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Extended Abstract
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Pharma Sci-Study the Photochemical of Fragrance Allergens of Eugenol Derivatives in Commercial Essential Oils and Containing Clove Drugs Using Gas Chromatography and Liquid Chromatography-Mass Spectrometry- Lai-Hao Wang- Chia Nan University of Pharmacy and Science

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Introduction

Fragrances may contain synthetic aromatic compounds (such as coumarin, nitro musks) as well as compounds of natural origin. Fragrances/flavors are used in a wide selection of products such as foods, medicines, cosmetics, and household products. Approximately 20% of essential oils consumed as fragrances in perfumery and cosmetics, 15% for the isolation of components and 5% medicine for aromatherapy. The majority (55%) of essential oils are utilized by the food industry for the production of aromatic extracts. The analytical control of essential oils is necessary to ensure the quality, shelf-life and storage conditions of products. Today, the gas chromatographic technique is almost exclusively used for the determination of essential oils. The scientific committee on cosmetic products and non-food products (SCCNFP) has selected 26 compounds causing contact allergies. In the European Union (EU) these “26 specific fragrance ingredients” require labeling on cosmetic and detergent products. Several methods have been proposed to identify fragrance allergens and determine their levels in essential oils, cosmetic products, and environmental samples (water and indoor air). Fragrances that are air-sensitive may form peroxides, respiratory irritants, and aerosol particles that cause inflammatory responses in the lungs. Exposure to fine particulate matter (aerosol) poses a significant health concern by increasing mortality and morbidity. According to previous studies, the terpenoids from essential oils emission participate in photochemistry reactions caused by aerosol products. Because essential oil contain unsaturated and cyclic terpenes, including alpha-pinene, d- limonene, linalool and geraniol which are found in detergents, fragrances and some other consumer products have been detected in indoor air. They can also form secondary organic aerosol (SOA) via reaction with ozone. Photochemistry of some allylic compounds such as cis-trans isoeugenol and citral, were oxidized in the presence of atmospheric oxygen or photosensitized species (O3, OH, NO3 etc). However, most reports have focused on photochemical reaction of eugenol derivatives to synthesis of new flavors chemicals. The intermediates of these photochemical products can percutaneous absorption or inhalation into human body. Liquid chromatography-mass spectrometry used in order to identify major metabolites of eugenol ester in rats. This study aimed to find out richer content suspected fragrance allergens in commercial essential oils and containing essential oil drugs. Then to study the effect of UV-B light irradiation of essential oils substrate, and the antioxidation effectiveness of eugenol derivatives in this process. Also, we investigated possible intermediates of photochemical reaction under the irradiation of the ultraviolet light.

This work is partly presented at World Congress on Advances in Pharmaceutical Sciences

Experimental Section

Materials and Apparatus

Sample material and fragrances: The commercial essential oils investigated were purchased at a local department store or retail grocery and drug food cosmetic outlet. Pure standard substances were purchased from biomedical supply houses: eugenol, isoeugenol and dihydrocoumarin from Acros Organics (Geel, Belgium); and trans-cinnamaldehyde, methyl eugenol, acetate eugenol and α-hexylcinnamadehyde, from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Cinnamyl alcohol and citral were obtained from Chem Service, Inc, West Chester, PA. Geraniol and coumarin were purchased from Fluka and Sigma-Aldrich, respectively. Hydroxycitronellal were purchased from MP Bio medicals, LLC.

The mixture fragrances were determined using a gas chromatography-flame ionization detector (GC-FID) (Model GC-2014 GC; Shimadzu Technologies). Fragrances were separated using an Agilent HP-5 column (60 m × 0.32 mm, film thickness 0.25 µm). A high-performance liquid chromatography (HPLC) system (Simadzu pump Model LC-10AD); Kyoto, Japan) containing a Rheodine 7125 injection valve with a 20 µl sample loop coupled to a high capacity ion trap mass spectrometer (HCT/MS) and high performane ESI (Electrospray ionization)-Ion Trap MSn system (Bruker corporation) and a spectrophotometric detector (L- 7420; Hitachi, Japan) was used.

Determining fragrance allergens using GC: The analysis of the essential oils and drug was carried out GC. The Shimadzu GC-2014 chromatograph equipped with FID and Agilent HP-5 column (60 m × 0.32 mm, film thickness 0.25 µm) was used to for quantitative analysis. The oven temperature was programmed: held isothermal at 100ºC for 2 min, then from 100°C-140°C at 5°C/min (stay in 130°C for 4 min, 140°C for 5 min), finally 10°C/min up to 200°C, injector temperature, 300°C; detector temperature, 300°C.

Photochemical reaction of isoeugenol: The solutions (10 ml) containing isoeugenol placed in photochemical reactor (SYSTEMS PR-2000), were irradiated for various periods (0-10 hr) in a container (glass, pyrex glass and quartz) by using a 310 nm lamp.

Results and Discussion

Screening of Commercial Essential Oils and Containing Clove Drugs

11 well-known sensitizing fragrance substances were selected: Eugenol, isoeugenol, geraniol, hydroxycitronellal, α-amylcinnamic aldehyde, cinnamaldehyde, cinnamic alcohol, citral, coumarin, dihydrocoumarin, and α-hexylcinnamaldehyde. They had been investigated 42 cosmetics products and caused of allergic contact dermatitis. In addition, suspected allergens such as methyl eugenol were tested for toxicity/carcinogenicity. The 12 studied fragrance allergens (SCCNFP/0017/98- most frequently reported allergens), their calibration data as well as the retention times are summarized in (Table 1). The GC method was applied to the analysis of 16 essential oils and 5 containing clove oil drugs of fragrance allergens in commercial samples and identified by comparing the retention time of the chromatographic peaks with those of authentic standards. In the essential oil, whose chromatogram is depicted in (Figure 1), five labeled allergens were detected, corresponding to eugenol (peak 7), methyl eugenol (peak 9), cis-isoeugenol (peak 10), trans-isoeugenol (peak 12), eugenyl acetate (peak 13). Chromatographic measurements were performed using sample by acquiring a regression equation (Table 1) which represents the relationship between the peak

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intensity (peak area) in potential mode of target analyte and concentration from chromatogram of fragrances standards whose concentration are already known. The analytical results were given in (Tables 2 and 3).

Table 1. Statistical evaluation of the calibration data obtained by GC-FID.

Fragrances	y=a+bX'	r §	Retention time
cis-Citral	y=3853+2939 x	0.9998	10.55
trans-Citral	y=-1167+5505 x	0.9995	11.44
Geraniol	y=-5439+11356 x	0.9997	10.84
trans-cinnamaldehyde	y=9255+10499 x	0.9988	11.64
Hydroxycitronellal	y=-2710+9684 x	0.9999	11.95
Cinnamic alcohol	y=-13900+12902 x	0.9993	12.77
eugenol	y=- 586 +11325 x	0.9999	14.62
Dihydrocamarin	y=-1005 +10279 x	0.9999	15.85
Methyl eugenol	y=- 9097 +13277 x	0.9998	16.41
cis-isoeugenol	y=-4057+877 x	0.9983	16.72
trans-isoeugenol	y=3475+8045 x	0.9995	18.75
Coumarin	y=-4241.6+11790 x	0.9999	18.37
Eugenyl acetate	y=-6601+10199 x	0.9999	21.93
α-Hexylcinnamaldehyde	y=154+13285 x	0.9999	27.64
N=Determinations, a=intercept on the ordinate, b=slope, §=correlation coefficient, '=range of linearity (µg mL^{-1}).			

Table 1. Statistical evaluation of the calibration data obtained by GC-FID.

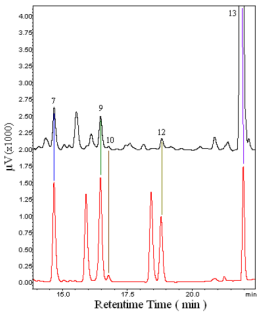

Figure 1. Typical gas chromatogram of non-irradiated myristica fragrans essential oil (upper) and standard solution (under). Peak identification: 7, Eugenol; 9, Methyl eugenol; 10, cis-isoeugenol; 12, trans-isoeugenol; 13, eugenyl acetate (peak over in Essential oil).

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Table 2. Analytical results of determining fragrances levels in commercial essential oils using gas chromatography with flame ionization detector (GC-FID).

Essential oils	Content (w/w, %) N=5										
	E	M.E	A.E	IsoE (cis)	IsoE (trans)	α-H	Ge	Co	C (cis)	C (trans)	Cin
Myristica fragrans (N1)	0.37 ± 0.03	0.23 ± 0.01	14.32 ± 1.32	----*	-----	----	----	----	----	----	----
Myristica fragrans (N2)	0. 47 ± 0. 03	35.0 ± 2.0	20.87 ± 0.47	0.24 ± 0.01	0. 25 ± 0.01	----	----	----	----	----	----
Cananga odorata (Y-W1)	0. 45 ± 0. 16	-----	6.40 ± 0. 25	0.12 ± 0.01	1.16 ± 0.04	----	0.57 ± 0.07	----	----	----	----
Cananga odorata (Y-W2)	1.81 ± 0. 91	-----	4.11 ± 0.05	0.46 ± 0.02	6.30 ± 3.96	----	0.22 ± 0.01	----	----	----	----
Cananga odorata (Y1)	0. 45 ± 0.03	-----	4.78 ± 0. 36	0.12 ± 0.02	3.52 ± 0.17	----	1.04 ± 0.06	----	----	----	----
Cananga odorata (Y2)	1.80 ± 0. 49	-----	2.47 ± 0. 06	0.17 ± 0.02	2.10 ± 0.03	----	0.40 ± 0.01	----	----	----	----
Ocimum basilicum (B1)	9.73 ± 0. 55	0. 27 ± 0. 01	0. 88 ± 0. 06	0.18 ± 0.002	0. 89 ± 0.11	----	----	----	0.85 ± 0.17	0.89 ± 0.12	----
Ocimum basilicum (B2)	-----	0. 54 ± 0. 06	-----	-----	-----	----	0.34 ± 0.02	0.86 ± 0.04	----	----	----
Cinnamonum zeylanicum (Cin)	5.51 ± 0. 01	2.81 ± 0. 12	0. 55 ± 0. 01	-----	-----	----	----	----	----	----	0.73 ± 0.03
Syzygium aromaticum (C1)	89.55 ± 0.76	0.08 ± 0.002	4.10 ± 0.11	3.23 ± 0.13	1.75 ± 0.11	----	0.08 ± 0.01	----	----	----	----
Syzygium aromaticum (C2)	98.67 ± 4.56	0. 36 ± 0. 03	4.33 ± 0. 22	2.53 ± 0.02	1.60 ± 0.06	----	0.15 ± 0.02	----	----	----	----
Zingiber officinalis (Gr1)	-----	-----	20.56 ± 0. 96	-----	-----	0.18 ± 0.03	0.44 ± 0.01	----	0.66 ± 0.03	0.62 ± 0.06	----
Zingiber officinalis (G2)	-----	-----	16.98 ± 0.41	-----	-----	0.11 ± 0.01	0.78 ± 0.03	----	0.32 ± 0.10	0.43 ± 0.03	----
Santalum album (S)	-----	-----	1.59 ± 0. 11	-----	-----	----	----	----	----	----	----
Melissa officinalis (M)	0.33 ± 0.02	-----	0.76 ± 0.04	-----	-----	----	8.73 ± 0.22	----	28.51 ± 9.65	8.47 ± 5.33	----
Rosa damascene (DR)	0.29 ± 0.01	0.25 ± 0.03	-----	-----	-----	----	2.11 ± 0.06	----	----	----	----
N=Determinations ---a: Not determined Nutmeg 1 and 2 (N1.N2): different lot with the same manufacturer; Y lang 1 and 2 (Y1.Y2): different lot with the same manufacturer; Basil 1 and 2 (B1. B2): different lot and manufacturer; Clove 1 and 2 (C1.C 2): different lot and manufacturer. Ylang-Wild (Y-W), Cinnamon (Cin), Clove (C), Ginger (G), Sandalwood (S), Melissa (M), Damask rose (DR), Eugenol (E), Methyl eugenol (ME), Acetyl eugenol (AE), Isoeugenol (cis), IsoE (cis), Isoeugenol (trans) IsoE (trans),α-Hexylcinnamal (α-H), Geraniol (Ge), Coumarin (Co), Citral (cis) C (cis), Citral (trans) C (trans), Cinnamic (Cin).										

Table 2. Analytical results of determining fragrances levels in commercial essential oils using gas chromatography with flame ionization detector (GC-FID).

Table 3. Analytical results of determining fragrances levels in commercial containing clove oil drugs using gas chromatography with flame ionization detector (GC-FID).

Containing clove oil drugs	Content (w/w, %) N =3				
	Eugenol	Methyl eugenol	Geraniol	Coumarin	trans-cinnamaldehyde
Toothache waters (0.4 mL/3 L)	20.94 ± 0.45	0.23 ± 0.01	----*	-----	-----
Herbal cream (40 mg/g)	2.68 ± 0. 07	-----	-----	-----	-----
Balm 1 (5 %/g)	4.69 ± 0. 38	-----	-----	-----	3.80 ± 0.34
Balm 2 (40 mg/g)	2.94 ± 0. 12	-----	-----	-----	-----
Armatic oil (12.5 mg/g)	0.95 ± 0.07	0.38 ± 0.03	0.78 ± 0.13	0.62 ± 0.04	-----
N=Determinations ---a: Not determined.					

Table 3. Analytical results of determining fragrances levels in commercial containing clove oil drugs using gas chromatography with flame ionization detector (GC-FID).

Various the extraction methods such as headspace, liquid extraction, solid-phase extraction, solid-phase micro extraction, sorptive tape extraction, ultrasound-assisted extraction, pressurized-liquid extraction and matrix solid phase dispersion extraction (MSPD) for determination of 26 fragrance allergens in essential oils, cosmetics, indoor air and environmental water samples. Mass spectrometry (MS) detectors are frequently

employed in perfume analysis laboratories when trace-level analysis is required. Therefore, GC or GC-GC in combination with MS has been investigated in the last decade for the detection of trace-level amounts of perfume in complex matrices. However, our purpose was to evaluate whether commercial essential oils and containing essential oil drug have present well-known or suspected sensitizing fragrance substances. Then, we selected 7 essential oils based on eugenol derivatives for more content can be used to process photo-irradiation.

Fragrances stability: Since, eugenol derivatives (eugenol and isoeugenol) are widely used as fragrances in cosmetics and perfumes and have antioxidant and antimicrobial properties, its quality at the time of use becomes extremely important. Eugenol is also a major Component of clove essential oil, which has been widely used as a herbal drug. It has antioxidant, anti-inflammatory and anesthetic activity, which could improve diabetic vascular and nerve function in streptozotocin-induced diabetic rat, and can be used as an analgesic in dental materials. Methyl eugenol occurs naturally in a variety of traditional foods, mainly in spices.

Storage conditions where elevated temperature, light, moisture and oxygen exist induce the auto-oxidation of fragrances. To ensure the stability of fragrances used in the product, it is also very important to previous understand the effect of the other ingredients used, as well as the effects of heat, light and temperature etc. Therefore, eugenol derivatives were kept for one month, at temperatures of 4°C and 28°C in the dark and chromatograms were recorded. Reverse phase (RP)-HPLC was done on a 5µm HPLC column (250 mm × 4.6 mm) (Luna C18; Phenomenex, Torrance, CA) eluted, during the mobile phase, with methanol-water ratios (40:60, v/v) containing 0.1% formic acid at a flow rate of 1 ml/min. After the sample components had been separated on the column, they were examined using an ultraviolet detector set at 240 nm. Eugenol derivatives were determined at defined time intervals (0, 1, 2, 3, 4, 5 weeks). The peak areas remained almost constant over the whole range of temperatures, expect eugenyl acetate which induced a major eugenol degradation product, and the amount of eugenol increased about 4-7 times at 28°C than that 4°C. To evaluate method linearity and precision, a calibration study was performed using UV and MS detector combined with LC pump to confirm peak. UV and MS detector are not entirely satisfactory for isomers such as isoeugenol, which have the same wavelength and mass spectra data (m/z). From (Figure 2) calibration graphs/curves for every analyte 5 overlapping peaks can be calculated to regression mode (the leastsquares line) for determining before and after storage of eugenol derivatives concentrations.

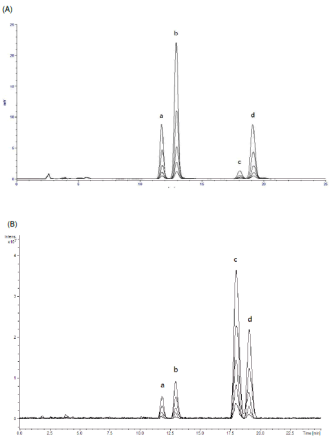

Figure 2. Chromatograms obtained by (A) LC-UV (B) LC-MS; (a) eugenol (b)isoeugenol ;(c) eugenyl acetate (d)methyl eugenol, Stationary phase, Phenomenex C18 column (250 mm x 4.6 mm i.d.); mobile phase, methanol- water (60 : 40, v / v); flow rate 1.0 mL/min.

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Isomerization was dependent upon the wavelength and intensity of the UV light. With 310 nm light

and three reactor (quartz, Pyrex glass, and glass container), respectively which had a very high peak area of cis-isoeugenol; isomerization took place between 5 and 10 min. The amount of cis-isoeugenol increased 6-10 times but trans-isoeugenol apparently decreased. When trans/cis – isomerization proceed between 20 and 30 min which cis-isoeugenol will not increase and the amount of trans-isoeugenol arrive at stability. From the (Figure 3) can been seen, the order of isomerization rate is quartz > Pyrex glass > glass.

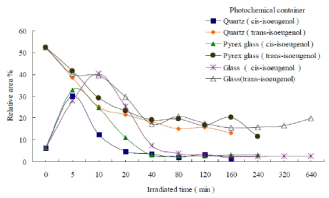

Figure 3. Quantitative changes of Z/E isomers in isoeugenol (1:10 mixture of cis and trans isomers) upon 310 nm irradiation over time.

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Radiation with eugenol derivatives and essential oils: Isoeugenol was found to be unstable to photochemical degradation as more than 50% degradation was seen after exposing to 310 nm (light UV-B) for 10 min forming major degradation (Figure 4A).

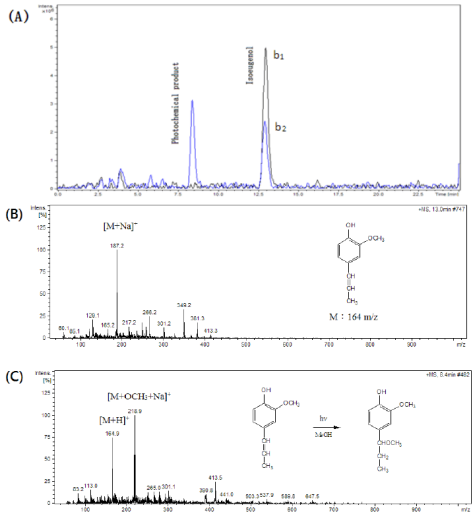

Figure 4. LC- MS chromatogram (A) isoeugenol before (b1) after (b2) 10 min upon 310 nm irradiation, and mass spectrome identify of (B) before Photochemical reaction; (C) after photochemical reaction. ESI–MS in positive mode.

Figure 4. LC- MS chromatogram (A) isoeugenol before (b1) after (b2) 10 min upon 310 nm irradiation, and mass spectrometry was used to identify of (B) before Photochemical reaction; (C) after photochemical reaction. ESI–MS in positive mode.

Hydroxylic solvents were found to add to the exocyclic double bond. Thus when isoeugenol was irradiated in methanol the major product was the methanol adducts m/z 219 (Figure 4C). Eugenol, methyleugenol and eugenyl acetate using irradiated conditions were the same as isoeugenol and shown in (Figures 5A-5C). The effect of light induced oxidation occur on myristica fragrans (Nutmeg 1), myristica fragrans (Nutmeg 2), cananga odorata (Ylang 2), ocimum basilicum (Basil 1), sygygium aromaticum (Clove 1), cinnamonum zeylanicum (Cinnamon) and cananga odorata (Ylang 1) when the light source turned on. From (Figures 6A-6D) can been seen, eugenol derivatives in essential oils have the same as their photochemical products with standard eugenol derivatives. GC analyses proved that clove oil is richer in eugenol (89%) than nutmeg oil (0.47%). The content of eugenol apparently decreased with photo-irradiated time in nutmeg oil than that clove oil. The content of photochemical products limited increased with photo-irradiated time in both essential oils. The content of eugenol and product were almost equal at 320 min in nutmeg oil (Figure 7A). However, the content of isoeugenol (no matter cis/trans) was higher than that product when cis form transferred to Trans form then not easily further degradation (Figure 7B). Compare with these data showed that eugenol was easily degradation than that isoeugenol during the irradiation. From Figure 7C can been seen methyleugenol and product were bitty change in nutmeg oil.

Conclusions

On the basis of the kinetic data of accelerated oxidation (UV light irradiation) of essential oils it is found out that clove oil contain two natural inhibitors- eugenol and isoeugenol. It has been shown that both inhibitions have photo stabilizing properties, but isoeugenol is better than eugenol and the same as in the literature. Therefore, clove oil was suitable as active substance in drug formulation.

Acknowledgements

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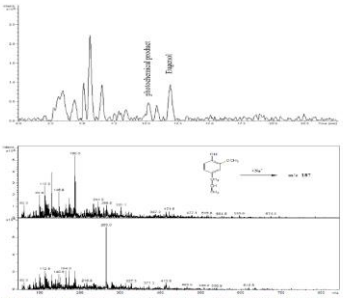

Figure 5A. Eugenol after 120 min photochemical reaction (upper) LC- MS chromatogram; Peaks, (a) eugenol (RT 11.9 min), photochemical product (RT10.2min.); (under) mass spectrometry of eugenol and photochemical product.

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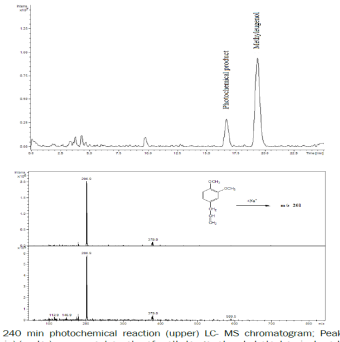


Figure 5B. Methyleugenol after 240 min photochemical reaction (upper) LC- MS chromatogram; Peaks, methyleugenol (RT 19.4 min), photochemical product (RT 16.6 min) (under) mass spectrometry of methyleugenol and photochemical product.

Figure 5B. Methyleugenol after 240 min photochemical reaction (upper) LC- MS chromatogram; Peaks, methyleugenol (RT 19.4 min), photochemical product (RT 16.6 min) (under) mass spectrometry of methyleugenol and photochemical product.

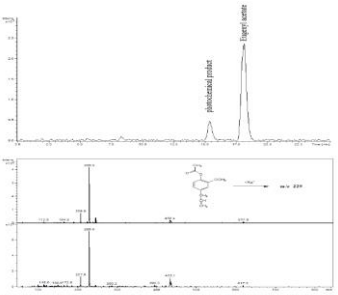


Figure 5C. Eugenyl acetate after 20 min photochemical reaction (upper) LC-MS chromatogram; Peaks, eugenyl acetate (RT 18.1 min), photochemical product (RT 8.4 min (under) mass spectrometry of eugenyl acetate and photochemical product.

Figure 5C. Eugenyl acetate after 20 min photochemical reaction (upper) LC-MS chromatogram; Peaks, eugenyl acetate (RT 18.1 min), photochemical product (RT 8.4 min (under) mass spectrometry of eugenyl acetate and photochemical product.

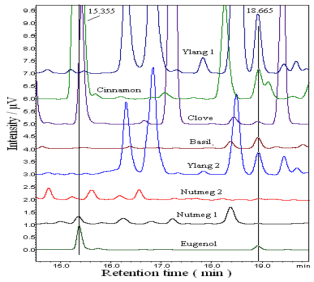
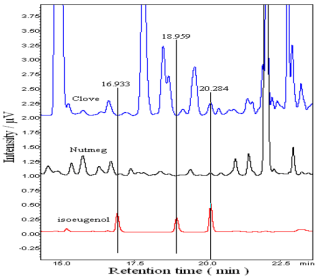


Figure 6A. Gas chromatograms after photo- irradiation of commercial essential oils and eugenol; Peaks: eugenol (RT 15.355 min); photochemical product (RT 18.665 min).

Figure 6A. Gas chromatograms after photo-irradiation of commercial essential oils and eugenol; Peaks: eugenol (RT 15.355 min); photochemical product (RT 18.665 min).



igure 6B. Gas chromatograms after photo- irradiation of commmercial essential oils and isoeugenol ; Peaks: cis-isoeugenol (RT 16.933 min), rans-isoeugenol (RT18.959 min), photochemical product (RT 20.284 min).

Figure 6B. Gas chromatograms after photo-irradiation of commmercial essential oils and

isoeugenol ; Peaks: cis-isoeugenol (RT 16.933 min), trans-isoeugenol (RT18.959 min), photochemical product (RT 20.284 min).

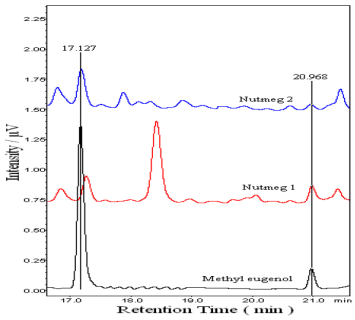


Figure 6C. Gas chromatograms after photo- irradiation of commercial essential oils and methyleugenol Peaks: methyleugenol (RT 17.1 photochemical product (RT 20.968 min).

Figure 6C. Gas chromatograms after photo-irradiation of commercial essential oils and methyleugenol Peaks: methyleugenol (RT 17.127 min), photochemical product (RT 20.968 min).

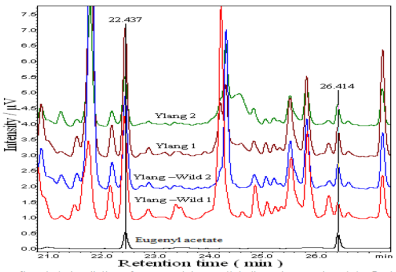


Figure 6D. Gas chromatograms after photo-irradiation of commercial essential oils and eugenyl acetate; Peaks: eugenyl acetate (RT 22. min); photochemical product (RT 26.414 min).

Figure 6D. Gas chromatograms after photo-irradiation of commercial essential oils and eugenyl acetate; Peaks: eugenyl acetate (RT 22.437 min); photochemical product (RT 26.414 min).

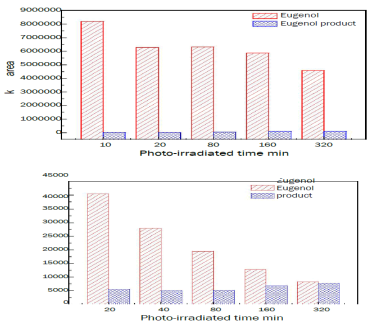


Figure 7A. Time course of content of eugenol and its photochemical product in (upper) clove oil (under) nutmeg oil after treatment with photo-irradiation.

Figure 7A. Time course of content of eugenol and its photochemical product in (upper) clove oil (under) nutmeg oil after treatment with photoirradiation.

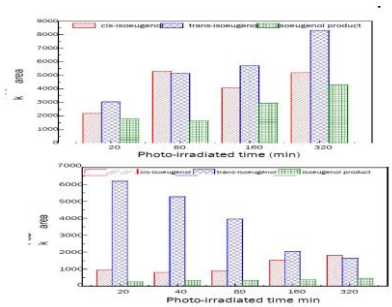


Figure 7B. Time course of content of isoeugenol and its photochemical product in (upper) clove oil (under) nutmeg oil after treatment with photo-irradiation.

Figure 7B. Time course of content of isoeugenol and its photochemical product in (upper) clove oil (under) nutmeg oil after treatment with photo-irradiation.

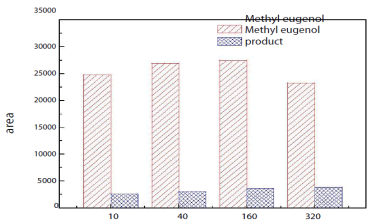


Figure 7C. Time course of content of methyl eugenol and its photochemical product in nutmeg oil after treatment with photo-irradiation.

Figure 7C. Time course of content of methyl eugenol and i ts photochemical product in nutmeg oil after treatment with photo-irradiation.

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