
β-Elemene	1393	2.17 (0.19)	–	0.05 (0.01)	1.15 (0.11)
Carveol	1225	–	–	–	1.14 (0.08)
β-Caryophyllene	1467	–	–	5.32 (0.47)	15.92 (1.28)
Longifolene	1398	6.22 (0.47)	0.07 (0.01)	5.11 (0.35)	1.03 (0.12)
β-Farnesol	1696	1.12 (0.14)	2.78 (0.31)	0.06 (0.01)	–
α-Gurjunene	1412	3.09 (0.28)	2.44 (0.22)	3.12 (0.26)	2.01 (0.24)
Aromadendrene	1496	10.76 (0.88)	10.23 (0.95)	6.45 (0.53)	15.02 (0.95)
γ-Muurolene	1475	1.05 (0.11)	1.70 (0.19)	0.52 (0.07)	1.41 (0.15)
Germacrene D	1482	–	–	–	0.47 (0.07)
β-Selinene	1479	–	–	–	1.20 (0.09)
Ledene	1485	6.72 (0.59)	1.90 (0.23)	2.00 (0.18)	0.94 (0.10)
γ-Elemene	1425	2.63 (0.19)	–	–	1.60 (0.14)
γ-Gurjunene	1477	9.40 (1.02)	9.97 (0.81)	8.40 (0.91)	10.08 (0.65)
Cadinene	1519	0.74 (0.08)	0.49 (0.06)	0.69 (0.08)	–
epi-Globulol	1564	–	0.81 (0.09)	1.26 (0.09)	–
α-Guaiene	1453	0.26 (0.03)	0.32 (0.05)	1.56 (0.11)	0.09 (0.02)
Spathulenol	1619	8.34 (0.51)	1.78 (0.23)	1.42 (0.17)	4.20 (0.51)
Valencene	1490	–	–	0.81 (0.09)	–
α-Muurolene	1523	1.38 (0.16)	0.42 (0.06)	0.49 (0.07)	0.17 (0.05)
Cedrenol	1604	1.53 (0.11)	–	–	–
β-Eudesmol	1654	–	–	2.14 (0.33)	–
γ-Cadinol	1658	0.32 (0.04)	–	–	0.66 (0.09)
Allo-aromadendrene	1546	1.44 (0.16)	1.02 (0.09)	1.21 (0.21)	–
Tetradecanoic acid	1767	–	–	0.34 (0.06)	0.05 (0.02)
Longipinocarvone	1559	1.32 (0.09)	–	–	1.48 (0.16)
n-Hexadecanoic acid	2380	–	–	0.13 (0.03)	0.08 (0.02)
Unknown	–	5.43 (0.46)	4.32 (0.31)	6.01 (0.59)	5.12 (0.44)

* As identified by GC×GC–TOF-MS software; names according to NIST mass spectral library, and by comparing their Kovats retention indices.
† RI = Retention index; Kovats retention indices (column: DB5).
‡ Percentage of each component is calculated as peak area of analyte divided by peak area of total ion chromatogram times 100 (In the case of multiple identification, the areas of the peaks that belong to one analyte were combined to find the total area for this particular analyte). The results are the mean of five experiments and the data mentioned in parentheses are the corresponding standard deviations (±) of the readings.
§ – = Not detected or percentage of the component is lower than 0.05%.

Conclusion

A wide range of organic compounds from fruit of *E. camaldulensis* trees, collected from four different geographical areas, were qualitatively and quantitatively analyzed using DTD coupled with GC×GC–TOF-MS. The highest volatiles yield (1.18%) was found in samples from Adrasan and the samples from Cesme contained the highest number of volatile compounds (59) found. It should be noted that DTD is not a feasible production technique for essential oils and/or flavour compounds. However it can be used to determine content in a production process very quickly using only a small amount of the starting sample and no pre-preparation. Commercial producers of essential oils could, therefore, apply this technique in selecting which part of the tree (leaf, flower, bark) at which time of year would produce the highest quality oil, as they would be able to see quickly if their desired components were present and in what quantity. The use of DTD for quantitation as well as qualification would represent a major and useful advancement in industry.

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