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Simultaneous Determination of Alkenyl Benzenes in Essential Oils and Human Serum by Gas Chromatrography and GC-MS

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A gas chromatrography and GC-MS method have been developed for the simultaneous determination of fragrance containing alkenyl benzenes in commercial oils, as well as percutaneous absorption in humans after the application of an essential oil formulation. Nine alkenyl benzenes derivatives (estragole, *trans*-anethole, safrole, eugenol, methyl eugenol, isoeugenol, eugenyl acetate, myristicin and α-asarone) and seven other fragrances could be separation on an DB-5 column. A hierarchical cluster analysis established based on the GC-MS data from 23 samples essential oils.

Key Words: Fragrance alkenyl benzenes, Essential oils, Hierarchical cluster analysis, Human serum.

INTRODUCTION

The structurally related substituted alkenyl benzene derivatives eugenol, methyl eugenol, estragole, safrole and myristicin, occur naturally in a variety of traditional food, particularly in spies such as clove,cinnamon and basil[1]. Some of them demonstrated to be an effective, inexpensive anesthetic agent, antioxidants and blood circulation enhancers[2,3]. The estragole, anethole and safrole determination were studied by headspace gas chromatograpgy (HS-GC)[4,5]. GC/mass spectrometry analysis of alkenyl benzenes such as asarone, methyl eugenol were studied[6-11]. Essential oils on the market were analyzed using GC and GC-MS and the main ingredients of each essential oil were quantified[12,13]. The hierarchic analysis of variance design was used to estimate variance components for essential oil have been investigated[14-20] based on GC and GC-MS data. The aim of this work is to determine the chemical composition and quality of the oils of commercial and the simultaneous determination of *p*-allyl benzenes derivatives in human serum. In addition, to examine the relationship between alkenyl benzenes in various species of essential oils.

EXPERIMENTAL

The substituted alkenyl benzene derivatives were obtained as following: Methyl eugenol (1,2-dimethoxyl-4-allylbenzene, Chem. Service, Inc., West Chester, PA);

Eugenol (1-hydroxy-2-methoxyl-4-allylbenzene, Acros Organics, Geel, Belgium); isoeugenol (1-hydroxy-2-methoxyl-4-propenyl benzene, Acros Organics, Geel, Belgium); *trans*-anethole (1-methoxyl-4-propenyl benzene, Acros Organics, Geel, Belgium); α-asarone (1,3,6-trimethoxyl-4-propenyl benzene, Acros Organics, Geel, Belgium); myristicin (6-methoxy-1,2-methylenedioxy-4-allyl benzene, Acros Organics, Geel, Belgium); safrole (1,2-methylenedioxy-4-allyl benzene, Fluka, Buchs, Switzerland); estrogole (1-methoxy-4-allylbenzene, Aldrich Chemical Co. Inc., Milwaukee, WI); acetyl eugenol (phenol, 2-methoxy-4-allyl acetate, TCI, Tokyokasei Co., Japan).

Cinnamyl alcohol and citral were obtained from Chem. Service, Inc., West Chester, PA. Geraniol and α-amylcinnamaldehyde were purchased from Fluka and Aldrich, respectively. Samples of 23 essential oils obtained local department store or retail grocern and drug food cosmetic outlet. They were labeled as 100 % natural products.

The analysis of the essential oils was carried out GC and GC-MS. The Hewlett-Packard Agilent 6890 chromatograph equipped with FID and capillary column (30 m × 0.32 mm, film thickness 0.25 μm) was used to for quantitative analysis. The oven temperature was programmed: held isothermal at 40 ºC for 2 min, then from 40-200 ºC at 10 ºC/min (stay in 80, 120, 140 and 200 ºC for 2 min), finally 20 ºC/min up to 300 ºC. Injector temperature, 300 ºC; detector temperature, 325 ºC; carrier gas nitrogen at a 2.1 mL/min; split, 1:50. Analysis by GC-MS was performed using a chromatograph Shimadzu QP-2010 mass spectrometer instrument at 70 eV and 250 ºC. GC column: DB-5 (5 % phenyl-methylpolysiloxane), fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The temperature was from 45-250 ºC at 5 mL/min. Helium gas was used as carrier at a flow rate of 0.82 mL/min. The percentrage composition of essential oils was computed from GC peak areas.

Cluster analysis: Cluster analysis was used to classify and group all the landraces according to their main components. Cluster analysis based on selected components (Table-1) was calculated using the Euclidean distance measure.

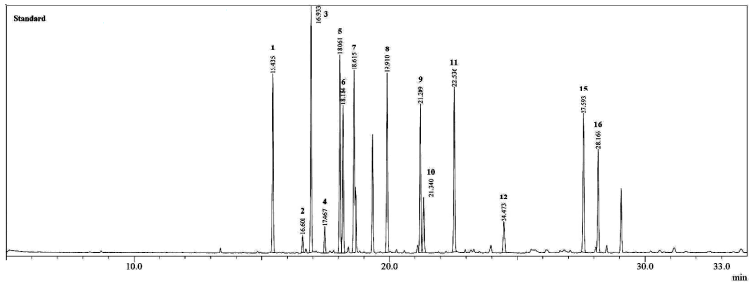
RESULTS AND DISCUSSION

Identification of components: Sixteen components of essential oils fragrances were identified by comparing the retention time and comparision of the obtained mass spectra of the chromatographic peaks with those of authentic standards and with spectra of the NIST 05 libraary. Quantitation ions and retention times are listed in Table-1. The chromatogram for the standard mixture is shown in Fig. 1(A). A representative clove essential oil was shown in Fig. 1(B). It shows one major peaks and six minor peaks. Identification of eugenol and other geraniol, methyl eugenol, *cis*-isoeugenol, acetyl eugenol, myristicin and α-asarone was made by comparing the retention times with those of authentic standards and by GC-MS quantitation ions (m/z).

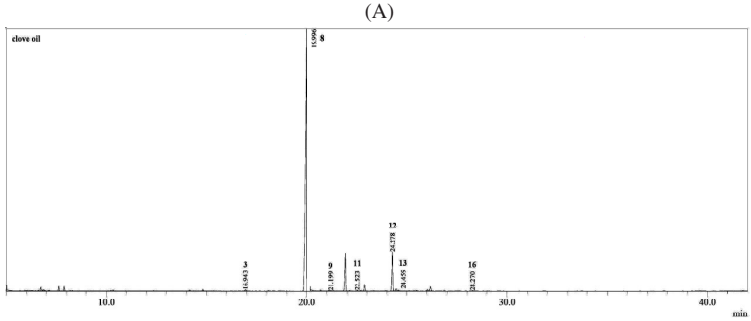
TABLE-1
ALKENYL BENZENES COMPONENTS OF ESSENTIAL OIL

Peak numbers	Fragrances	Retention times (min)	Relative content (%)	Quantitation ions (m/z)	Identification methods
1	Estragole	15.434	ND	148	GC, GC/MS
2	β-Citral	16.601	ND	154	GC, GC/MS
3	Geraniol	16.933	0.36	148	GC ,GC/MS
4	α-Citral	17.466	ND	148	GC, GC/MS
5	*trans*-Anethole	18.061	ND	148	GC, GC/MS
6	Safrole	18.184	Trace	162	GC, GC/MS
7	Cinnamyl alcohol	18.615	ND	92	GC, GC/MS
8	Eugenol	19.910	76.28	164	GC, GC/MS
9	Methyl eugenol	21.209	0.09	178	GC, GC/MS
10	*trans*-Isoeugenol	21.340	ND	164	GC, GC/MS
11	*cis*-Isoeugenol	22.532	0.14	164	GC, GC/MS
12	Acetyl eugenol	24.267	8.07	192	GC, GC/MS
13	Myristicin	24.476	0.44	208	GC/MS
14	Elemicin	25.080	ND	208	GC/MS
15	α-Amyl cinnaldehyde	27.590	Trace	202	GC, GC/MS
16	α-Asarone	28.165	0.08	208	GC,GC/MS

ND: Not determined.



(A)



(B)

Fig. 1. GC/MS chromatograms from standard solution (A) and commercial essential oil sample (B) Peak identification: (1) estragole; (2) β-citral; (3) geraniol;(4) α-citral; (5) *trans*-anethole; (6) safrole;(7) cinnamyl alcohol; (8) eugenol; (9) methyl eugenol (10) *trans*-isoeugenol; (11) *cis*-isoeugenol; (12) acetyl eugenol; (15) α-amyl cinnaldehyde; (16) α-asarone

Essential oil composition: Table-2 shows, 16 fragrances of various group samples essential oils collected in 2006 and 2007. The relative content of the essential oils composition (percentage of peak area) obtained by GC-MS, were statistically analyzed using cluster analysis, calculated by STATISTICA 6.0 software. The Euclidean distance was used to measure the similarity between samples and the Ward's method was used as an agglomerative algorithm. The dendrogram (Fig. 2) suggests the existence of two clusters (cluster I and II). The main alkenyl benzenes of nutmeg

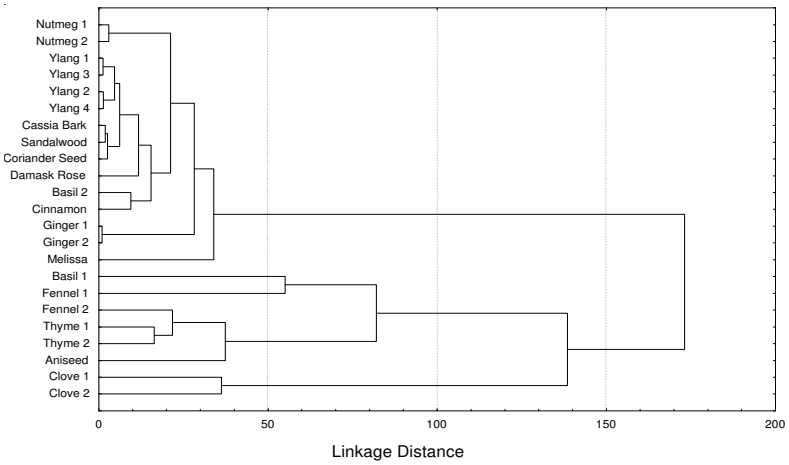


Fig. 2. Dendrogram obtained by cluster analysis of percentage composition of 23 samples of essential oils

and ylang six landraces (nutmeg 1,2, ylang 1,2,3,4) belong to cluster I is myristicin. The average myristicin content was much higher than the other essential oils, in which myristicin 9.29 and 2.09 % for nutmeg and ylang, respectively. The essential oils of four *Cinnamonum cassia*, *Santalum album*, *Coriandrum sativum* and *Rosa damascene* were clearly distant from the oils of other nineteen oils investigated (Table-2). They were characterized by a low content and few kinds of alkenyl benzenes. Significant differences were observed in the chemical composition of these oils of alkenyl benzenes below 1 %. The essential oils of cluster II are characterized by a higher content alkenyl benzene such as estragole (16.3-45.8 %), safrole (22.0-41.05 %) and eugenol (41.0-76.3 %) compared to cluster I. Eight landraces basil, fennel 1 and 2, thyme 1 and 2, aniseed and clove 1 and 2 belonged to this chemotype. Estragole type has been reported as the major constituent of the essential of *Ocimum basilicum*[19]. Major volatile constituents present in fennel (*Foeniculum vulgare*) are *trans*-anethole and estragole[4] but *Foeniculum dulceare* are safrole and estragole. The main alkenyl benzenes of four landraces (fennel 2, thymus 1and 2, aniseed) was safrole, in which safrole contents ranged from 22.0-59.8 % and average content was 38.1 %. Table-2 showed 7 alkenyl benzenes (*trans*-anethole, eugenol, methyl eugenol, isoeugenol, acetyl eugenol, myristicin and α-asarone) in clove essential

TABLE-2
FRAGRANCE CONTAINING ALKENYL BENZENES COMPONENTS
IN ESSENTIAL OILS MEASURED BY GC/MS

Essential oils	Compositions (%)
Myristica fragrans (Nutmeg 1)	0.19 Estragole, 0.24 *trans*-anethole, 1.81 safrole, 0.21 eugenol, 0.33 methyl eugenol, 0.09 *cis*-isoeugenol, 10.73 myristicin, 0.17 elemicin
Myristica fragrans (Nutmeg 2)	0.30 Estragole, 1.64 safrole, 0.24 eugenol, 0.30 methyl eugenol, 0.36 acetyl eugenol, 7.86 myristicin,0.12 elemicin
Cananga odorata (Ylang-Wild 1)	0.28 Eugenol, 0.15 acetyl eugenol, 2.91 myristicin, 1.29 α-amyl cinnaldehyde, 0.25 elemicin
Cananga odorata (Ylang 3)	0.85 Geraniol, 0.32 eugenol, 0.14 *cis*-isoeugenol, 2.03 myristicin, 1.27 α-amyl cinnaldehyde
Cananga odorata (Ylang-Wild 2)	0.11 Geraniol, 0.11 *trans*-anethole, 2.24 *cis*-isoeugenol, 2.04 myristicin, 1.18 α-amyl cinnaldehyde, 0.17 α-asarone
Cananga odorata (Ylang 4)	0.37 Geraniol, 0.16 *trans*-anethole, 3.38 *cis*-isoeugenoll, 1.39 myristicin, 1.00 α-amyl cinnaldehyde
Cinnamonum cassia (Cassia Bark)	0.68 β-Citral, 0.15 *trans*-anethole, 0.09 cinnamyl alcohol, 0.51 *cis*-isoeugenol
Santalum album (Sandalwood)	0.31 Acetyl eugenol, 1.65 α-asarone
Coriandrum sativum (Coriander Seed)	0.40 Estragole, 1.93 geraniol
Rosa damascene (Damask Rose)	0.19 β-Citral, 7.97 geraniol, 0.17 α-citral, 0.75 eugenol, 0.80 methyl eugenol
Ocimum basilicum (Basil 2)	1.15 Estragole, 0.26 geraniol, 1.26 *trans*-anethole, 9.24 eugenol, 3.26 α-amyl cinnaldehyde
Cinnamonum zeylanicum (Cinnamon)	1.87 Estragole, 0.20 α-citral, 0.32 cinnamyl alcohol, 3.58 eugenol, 6.68 *cis*-isoeugenol, 0.24 acetyleugenol
Zingiber officinalis (Ginger root1)	0.55 Estragole, 0.29 geraniol, 0.35 α-citral, 0.26 safrole, 0.33 *trans*-isoeugenol, 9.86 acetyl eugenol, 0.35 myristicin
Zingiber officinalis (Ginger 2)	0.72 Estragole, 0.29 α-citral, 0.27 *trans*-anethole, 0.28 Safrole, 0.39 *trans*-isoeugenol, 10.68 acetyl eugenol, 0.37 myristicin
Melissa officinalis (Melissa)	0.26 Estragole, 12.33 α-citral, 7.27 geraniol, 15.86 α-citral, 0.39 *cis*-isoeugenol
Ocimum basilicum (Basil sweet 1)	45.81 Estragole, 0.48 α-citral, 0.08 geraniol, 0.71 α-citral, 0.07 eugenol,
Foeniculum vulgare (fennel 1)	4.52 Estrogle, 0.12 geraniol, 36.46 *trans*-anethole
Foeniculum dulce (fennel 2)	1.35 Estragole, 0.16 geraniol, 41.29 safrole
Thymus vulgaris (Thyme 1)	19.02 Estragole, 29.39 safrole, 0.11 eugenol
Thymus vulgaris (Thyme 2)	4.45 Estragole, 0.24 geraniol, 22.02 safrole
Pimpinella anisum (Aniseed)	16.27 Estragole, 59.77 safrole, 0.25 myristicin
Eugenia caryophyllata (Clove 1)	0.07 Geraniol, 0.03 *trans*-anethole, 41.13 eugenol, 0.03 methyl eugenol, 0.23 *trans*-isoeugenol, 0.08 *cis*-isoeugenol, 0.01 acetyl eugenol, 3.94 myristicin, 0.02 α-amyl cinnaldehyde, 0.08 α-asarone
Sygygium aromaticum (Clove 2)	0.36 Geraniol, 76.28 eugenol, 0.09 methyl eugenol, 0.14 *cis*-isoeugenol, 8.07 acetyl eugenol, 0.44 myristicin

Nutmeg 1 and 2: different lot with the same manufacturer; Ylang 1 and 2: different lot with the same manufacturer; Ylang 3 and 4: different lot and manufacturer; Basil 1 and 2: different lot and manufacturer; Ginger 1 and 2: different lot and manufacturer; Fennel 1 and 2: different lot and manufacturer; Thyme 1 and 2: different lot and manufacturer; Clove 1 and 2: different lot and manufacturer.

oil (*Eugenia caryophyllata*). Eugenol has been detected as the main component in clove essential oil (41.1-76.3 %). This was in agreement with previous data on the content of eugenol in clove oil[21,22].

Serum analysis of alkenyl benzenes: After percutaneous absorption experiments with clove essential oil creams, main fragrance eugenol and low concentrations of geraniol, methyl eugenol, *cis*-isoeugenol, acetyl eugenol, myristicin could be detected in the serum, but only trace of α-asarone could not be detected and shown in Fig. 3. The following average concentrations in serum could be found as follows: eugenol 222 ± 34, geraniol 6.18 ± 0.67, methyl eugenol 0.74 ± 0.08, *cis*-isoeugenol 1.87 ± 0.69, acetyl eugenol 30.2 ± 11 and myristicin 12.8 ± 1.6 ng/mL, respectively. Fig. 4 shows the GC/MS chromatogram of human serum after treated of a clove essential oil creams to skin. The major three peaks, *i.e*. eugenol, acetyl eugenol and myristicin and two trace peaks, *i.e*. safrole and α-amyl cinnaldehyde, can be verified by their retention times and MS data. Fig. 5 shows the human serum eugenol concentration-times profiles after treated with a clove essential oil w/o micro-emulsion, o/w-emulsion and nano-emulsion. The percutaneous absorption most of eugenol resulted in a rise in serum levels, which reached a maximum concentration 120-180 min after cutaneous treatment with w/o, o/w and nano-emulsions of clove essential oil; serum levels then declined about 180 min. From the data, serum eugenol concentration in nano- emulsions is higher than the others. It is possible to conclude that the nano-emulsion formulation is an efficacious carrier for the *trans*-dermal delivery of eugenol.

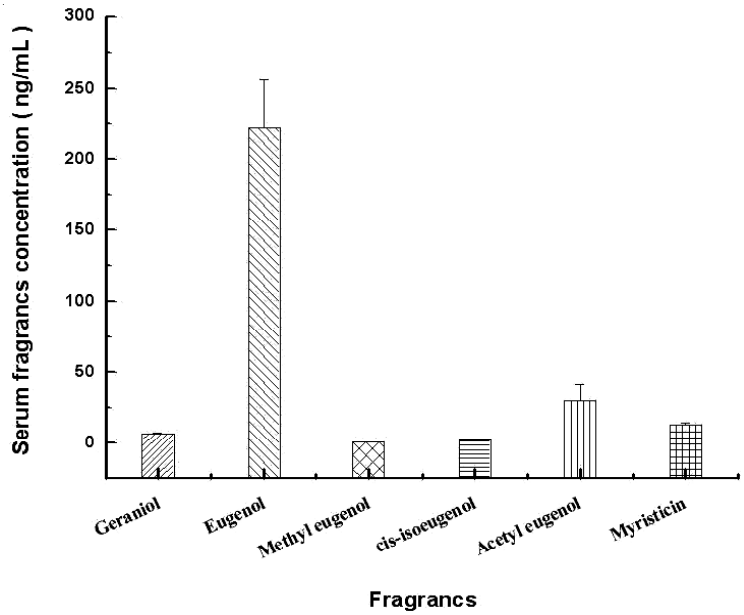


Fig. 3. Concentrations of fragrances in blood samples after application of clove essential oil formulation

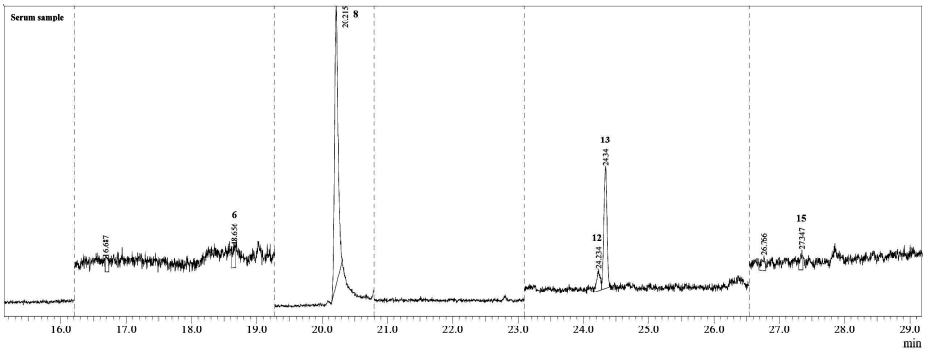


Fig. 4. GC/MS chromatogram of human serum after treated of a clove essential oil cream to skin

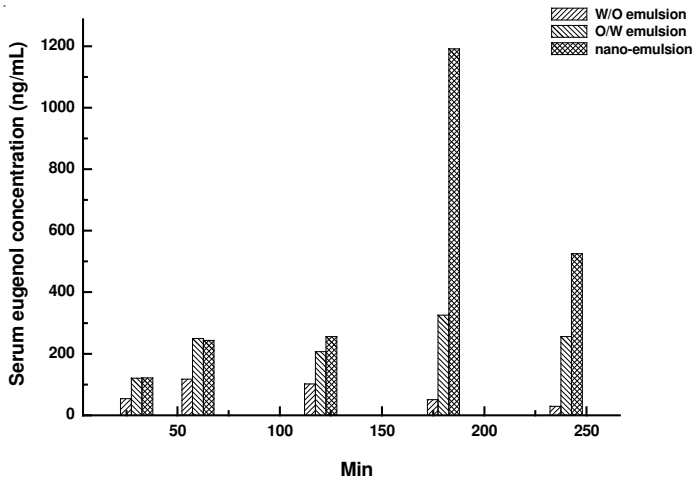


Fig. 5. Time course of percutaneous absorption of eugenol in human serum after treated with a clove essential oil w/o micro-emulsion, o/w-emulsion and nano-emulsion

Conclusion

In 23 essential oil samples, we identified seven different alkenyl benzenes (*trans*-anethole, eugenol, methyl eugenol, isoeugenol, acetyl eugenol, myristicin and α-asarone) and three sensitizing fragrances including geraniol, α-amylcinnamic aldehyde and citral. Low contents of alkenyl benzenes in *Cinnamonum cassia*, *Santalum album*, *Coriandrum sativum* and *Rosa damascene* indicated that these essential oils could be considered as a flowering and fragrance agent in food and perfume industries.

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