

Thermo-Oxidative Decomposition of Lovage (*Levisticum officinale*) and Davana (*Artemisia pallens*) Essential Oils under Simulated Tobacco Heating Product Conditions *

by

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SUMMARY

The thermo-oxidative decomposition of lovage (*Levisticum officinale*) and davana (*Artemisia pallens*) essential oils has been studied by pyrolysis-gas chromatography/mass spectrometry in 9% oxygen and 91% nitrogen atmosphere at 300 °C to simulate low-temperature tobacco heating conditions. Both lovage and davana oils contain numerous chemical substances; the main components of both oils are various oxygen-containing compounds. Isobenzofuranones are the most important constituents of lovage oil, and their relative intensity changed significantly during oxidative pyrolysis. (Z)-ligustilide underwent two kinds of decomposition reactions: an aromatization reaction resulting in the formation of butylidenephthalide and the scission of the lactone ring with the elimination of carbon dioxide or carbon monoxide. Davanone is the main component of davana oil, which did not decompose considerably during low-temperature oxidative pyrolysis. However, the relative yield of the second most intensive component, bicyclogermacrene, reduced markedly due to bond rearrangement reactions. Davana ether underwent oxidation reactions leading to the formation of various furanic compounds. The changes in the composition of both essential oils could be

interpreted in terms of bond splitting, intramolecular rearrangement mechanisms and oxidation reactions of several constituents during low-temperature oxidative pyrolysis. The applied thermo-oxidative method was found to be suitable to study the stability of the essential oils and monitor the decomposition products under simulated tobacco heating conditions. In spite of the complicated composition of the essential oils, no evidence for interaction between the oil components was found. [Beitr. Tabakforsch. Int. 29 (2020) 27–43]

KEY WORDS

Flavour; lovage oil; davana oil; gas chromatography/mass spectrometry (GC/MS); oxidative pyrolysis.

ZUSAMMENFASSUNG

Entsprechend den Bedingungen, die bei niederen Temperaturen beim Erhitzen von Tabak gegeben sind, wurde der thermisch-oxidative Abbau der ätherischen Öle des Liebstöckels (*Levisticum officinale*) und der Davana (*Artemisia*

pallens) in einer Atmosphäre von 9% Sauerstoff und 91% Stickstoff bei 300°C mittels der Kombination Pyrolyse-Gaschromatographie/Massenspektrometrie untersucht. Liebstöckel- und Davanaöl enthalten jeweils eine Vielzahl von Substanzen, wobei die Hauptkomponenten beider Öle verschiedene sauerstoffhaltige Verbindungen sind. Isobenzofuranone sind die wichtigsten Bestandteile des Liebstöckelöls. Ihre relative (geschmacklich/geruchliche) Intensität veränderte sich während der oxidativen Pyrolyse signifikant. Bei den (Z)-Ligustiliden wird auf zwei Wegen abgebaut: über eine Aromatisierungsreaktion, die zur Bildung von Butylidenephthalid führt, sowie eine Spaltung des Lactonrings mit Abspaltung von Kohlendioxid oder Kohlenmonoxid. Der Hauptbestandteil des Davanaöls ist Davanon. Bei der oxidativen Pyrolyse bei niedriger Temperatur wird Davanon nur geringfügig abgebaut. Im Gegensatz dazu reduzierte sich die relative Ausbeute von Bicyclogermacren, der Komponente mit der zweitstärksten geruchlich/geschmacklichen Intensität, aufgrund von Bindungs-umgruppierungen deutlich. Oxidation des Davan-Äthers führte zur Bildung verschiedener Furan-Derivate. Die Veränderungen in der Zusammensetzung beider essentiellen Öle bei der nieder-Temperatur Pyrolyse kann durch Bindungsspaltung, intramolekularer Umgruppierungen und Oxidationsreaktionen einer Vielzahl ihrer Bestandteilen erklärt werden. Die beschriebene, thermisch-oxidative Methode, erwies sich für die Untersuchung der Stabilität ätherischer Öle und zur Bewertung ihrer Abbauprodukte unter Bedingungen, die Gegebenheiten im Tabak bei mittelstarker Temperaturbelastung simulieren, als geeignet. Es wurde, trotz der komplexen Zusammensetzung der ätherischen Öle, kein Hinweis auf Wechselwirkungen zwischen den verschiedenen Komponenten der Öle gefunden. [Beitr. Tabakforsch. Int. 29 (2020) 27–43]

RESUME

La décomposition thermo-oxydative des huiles essentielles de livèche (*Levisticum officinale*) et de davana (*Artemisia pallens*) fut étudiée au travers d'une spectrométrie de masse accompagnée d'une chromatographie des gaz de pyrolyse dans une atmosphère à 9% d'oxygène et 91% d'azote portée à 300°C afin de simuler les conditions de chauffe à basse température du tabac. La composition chimique des huiles de livèche et de davana est complexe et inclut divers composés oxygénés parmi ses constituants principaux. Les isobenzofuranones sont les constituants principaux de l'huile de livèche et leur intensité relative changea de façon significative durant la pyrolyse oxydative. Le (Z)-ligustilide subit deux types de réactions de décomposition : Une réaction d'aromatisation menant à la formation du butylidenephthalide ainsi que la scission de l'anneau de lactone avec élimination du dioxyde ou du monoxyde de carbone. Le davanone est le constituant principal de l'huile de davana, qui ne subit aucune décomposition importante durant la pyrolyse oxydative à basse température. Toutefois, le rendement relatif du deuxième composé le plus intensif, à savoir le bicyclogermacrène, chuta de façon marquée en raison des réactions de réarrangement des liaisons. Le davana-éther subit des réactions d'oxydation provoquant la formation de divers dérivés furaniques. Les

modifications apportées à la composition des deux huiles essentielles purent être interprétées comme des clivages de liaisons, des mécanismes de réarrangement intramoléculaires et des réactions d'oxydation de divers constituants durant la pyrolyse oxydative à basse température. La méthode thermo-oxydative appliquée s'avéra utile à l'étude de la stabilité des huiles essentielles et à l'observation des produits de décomposition dans des conditions simulées de chauffe du tabac. En dépit de la composition complexe des huiles essentielles, aucune preuve d'interaction entre les constituants des huiles ne fut découverte. [Beitr. Tabakforsch. Int. 29 (2020) 27–43]

INTRODUCTION

Essential oils of plants are widely used to add fragrance to perfumes and cosmetics or as flavours in food, beverage, and tobacco industries (1–3). Essential oils are very complex mixtures; hence the identification of the numerous aroma components is a great challenge. Hundreds of components have been identified in various essential oils; a few hundred compounds are common in several oils (4). Lovage and davana are aromatic herbs, and the essential oils of both plants are applied in the tobacco industry (3). Lovage (*Levisticum officinale*) has been long cultivated in Europe and southwestern Asia, the leaves being used as an herb, the roots as a vegetable, and the seeds as a spice. The essential oil is mostly produced by steam distillation and solvent extraction from the roots and leaves of the plant, although the application of supercritical extraction was also suggested (1, 5). The essential oil from the leaves is fairly thin and has a sweet and spicy aroma, while the oil from the roots is quite resinous and has a strong floral aroma with a slight bitterness. The leaf oil is principally used as a fragrance in cosmetics, while the root oil is a flavouring agent in food, drinks, cigarettes etc. The compositions of the oils extracted from different anatomical parts of lovage are fairly different (6–9). The essential oil from the leaves contains alpha-terpinyl acetate, ligustilides, pentyl cyclohexadiene, α -phellandrene and α -terpineol, while the root oil has (Z)-ligustilide, senkyunolide, various phthalides, terpenoids and coumarins (9–12). (Z)-Ligustilide, which is known as a major lovage constituent, has been found in amounts of 37–68% in European lovage root oils (13). The content of (Z)-ligustilide in the leaf oil is lower compared with the root oil. The essential oil composition varies significantly on the production site and harvesting time (6, 13–14). Pure Z-ligustilide is a very unstable compound and it degrades rapidly in daylight. About 15% of it degraded when stored at 4 °C in the refrigerator for 15 days; however, the other components of the oil might have stabilising effects (15–16).

Davana (*Artemisia pallens*) is an aromatic herbaceous plant, which is native to the Southern part of India. Davana is commercially cultivated for its fragrant leaves and flowers. The essential oil is produced from the leaves and flowers by steam distillation and it has a pleasant herbal and fruity fragrance (17–18). Davana oil is used in the production of perfumes, cosmetics and is also widely used in the food industry for flavouring tobacco, cakes, pastries and some beverages. Davana oil has been widely investi-

gated by GC/MS, IR and NMR; however, its composition is so complex that not all of its major components have been identified (17, 19). The major component of davana oil is a sesquiterpene ketone, davanone, which represents 30–65% of the oil. Eight davanone isomers have been identified (20–21), but (+)-davanone dominates among them. The purified davanone compound is practically odourless; however, within the mixture it is thought to enhance the overall odour of the oil. The characteristic aroma of davana oil originates mainly from davana furan, but davana ether and other furan derivatives were also reported as odoriferous constituents (22). Davanone, bicyclogermacrene, davana ether, davana furan, cinnamates, and linalool are the major constituents (20, 23). The composition of the oil depends on the plant growth stage and the part of the plant; however, the essential oil yield and composition have been shown not to vary from year to year (20). Among the components of davana oil, bicyclogermacrene appears to be unstable. NJORGE *et al.* (24) observed that bicyclogermacrene was the main sesquiterpene hydrocarbon of the fresh cold-pressed yuzu oil; however, other authors reported bicycloelemene and sesquiterpene alcohols as the main sesquiterpenoid components. Bicyclogermacrene practically disappeared and was converted to spathulenol during the storage of yuzu oil at 25 °C for a few weeks (25).

The aim of the present work was to screen the thermal stability of the flavours to be applied in heated tobacco products, which is an important issue in the product safety assessment (26). Natural and synthetic flavouring compounds are applied during tobacco processing for cigarettes (27–28) and for low-temperature heated tobacco products (29–30). Oxidative pyrolysis can be performed to assess the thermal stability of the flavour compounds and to monitor the reaction products. In a previous paper, the thermo-oxidative degradation of lime, bergamot and cardamom essential oils has been described (31). In this study, we monitored the changes in the composition of lovage and davana essential oils during low-temperature oxidative decomposition in order to reveal the major reaction pathways under simulated conditions of low-temperature tobacco heating.

MATERIALS AND METHODS

Materials

Two flavour samples, lovage root oil (*Levisticum officinale*) and davana oil (*Artemisia pallens*) were obtained from Hertz Flavors GmbH & Co. KG (Reinbek, Germany). The samples were stored in amber essential oil dropper bottles at 4 °C in the refrigerator prior to analysis. All experiments were performed within a 10-day time window after first breaking the seal on the bottles, to minimize oxidation during refrigerated storage.

Analysis of the as-received samples

The gas chromatography/mass spectrometry (GC/MS) experiments were performed using an Agilent 6890 A/5973 GC/MS (Agilent Technologies, Santa Clara, CA,

USA). The essential oil samples were dissolved in dichloromethane to obtain 30 mg/mL concentration on the day of the measurements. Aliquots of 1 µL solution were injected to the GC/MS operating with helium carrier gas with a split ratio of 100:1.

The separation of the compounds was carried out on a DB-1701 capillary column (30 m × 0.25 mm, 0.25 µm film thickness). The GC oven was programmed to hold at 40 °C for 7 min before increase to 280 °C at a rate of 10 °C/min. The mass spectrometer was operated in electron impact ionization mode at 70 eV in a range of *m/z* 29–400. The identification of the compounds was performed using NIST 2011 and Wiley 2009 mass spectral libraries. For the correct identification of the isomers, literature data (4, 12–13, 19) were also taken into account. Five parallel experiments were carried out for both samples.

Low-temperature pyrolysis-GC/MS

The pyrolysis experiments were carried out using a Pyroprobe 2000 (CDS Analytical, Oxford, PA, USA) coupled on-line to the Agilent 6890/5973 GC/MS. The pyrolyser is equipped with a platinum coil and a quartz tube. Aliquot of 0.05 µL essential oil sample was dispensed on a piece of quartz wool placed in the middle of the quartz tube. The Pyroprobe was immediately inserted into the pyrolysis chamber preheated to 250 °C. The sample was then heated to 300 °C (calibrated temperature) and kept there for 5 min in 9% oxygen and 91% nitrogen atmosphere using 276 mL/min flow rate. The temperature was chosen as 300 °C in order to mimic the heating conditions for the low-temperature tobacco heating products (30). The gas mixture purged the volatile molecules to the DB-1701 capillary column and then the carrier gas was switched to helium. A solvent delay of 7 min was applied to protect the mass spectrometer from oxygen. The parameters of GC/MS analysis were the same as for the analysis of the essential oil solutions. Five parallel runs were performed on both samples.

RESULTS AND DISCUSSION

Lovage oil

Figure 1(a) presents the chromatogram of the lovage root oil solution. The identification of the compounds, the relative intensities (area %) and the standard deviations calculated from five parallel experiments are listed in Table 1. Compounds having relative intensities higher than 0.1% were considered for evaluation. The quantification of the oil components by using reference compounds was not the purpose of this study. Therefore, the relative intensity data calculated from the integrated total ion current values were used for evaluating the composition of the essential oils. The area percent-values of the chromatograms provide a good estimate of the changes in the compositions during the thermo-oxidative decomposition.

The main component of lovage root oil was (*Z*)-ligustilide [50], which had 58% relative intensity calculated as area-percentage. (*E*)-Ligustilide [56] represented only 4%, while the relative intensity of 2-pentylcyclohexa-1,3-diene [10]

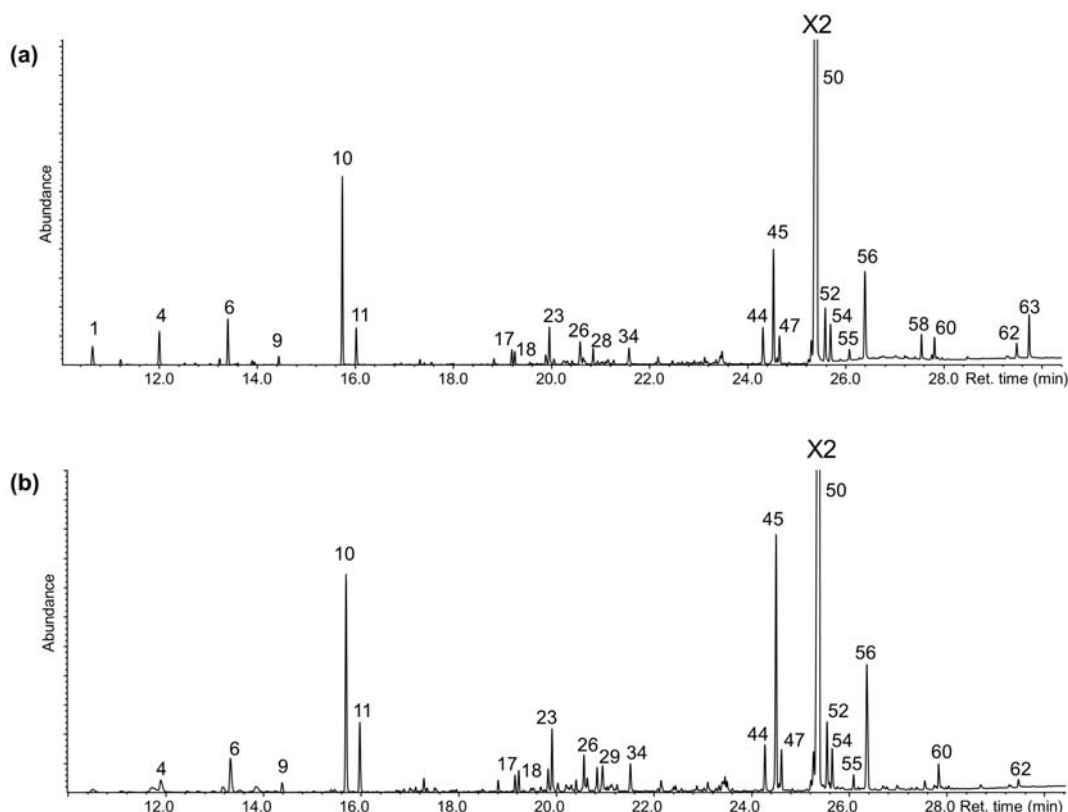


Figure 1. (a) GC/MS and (b) Py-GC/MS total ion chromatogram of lovage oil (numbered peak identities are given in Table 1 and are shown in square brackets in the text).

and (*Z*)-3-butyldenephthalide [45] amounted to 6 and 4%, respectively. Eleven other compounds had relative intensity between 1 and 2%, altogether representing 15% of the oil. These components belonged to various types of compounds: monoterpenes, sesquiterpenes, alkylbenzenes, isobenzofuranone derivatives, and long-chain alcohol and ester. All the other compounds were minor components of relative concentrations between 0.1 and 1%, altogether amounting to 13%.

The components of lovage oil have been categorized into classes, as shown in Table 2. It can be established that isobenzofuranones were the most significant constituents of lovage oil including alkyl- and alkenyl-dihydroisobenzofuranones, tetrahydroisobenzofuranones, and phthalides, altogether representing a relative intensity of 74%. Terpenoids constituted 9.5% of the oil; slightly more than half of them were monoterpenoids, with the rest being sesquiterpenoids. Hydrocarbon terpenes dominated among the terpenoids, oxygen-containing terpene alcohols and acetate constituted only about 11 and 24% of monoterpenoids and sesquiterpenoids, respectively. Cyclohexadiene and benzene with aliphatic side chains represented another important group of hydrocarbon components constituting about 7% of the oil. Lovage oil contained long-chain aliphatic compounds as well, such as fatty acid, fatty acid esters, and alcohol.

During oxidative pyrolysis at 300 °C, the relative composition of lovage oil altered, as the pyrolysis-gas chromatogram (pyrogram) illustrates in Figure 1(b). The differences in the relative intensity data of the as-received and the pyrolysed oil are shown in Table 1. It should be noted that the

Table 2. Relative intensities (area-%) of the compound classes of lovage oil as received (injection) and after Py-GC/MS experiment (pyrolysis).

Compound classes	Injection area-%	Pyrolysis area-%	Difference in area-%
Monoterpenoids	5.05	4.02	-1.03
Alkyl-, alkenyl-benzenes and cyclohexadienes	7.26	9.01	+1.75
Alkyl-, alkenyl-dihydroisobenzofuranones	65.00	59.60	-5.40
Alkyl-, alkenyl-phthalides	6.57	11.06	+4.49
Alkyl-, alkenyl-tetrahydroisobenzofuranones	2.46	2.58	+0.12
Cyclic anhydrides (benzene, cyclohexadiene)	0.86	1.74	+0.88
Sesquiterpenoids	4.46	5.32	+0.86
Fatty acid and alcohol derivatives	4.36	1.71	-2.65
Alkenes and alkenyl oxo compounds	0.17	0.83	+0.66

evaporation of the compounds played a considerable role due to the volatility of the oil components, the moderate pyrolysis temperature, and the high gas flow rate. Nevertheless, the relative intensities of numerous compounds were changed and a few new products were formed during pyrolysis. Changes in composition were assessed for statistical significance using two tailed, two-sample t-tests with an alpha risk of 0.05. For instances, where a compound was absent in either sample group, significance was evaluated using a one-sample t-test with an alpha risk of 0.05.

Table 1. Composition of lovage oil and pyrolysis products at 300 °C in 9% oxygen / nitrogen atmosphere.

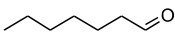
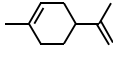
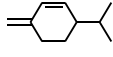
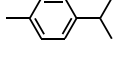
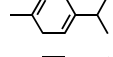
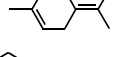
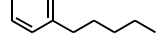
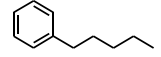
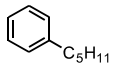
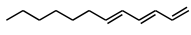
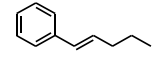
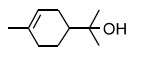
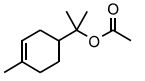
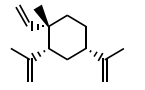
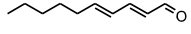
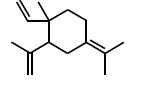
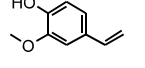
No.	t_{ret} /min	MW	Structure	Compound	Injection /area-% aver. $\pm$ std. dev.	Pyrolysis /area-% aver. $\pm$ std. dev.	Difference in area-%	Significance level ^a
1	10.45	136		α -Pinene	0.74 ± 0.10	0.22 ± 0.08	-0.52	***
2	11.03	136		Camphene	0.21 ± 0.03	0.10 ± 0.03	-0.11	***
3	11.74	114		Heptanal	0	0.49 ± 0.10	+0.49	***
4	11.86	136		β -Pinene	1.23 ± 0.15	0.66 ± 0.18	-0.57	***
5	13.14	136		D-Limonene	0.22 ± 0.03	0.33 ± 0.06	+0.11	*
6	13.31	136		β -Phellandrene	1.57 ± 0.14	1.42 ± 0.20	-0.15	N
7	13.51	134		p-Cymene	0.07 ± 0.00	0.14 ± 0.02	+0.07	**
8	13.85	136		γ -Terpinene	0.15 ± 0.01	0.29 ± 0.05	+0.14	**
9	14.37	136		Terpinolene	0.29 ± 0.02	0.32 ± 0.04	+0.03	N
10	15.69	150		2-Pentylcyclohexa-1,3-diene	6.00 ± 0.29	6.56 ± 0.52	+0.56	N
11	15.97	148		n-Pentyl-benzene	1.22 ± 0.05	2.12 ± 0.13	+0.90	***
12	17.12	148		C ₅ -benzene	0.01 ± 0.00	0.13 ± 0.01	+0.12	***
13	17.28	164		1,3,5-Dodecatriene	0.17 ± 0.01	0.20 ± 0.01	+0.03	*
14	17.30	146		1-Phenyl-1-pentene	0.02 ± 0.00	0.20 ± 0.01	+0.18	***
15	17.35	154		α -Terpineol	0.08 ± 0.01	0.12 ± 0.01	+0.04	***
16	18.81	204		α -Copaene	0.20 ± 0.01	0.32 ± 0.03	+0.12	***
17	19.15	196		α -Terpinyol acetate	0.49 ± 0.03	0.44 ± 0.09	-0.05	N
18	19.22	204		β -Elementene	0.44 ± 0.02	0.55 ± 0.06	+0.11	*
19	19.48	152		2,4-Decadienal	0	0.14 ± 0.01	+0.14	**
20	19.54	204		β -Funebrene	0.10 ± 0.01	0.16 ± 0.02	+0.06	***
21	19.68	204		γ -Elementene	0.08 ± 0.01	0.17 ± 0.02	+0.09	***
22	19.83	150		2-Methoxy-4-vinylphenol	0.41 ± 0.09	0.67 ± 0.12	+0.26	**

Table 1. Continued

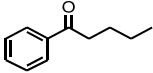
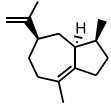
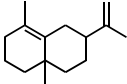
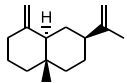
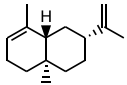
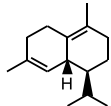
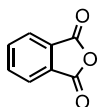
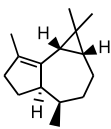
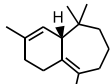
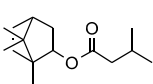
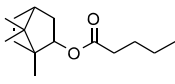
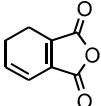
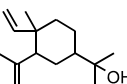
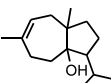
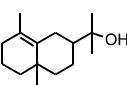
No.	t_{ret} /min	MW	Structure	Compound	Injection /area-% aver. $\pm$ std. dev.	Pyrolysis /area-% aver. $\pm$ std. dev.	Difference in area-%	Significance level ^a
23	19.91	162		Valerophenone	1.32 ± 0.06	1.77 ± 0.14	+0.45	***
24	20.03	204		α -Bulnesene	0.23 ± 0.01	0.34 ± 0.02	+0.11	***
25	20.40	204		2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	0.12 ± 0.01	0.30 ± 0.03	+0.18	***
26	20.56	204		β -Selinene	1.00 ± 0.06	1.04 ± 0.11	+0.04	N
27	20.63	204		α -Selinene	0.39 ± 0.02	0.41 ± 0.04	+0.02	N
28	20.83	204		δ -Cadinene	0.66 ± 0.04	0.64 ± 0.05	-0.02	N
29	20.95	148		Phthalic anhydride	0.08 ± 0.00	0.85 ± 0.11	+0.77	***
30	21.03	204		α -Gurjunene	0.07 ± 0.01	0.12 ± 0.03	+0.05	**
31	21.09	204		β -Himachalene	0.10 ± 0.01	0.15 ± 0.02	+0.05	**
32	21.12	238		Bornyl isovalerate	0.20 ± 0.02	0.19 ± 0.02	-0.01	N
33	21.24	238		Bornyl pentanoate	0.17 ± 0.01	0.19 ± 0.01	+0.02	N
34	21.51	150		1,3-Cyclohexadiene-1,2-dicarboxylic anhydride	0.77 ± 0.04	0.89 ± 0.08	+0.12	*
35	22.14	222		Elemol	0.28 ± 0.03	0.33 ± 0.05	+0.05	N
36	22.44	222		Carotol	0.13 ± 0.01	0.17 ± 0.02	+0.04	*
37	23.02	222		γ -Eudesmol	0.13 ± 0.01	0.12 ± 0.02	-0.01	N

Table 1. Continued

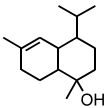
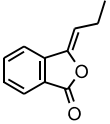
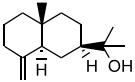
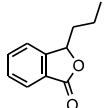
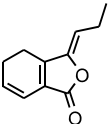
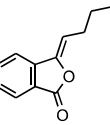
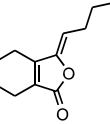
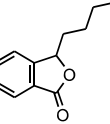
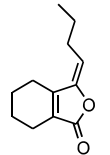
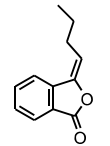
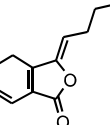
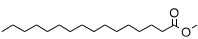
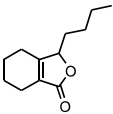
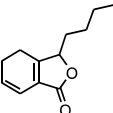
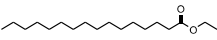
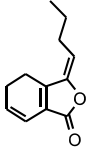
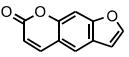
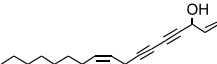
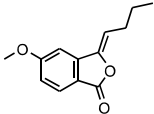
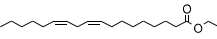
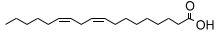
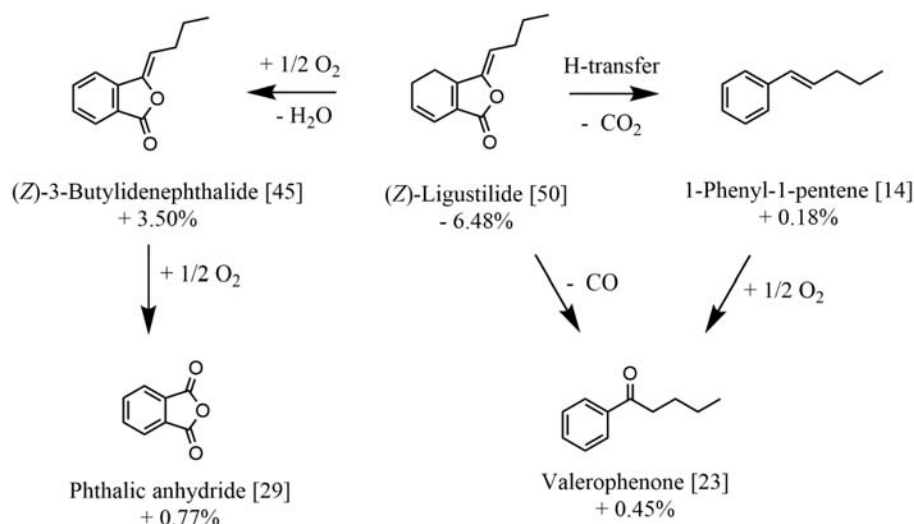
No.	t_{ret} /min	MW	Structure	Compound	Injection /area-% aver. $\pm$ std. dev.	Pyrolysis /area-% aver. $\pm$ std. dev.	Difference in area-%	Significance level ^a
38	23.10	222		α -Muurolol	0.25 ± 0.02	0.27 ± 0.05	+0.02	N
39	23.33	222		Unidentified sesquiterpene	0.19 ± 0.01	0.18 ± 0.03	-0.01	N
40	23.40	174		3-Propylidenephthalide	0.15 ± 0.01	0.27 ± 0.04	+0.12	**
41	23.42	222		β -Eudesmol	0.29 ± 0.02	0.24 ± 0.05	-0.05	N
42	23.45	222		Unidentified	0.54 ± 0.05	0.43 ± 0.06	-0.11	*
43	23.50	176		3-Propylphthalide	0.06 ± 0.00	0.33 ± 0.05	+0.27	***
44	24.27	176		(Z)-3-propylidene-4,5-dihydroisobenzofuran-1(3H)-one	1.45 ± 0.12	1.50 ± 0.09	+0.05	N
45	24.49	188		(Z)-3-Butylidenephthalide	4.19 ± 0.30	7.69 ± 0.44	+3.50	***
46	24.56	192		(Z)-Sedanonic acid lactone	0.20 ± 0.04	0.17 ± 0.03	-0.03	N
47	24.61	190		3-n-Butylphthalide	1.04 ± 0.07	1.22 ± 0.08	+0.18	**
48	25.21	192		(E)-Sedanonic acid lactone	0.20 ± 0.01	0.36 ± 0.03	+0.16	***
49	25.26	188		(E)-3-Butylidenephthalide	0.94 ± 0.06	1.42 ± 0.08	+0.48	***
50	25.37	190		(Z)-Ligustilide	58.05 ± 1.90	51.57 ± 2.14	-6.48	**

Table 1. Continued

No.	t _{ret} /min	MW	Structure	Compound	Injection /area-% aver. ± std. dev.	Pyrolysis /area-% aver. ± std. dev.	Difference in area-%	Significance level ^a
51	25.47	270		Hexadecanoic acid methyl ester	0	0.20 ± 0.03	+0.20	***
52	25.54	194		3-Butyl-4,5,6,7- tetrahydroisobenzofuran- 1(3H)-one	2.07 ± 0.16	2.05 ± 0.10	-0.02	N
53	25.59	218		Isobenzofuranone derivative	0	0.18 ± 0.02	+0.18	***
54	25.65	192		Senkyunolide	1.45 ± 0.09	1.29 ± 0.09	-0.16	*
55	26.09	284		Hexadecanoic acid, ethyl ester	0.42 ± 0.03	0.41 ± 0.04	-0.01	N
56	26.36	190		(E)-Ligustilide	4.06 ± 0.24	5.23 ± 0.22	+1.17	***
57	26.99	186		Ficusin (Furocoumarin)	0.22 ± 0.07	0.21 ± 0.03	-0.01	N
58	27.55	244		Falcarinol	1.02 ± 0.22	0.34 ± 0.10	-0.68	**
59	27.74	218		5-Methoxy-3- butylidenephthalide	0.19 ± 0.01	0.13 ± 0.01	-0.06	***
60	27.83	308		Linoleic acid ethyl ester	0.79 ± 0.07	0.76 ± 0.11	-0.03	N
61	28.47	280		Linoleic acid	0.37 ± 0.25	0	-0.37	*
62	29.47	224		Unidentified	0.75 ± 0.14	0.31 ± 0.11	-0.44	***
63	29.72			Fatty acid ester	1.76 ± 0.35	0	-1.76	***

^a p-values (t-test): N, no significant change ($p > 0.05$); *, $0.05 \geq p > 0.01$; **, $0.01 \geq p > 0.001$; ***, $p \leq 0.001$.



Scheme 1. Proposed conversion reactions of (Z)-ligustilide during low temperature pyrolysis of lovage oil. Compound numbers correspond to those in Table 1. The percentage data indicate the change in composition (area-%) (Table 1).

The significance of the changes in the area-percent values of the components is listed in Table 1. The relative intensity of the main component, (Z)-ligustilide [50] reduced from 58.1 to 51.6% ($0.01 \leq p < 0.001$). The levels of (Z)-3-butylidenephthalide [45] and other alkyl- and alkylidenephthalides were enhanced during pyrolysis. Scheme 1 presents the reaction routes proposed for the conversion of (Z)-ligustilide [50] during low-temperature oxidative pyrolysis. The cyclohexadiene ring of (Z)-ligustilide was transformed (aromatised) via dehydrogenation leading to the formation of (Z)-3-butylidenephthalide [45]. Similarly to our previous studies (30–32), it is likely that oxygen radicals initiated the cleavage of the C–H bonds, resulting in the release of water molecules, because the pyrolysis temperature was considered low for dehydrogenation with the formation of hydrogen molecules. Our previous study on flavour ingredients (30) demonstrated that the dehydrogenation reactions were more pronounced in the oxidative than in the inert atmosphere. (Z)-3-Butylidenephthalide [45] could be further oxidized at the alkylidene group forming phthalic anhydride [29]. The scission of the furanone ring of (Z)-ligustilide [50] also took place as the increased yields of valerophenone [23] and 1-phenyl-1-pentene [14] indicated. The elimination of carbon dioxide from the furanone ring followed by hydrogen transfer could result in the formation of 1-phenyl-1-pentene [14]. The removal of CO groups and hydrogen transfer led to the release of valerophenone [23]. Valerophenone could also be formed via the oxidation of 1-phenyl-1-pentene [14]. Similar reactions were observed in the case of alkyl cinnamate compounds during oxidative pyrolysis: the double bonds were oxidised forming oxo-compounds (32). Cinnamaldehyde underwent transformation to benzaldehyde at as low a temperature as 60 °C, as described by FRIEDMAN (33).

As Table 2 shows, the relative intensity of dihydroisobenzofuranones generally decreased due to the aromatisation of the cyclohexadiene rings; however, the cyclohexene rings of alkyl and alkenyl-tetrahydroisobenzofuranones did not undergo aromatisation. The relative intensity of fatty

alcohol, acid and esters decreased, while the intensity of shorter-chain alkenes and alkenyl-oxo compounds increased, indicating oxidation reactions and chain scissions. Small changes took place in the distribution of cyclic monoterpenes during low-temperature pyrolysis (Table 1). The total intensity of the bicyclic monoterpenes like α - and β -pinene [1 and 4] was reduced, while the relative yield of monocyclic monoterpenes slightly increased. The four-membered ring of pinenes was cleaved next to the tertiary carbon atom, resulting in the formation of limonene [5] and γ -terpinene [8] similarly to what we observed in lime essential oil previously (31).

Davana oil

The chromatogram of davana oil solution can be seen in Figure 2(a) and the identification of the compounds is found in Table 3. Furthermore, Table 3 lists the relative intensities and standard deviations considering the peaks having > 0.1 area-percent either in the as-received oil or in the pyrolysate. In the as-received oil, 75 peaks were identified, two peaks [42 and 63] among those contained two unresolved compounds and one peak [24] contained three unresolved compounds. Five peaks contained two slightly resolved compounds each [16–17, 19–20, 32–33, 34–35, and 43–44] and their intensity ratios were estimated based on the selected ion chromatograms. Additionally, 29 unidentified peaks were taken into account in the area-percent calculations, which had a relative intensity of $> 0.1\%$. As Table 3 indicates, the majority of the constituents belonged to furan derivatives of different oxygen contents as well as to sesquiterpenes and oxygen- and sulphur-containing sesquiterpenoids. (+)-Davanone [59] was the main component of davana oil representing 29.5 area-percent of the total ion chromatogram. Other davanone isomers [54, 55 and 56] represented only a total of 3.4 area-percent. The second-most intense peak in the chromatogram was bicyclogermacrene [41], amounting to 11.7 area-percent. The intensity of bicyclogermacrene was relatively high, while that of the two bicycloelemene isomers [16 and 18]

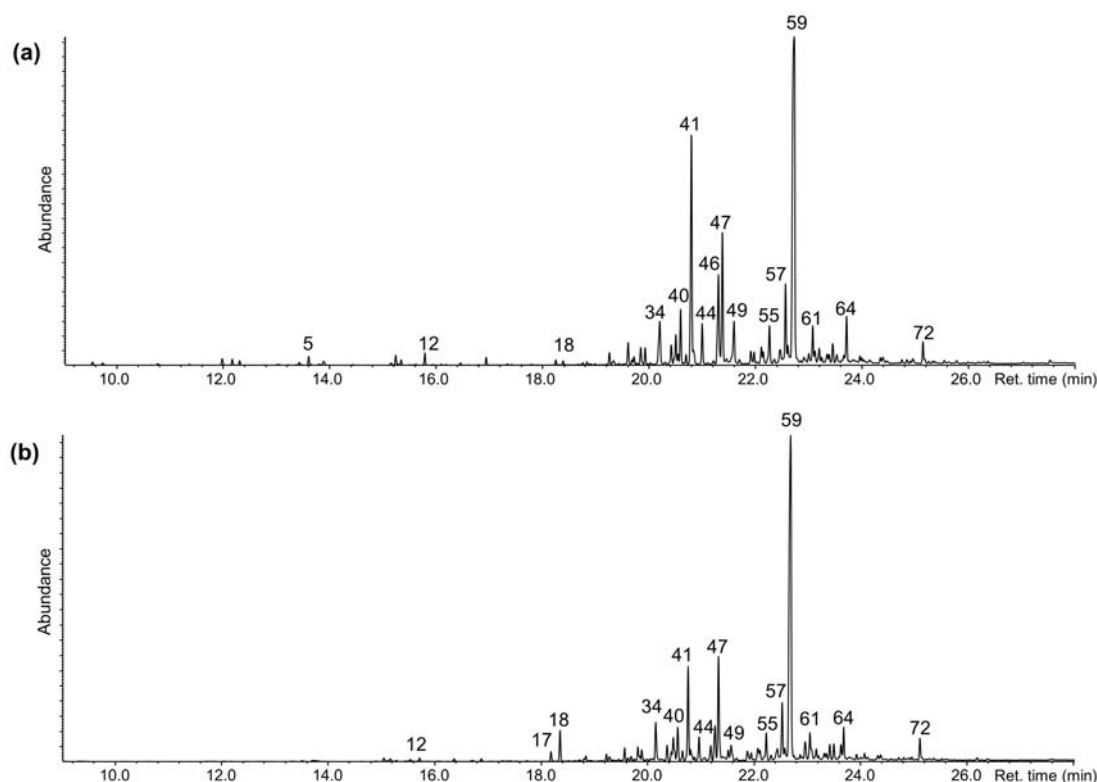


Figure 2. (a) GC/MS and (b) Py-GC/MS total ion chromatogram of davana oil (numbered peak identities are given in Table 3).

was very low (below 0.2 area-percent) indicating that the as-received davana oil had been kept fresh (24).

The oil components were categorised into groups according to their chemical structure; and the summed intensities of the compounds in the groups are listed in Table 4. The furan derivatives were grouped into davanones, hydroxyisodavanones, davana ethers and the remaining compounds of smaller molecular mass were referred to as 'other furan derivatives'.

The furan derivatives constituted about 48% of davana oil, while sesquiterpenes and sesquiterpenoids were the other main components amounting to about 27%. Davana oil contained several aliphatic and aromatic esters; however, most of them at low concentrations with the exceptions of

geranyl acetate [25] and methyl [37] and ethyl cinnamates [34 and 47], representing about 1, 1 and 8%, respectively. Ethyl *trans*-cinnamate [47] was the third-most intense peak in the chromatogram, amounting to 6.3%.

During oxidative pyrolysis at 300 °C, the majority of davana oil components evaporated unchanged due to the moderate temperature and the high flow rate. Nevertheless, there were substantial alterations in the intensity of several compounds, as Figure 2b and Table 3 demonstrate. The significance of the intensity increase of the main constituent, (+)-davanone [59] appears marginal as determined by t-test. However, the second-most important component of davana oil, bicyclogermacrene [41] underwent a substantial decrease in its relative intensity from 11.7 to 6.5%, while the relative concentrations of other sesquiterpenes increased (Table 4). These observations can be explained by rearrangement reactions of bicyclogermacrene [41] as illustrated in Scheme 2a. Since the intensity of a bicycloelemene isomer [18] was increased from 0.18 to 2.03% and another isomer [16] was formed as a result of pyrolysis, these appear logical candidates for the products from conversion of bicyclogermacrene. It is proposed that the rearrangement reaction of bicyclogermacrene [41] took place by a pericyclic reaction referred to as sigmatropic shift. A σ -bond was formed between the tertiary and secondary carbon atoms at the double bonds, a double bond was shifted, and a single bond was broken leading to the formation of bicycloelemene, as demonstrated in Scheme 2a. Other rearrangement reactions of bicyclogermacrene [41] occurred to a smaller degree. The scission of the strained 3-membered ring probably took place with the creation of a

Table 4. Relative intensities (area-%) of the compound classes of davana oil as received (injection) and after Py-GC/MS experiment (pyrolysis).

Compound classes	Injection area-%	Pyrolysis area-%	Difference area-%
Esters	12.56	12.49	-0.07
Bicyclogermacrene	11.68	6.46	-5.22
Other sesquiterpenes	7.59	10.45	+2.86
O-,S-containing sesquiterpenoids	8.09	8.72	+0.63
Davanones	32.95	35.72	+2.77
Hydroxyisodavanones	1.75	2.00	+0.25
Davana ethers	10.43	5.83	-4.60
Other furan derivatives	3.13	4.43	+1.30

Table 3. Composition of davana oil and pyrolysis products at 300 °C in 9% oxygen / nitrogen atmosphere.

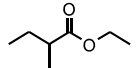
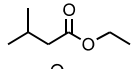
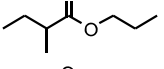
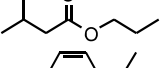
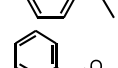
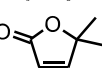
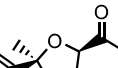
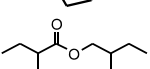
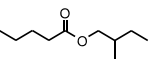
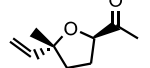
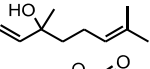
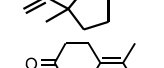
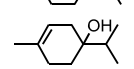
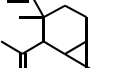
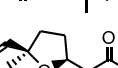
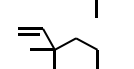
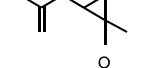
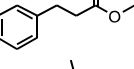
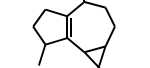
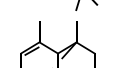
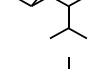
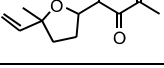
No.	t_{ret} /min	MW	Structure	Compound	Injection /area-% aver. $\pm$ std. dev.	Pyrolysis /area-% aver. $\pm$ std. dev.	Difference in area-%	Significance level ^a
1	9.40	130		Ethyl 2-methylbutanoate	0.20 $\pm$ 0.03	0	-0.20	***
2	9.60	130		Ethyl 3-methylbutanoate	0.14 $\pm$ 0.02	0	-0.14	***
3	12.04	144		Propyl 2-methylbutanoate	0.29 $\pm$ 0.03	0.06 $\pm$ 0.04	-0.23	***
4	12.18	144		Propyl 3-methylbutanoate	0.21 $\pm$ 0.03	0.03 $\pm$ 0.03	-0.18	***
5	13.51	134		<i>p</i> -Cymene	0.41 $\pm$ 0.04	0.13 $\pm$ 0.09	-0.28	**
6	13.71	106		Benzaldehyde	0.07 $\pm$ 0.00	0.18 $\pm$ 0.06	+0.11	*
7	15.04	112		5,5-Dimethylfuran-2(5 <i>H</i>)-one	0.13 $\pm$ 0.01	0.37 $\pm$ 0.04	+0.24	***
8	15.16	154		<i>cis</i> -Arbusculone	0	0.12 $\pm$ 0.02	+0.12	***
9	15.17	172		2-Methylbutyl 2-methylbutanoate	0.46 $\pm$ 0.03	0.23 $\pm$ 0.10	-0.23	**
10	15.28	172		2-Methylbutyl pentanoate	0.23 $\pm$ 0.01	0.10 $\pm$ 0.05	-0.13	**
11	15.54	154		<i>trans</i> -Arbusculone	0.03 $\pm$ 0.00	0.20 $\pm$ 0.02	+0.17	***
12	15.72	154		Linalool	0.50 $\pm$ 0.02	0.28 $\pm$ 0.10	-0.22	**
13	16.36	126		5-Ethenyl-5-methyl-dihydrofuran-2(3 <i>H</i>)-one	0.13 $\pm$ 0.01	0.25 $\pm$ 0.03	+0.12	***
14	16.71	138		4-(Propan-2-ylidene)cyclohexanone	0	0.11 $\pm$ 0.03	+0.11	***
15	16.88	154		Terpinen-4-ol	0.35 $\pm$ 0.01	0.24 $\pm$ 0.06	-0.11	*
16	18.18	204		Bicycloelemene (isomer 1) ^b	0	0.37 $\pm$ 0.04	+0.37	***
17	18.19	182		Nordavanone ^b	0.24 $\pm$ 0.01	0.31 $\pm$ 0.03	+0.07	**
18	18.36	204		Bicycloelemene (isomer 2)	0.18 $\pm$ 0.01	2.03 $\pm$ 0.10	+1.85	***
19	18.71	164		Methyl 3-phenylpropanoate ^b	0	0.12 $\pm$ 0.01	+0.12	***
20	18.73	204		Isoledene ^b	0.12 $\pm$ 0.00	0.04 $\pm$ 0.00	-0.08	***
21	18.81	204		α -Copaene	0.17 $\pm$ 0.00	0.18 $\pm$ 0.02	+0.01	N
22	18.84	210		4-(5-Ethenyl-5-methyltetrahydrofuran-2-yl)pentan-2,3-dione	0.05 $\pm$ 0.01	0.37 $\pm$ 0.04	+0.32	***

Table 3. Continued

No.	t_{ret} /min	MW	Structure	Compound	Injection /area-% aver. $\pm$ std. dev.	Pyrolysis /area-% aver. $\pm$ std. dev.	Difference in area-%	Significance level ^a
23	19.23	204		β -Elementene	0.52 ± 0.01	0.49 ± 0.04	-0.03	N
		162		Methyl <i>cis</i> -cinnamate +				
24	19.28	204		α -Gurjunene +	0.19 ± 0.09	0.34 ± 0.04	+0.15	*
		204		δ -Selinene				
25	19.57	196		Geranyl acetate	1.03 ± 0.02	0.82 ± 0.07	-0.21	**
26	19.63	178		Ethyl 3-phenylpropanoate	0.20 ± 0.00	0.19 ± 0.03	-0.01	N
27	19.69	204		β -Caryophyllene	0.33 ± 0.01	0.33 ± 0.02	0	N
28	19.74	204		Eudesma-3,7(11)-diene	0.06 ± 0.01	0.13 ± 0.01	+0.07	***
29	19.82	204		Aromadendrene (isomer 1)	0.75 ± 0.03	0.91 ± 0.06	+0.16	**
30	19.85	204		Eudesma-5,11-diene	0.05 ± 0.00	0.23 ± 0.07	+0.18	**
31	19.90	220		Davana furan	0.70 ± 0.01	0.67 ± 0.05	-0.03	N
32	20.10	192		Benzyl isovalerate ^b	0.20 ± 0.03	0.15 ± 0.05	-0.05	N
33	20.12	204		Aromadendrene (isomer 2) ^b	0.51 ± 0.08	0	-0.51	***
34	20.15	176		Ethyl <i>cis</i> -cinnamate ^b	2.02 ± 0.13	2.45 ± 0.15	+0.43	**
35	20.16	204		α -Humulene ^b	0	0.24 ± 0.01	+0.24	***
36	20.28	204		γ -Murolene	0.13 ± 0.00	0.10 ± 0.02	-0.03	*
37	20.37	162		Methyl <i>trans</i> -cinnamate	1.02 ± 0.02	1.05 ± 0.04	+0.03	N
38	20.45	204		Isobicyclogermacrene	0.23 ± 0.04	0.66 ± 0.14	+0.43	**

Table 3. Continued

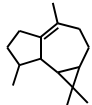
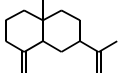
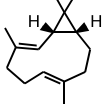
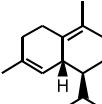
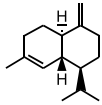
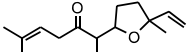
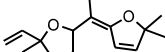
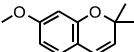
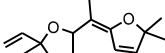
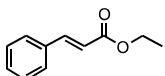
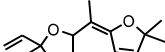
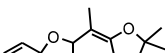
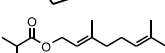
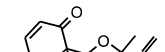
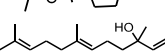
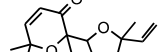
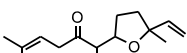
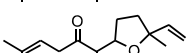
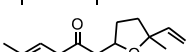
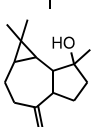
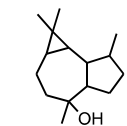
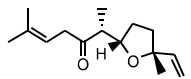
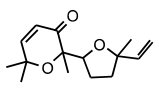
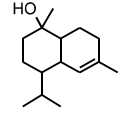
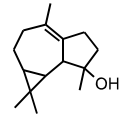
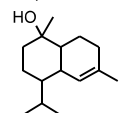
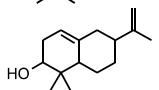
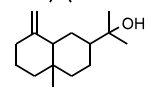
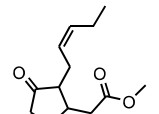
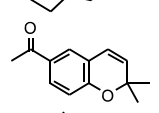
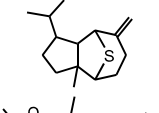
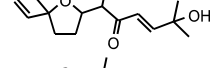
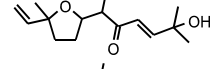
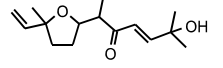
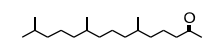
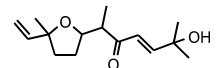
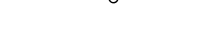
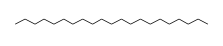
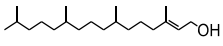
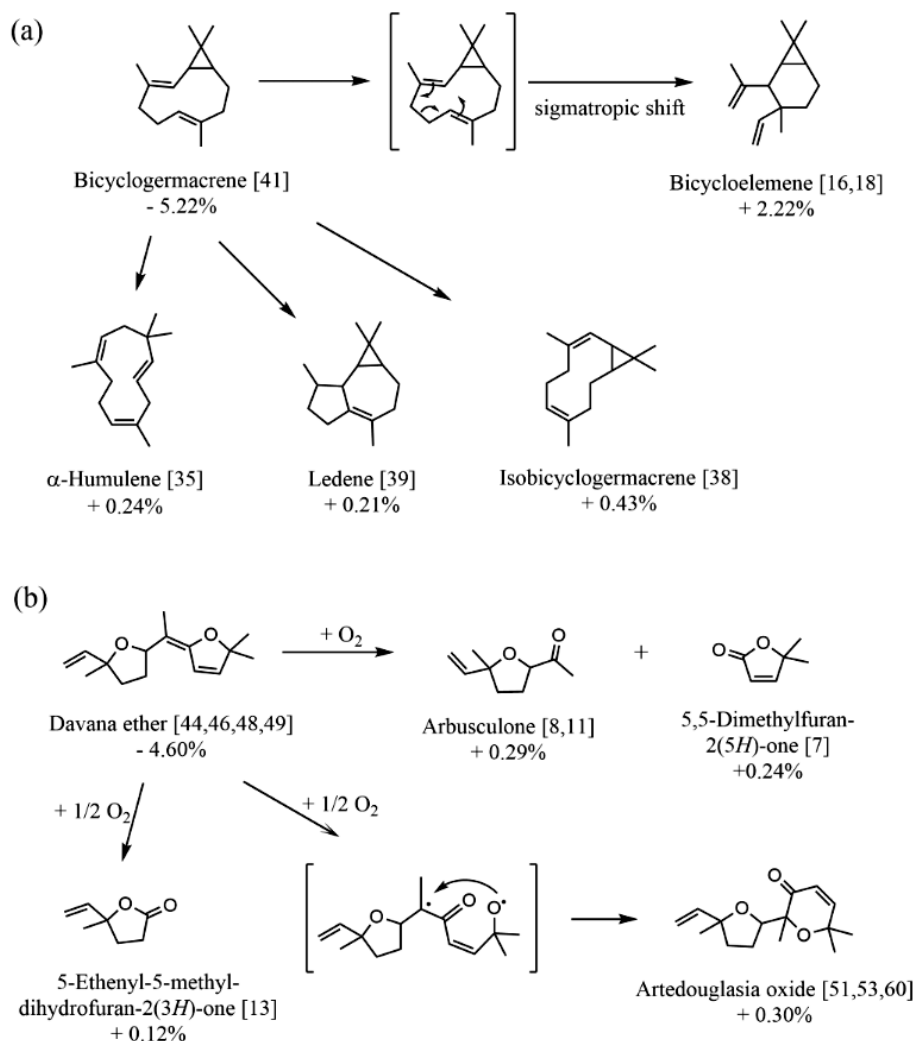
No.	t _{ret} /min	MW	Structure	Compound	Injection /area-% aver. ± std. dev.	Pyrolysis /area-% aver. ± std. dev.	Difference in area-%	Significance level ^a
39	20.49	204		Ledene	1.27 ± 0.03	1.48 ± 0.06	+0.21	***
40	20.57	204		β-Selinene	2.50 ± 0.05	2.30 ± 0.08	-0.20	**
41	20.76	204		Bicyclogermacrene	11.68 ± 0.10	6.46 ± 0.26	-5.22	***
42	20.81	204		δ-Cadinene +	0.77 ± 0.05	0.96 ± 0.06	+0.19	***
		204		γ-Cadinene				
43	20.96	236		Davanone (isomer 1) ^b	0	0.55 ± 0.01	+0.55	***
44	20.97	234		Davana ether (isomer 1) ^b	1.94 ± 0.06	1.10 ± 0.03	-0.84	***
45	21.08	190		Precocene	0.04 ± 0.00	0.17 ± 0.02	+0.13	***
46	21.27	234		Davana ether (isomer 2)	5.41 ± 0.20	2.78 ± 0.12	-2.63	***
47	21.34	176		Ethyl <i>trans</i> -cinnamate	6.34 ± 0.14	7.12 ± 0.04	+0.78	***
48	21.45	234		Davana ether (isomer 3)	0.24 ± 0.07	0.16 ± 0.04	-0.08	*
49	21.55	234		Davana ether (isomer 4, 5)	2.84 ± 0.07	1.79 ± 0.11	-1.05	***
50	21.67	224		Geranyl isobutyrate	0.23 ± 0.01	0.16 ± 0.05	-0.07	*
51	21.87	250		Artedouglasia oxide (isomer 1)	0.70 ± 0.02	0.82 ± 0.05	+0.12	**
52	21.95	222		Nerolidol	0.50 ± 0.04	0.45 ± 0.05	-0.05	N
53	22.07	250		Artedouglasia oxide (isomer 2)	0.88 ± 0.03	0.99 ± 0.01	+0.11	***
54	22.11	236		Davanone (isomer 2)	0.50 ± 0.03	0.61 ± 0.05	+0.11	**
55	22.23	236		Davanone (isomer 3)	1.83 ± 0.15	1.69 ± 0.09	-0.14	N
56	22.43	236		Davanone (isomer 4)	1.11 ± 0.02	1.09 ± 0.10	-0.02	N
57	22.53	220		Spathulenol	3.67 ± 0.18	3.90 ± 0.22	+0.23	N

Table 3. Continued

No.	t _{ret} /min	MW	Structure	Compound	Injection /area-% aver. ± std. dev.	Pyrolysis /area-% aver. ± std. dev.	Difference in area-%	Significance level ^a
58	22.58	222		Viridiflorol	0.62 ± 0.08	0.95 ± 0.05	+0.33	***
59	22.69	236		(+)-Davanone	29.50 ± 0.63	31.77 ± 1.72	+2.27	*
60	22.87	250		Artedouglasia oxide (isomer 3)	0.26 ± 0.02	0.33 ± 0.03	+0.07	**
61	23.05	222		Cadinol (isomer 1)	1.77 ± 0.03	1.77 ± 0.06	0	N
62	23.17	220		Isospathulenol	0.58 ± 0.05	0.62 ± 0.03	+0.04	N
63	23.32	222		Cadinol (isomer 2) +	0.51 ± 0.02	0.49 ± 0.04	-0.02	N
		220		4,8a-Dimethyl-6-(prop-1-en-2-yl)-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-ol				
64	23.42	222		β-Eudesmol	0.94 ± 0.02	0.99 ± 0.03	+0.05	*
65	23.92	224		Methyl jasmonate	0.28 ± 0.01	0.33 ± 0.03	+0.05	**
66	24.00	202		Desmethoxy enecalinal	0.09 ± 0.02	0.20 ± 0.07	+0.11	*
67	24.13	236		Mintsulfide	0.12 ± 0.01	0.18 ± 0.01	+0.06	***
68	24.72	252		2-Hydroxyisodavanone (isomer 1)	0.18 ± 0.02	0.17 ± 0.03	-0.01	N
69	24.81	252		2-Hydroxyisodavanone (isomer 2)	0.19 ± 0.01	0.18 ± 0.03	-0.01	N
70	24.93	252		2-Hydroxyisodavanone (isomer 3) ^b	0.14 ± 0.01	0.13 ± 0.01	-0.01	N
71	24.96	268		Hexahydrofarnesyl acetone ^b	0.14 ± 0.01	0.18 ± 0.01	+0.04	***
72	25.11	252		2-Hydroxyisodavanone (isomer 4)	1.08 ± 0.03	1.36 ± 0.15	+0.28	*
73	25.14	252		Hydroxyisodavanone	0.16 ± 0.01	0.15 ± 0.03	-0.01	N
74	26.40	296		Heneicosane	0.12 ± 0.01	0.06 ± 0.05	-0.06	N
75	27.50	296		Phytol	0.31 ± 0.03	0	-0.31	***
29 unidentified peaks					8.68	11.06	+2.38	

^a p-values (t-test): N, no significant change ($p > 0.05$); *, $0.05 \geq p > 0.01$; **, $0.01 \geq p > 0.001$; ***, $p \leq 0.001$.

^b The intensities of the slightly resolved peaks were estimated based on the selected ion chromatograms.



Scheme 2. Proposed reactions occurring in davana oil during oxidative pyrolysis. (a) Conversion of bicyclogermacrene and (b) oxidation reactions of davana ether. Compound numbers correspond to those in Table 3. The percentage data indicate the change in composition (area-%) (Table 3).

new double bond resulting in α -humulene [35]. A σ -bond could be formed between the two secondary carbon atoms at the double bonds leading to the formation of ledene [39]. The intensity of isobicyclogermacrene [38] was increased from 0.23 to 0.66%. Both isobicyclogermacrene and bicyclogermacrene are stereoisomers of 3,7,11,11-tetramethylbicyclo[8.1.0]undeca-2,6-diene, so the transformation between them can be explained by ring-opening and -closing reactions. The relative intensity of a few sesquiterpene alcohols, e.g., [58] and [64] slightly increased, indicating that sesquiterpenes undergo certain oxidation reactions during pyrolysis. The depletion of bicyclogermacrene and the increased concentration of bicycloelemene and sesquiterpene alcohols are in agreement with the observations of SAWAMURA and co-workers (24, 25), who detected similar changes in the composition of yuzu oil during aging.

Five davana ether stereoisomers [44, 46, 48–49] were found in the chromatogram, which had altogether 10.4% relative intensity in the as-received davana oil (Tables 3 and 4). The intensity of each isomer was reduced, representing only a

total of 5.8 area-percent in the pyrolysate. On the other hand, the intensity of other furan derivatives increased. These changes can be mostly explained by the oxidation of davana ethers, as illustrated in Scheme 2b. *cis*-Arbusculone [8] was not detected and *trans*-arbusculone [11] had a minor concentration in the as-received oil; however, their amount was increased by 0.12 and 0.17 area-percent in the pyrolysate, respectively. The relative intensity of 5,5-dimethylfuran-2(5H)-one [7] was almost tripled during pyrolysis. It is assumed that these compounds were formed in an oxidation reaction as depicted in Scheme 2b. The electron withdrawing capability of the oxygen atom made the double bond between the two furanic rings of davana ether more reactive; hence it could take up two oxygen atoms, while the C–C bond was cleaved, resulting in the formation of arbusculone and dimethylfuranone. The increased yield of 5-ethenyl-5-methyl-dihydrofuran-2(3H)-one [13] could be explained by scission of davana ether and oxygen uptake of the tetrahydrofuranic ring. The formation of artedouglasia oxide [51, 53, 60] can be rationalised by the following mechanism: An O-atom reacted with the reactive double-bond followed by the

opening of the furanic ring and the ring closure took place to the tertiary carbon atom.

Several components of davana oil did not change significantly during pyrolysis. Although the relative intensity of a few esters changed, the total intensity of esters did not alter (Table 4).

CONCLUSION

The composition of the as-received oils was characterised by GC/MS. In lovage oil, nearly 60 compounds were identified having relative intensities higher than 0.1% either in the original oil or in the pyrolysate. (Z)-Ligustilide was the major component with relative concentration of 58 area-percent and dihydro- and tetrahydroisobenzofuranones altogether represented 67.5 area-percent. The relative amount of (Z)-Ligustilide was lower in the pyrolysate, while the yield of alkenyl-phthalides was increased. This observation was explained by the dehydrogenation reaction of the cyclohexadiene ring, probably initiated by oxygen and followed by water release, resulting in the formation of the aromatic ring (butylidenephthalide). Butylidenephthalide was prone to oxidation at the double-bond, leading to the release of phthalic anhydride. The scission of the lactone ring probably took place with the evolution of carbon monoxide and carbon dioxide as the increase of valerophenone as well as alkenyl- and alkylbenzenes indicated.

More than 70 compounds were identified in davana oil. The major component, (+)-davanone constituted nearly 30 area-percent of the total ion chromatogram. Bicyclogermacrene represented almost 12%, while bicycloelemene had relative concentration below 0.2%. The concentration ratio of these compounds was indicative of the freshness of davana oil. During pyrolysis, the relative concentration of davanone did not change significantly. Nevertheless, the intensity of bicyclogermacrene decreased markedly, while that of two bicycloelemene isomers increased considerably, which can be explained by a sigmatropic reaction. Probably, other rearrangement reactions of bicyclogermacrene also occurred as shown by the increased yield of several cyclic sesquiterpenes. Furthermore, oxidation of bicyclogermacrene or other sesquiterpenes might take place, as the increased intensity of sesquiterpene alcohols indicated. Five stereoisomers of davana ether were found in the as-received davana oil, representing altogether more than 10 area-percent. During pyrolysis, the relative concentration of davana ethers was reduced, probably due to oxidation reactions. The double-bond next to the ether-bond appeared to be susceptible to oxidation, leading to the formation of various monocyclic or bicyclic furanic compounds.

In summary, the applied method is suitable for the first-pass screening of flavour ingredients under simulated tobacco heating conditions, as part of a multi-tiered product assessment approach. It is concluded, that several products were detected due to thermo-oxidative decomposition. Although various intramolecular reactions had likely taken place, no evidence was observed for interactions between components resulting in dimer or intermolecular adduct formation.

REFERENCES

1. Başer, K.H.C. and G. Buchbauer (Editors): Handbook of Essential Oils; Science, Technology, and Applications; 2nd Edition, CRC Press, Taylor & Francis Group, Boca Raton, FL, USA, 2015. DOI: 10.1201/b19393
2. Hashemi, S.M.B., A.M. Khaneghah, and M.S. Sant'Ana (Editors): Essential Oils in Food Processing: Chemistry, Safety and Applications; John Wiley & Sons Ltd, Hoboken, NJ, 2017. ISBN: 978-1-119-14934-7
3. Rodgman, A.: Some Studies of the Effects of Additives on Cigarette Mainstream Smoke Properties. III. Ingredients Reportedly Used in Various Commercial Cigarette Products in the USA and Elsewhere; Beitr. Tabakforsch. Int. 21 (2004) 47–104. DOI: 10.2478/cttr-2013-0771
4. Adams, R.P.: Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry; 4th Edition, Allured Publishing Corp., Carol Stream, IL, USA, 2007. ISBN: 1932633219
5. Daukšas, E., P.R. Venskutonis, B. Sivik, and T. Nillson: Effect of Fast CO₂ Pressure Changes on the Yield of Lovage (*Levisticum officinale* Koch.) and Celery (*Apium graveolens* L.) Extracts; J. Supercrit. Fluids 22 (2002) 201–210. DOI: 10.1016/S0896-8446(01)00115-2
6. Bylaité, E., R.P. Venskutonis, and J.P. Roozen: Influence of Harvesting Time on the Composition of Volatile Components in Different Anatomical Parts of Lovage (*Levisticum officinale* Koch.); J. Agric. Food Chem. 46 (1998) 3735–3740. DOI: 10.1021/jf9800559
7. Bylaité, E., J.P. Roozen, A. Legger, R.P. Venskutonis, and M.A. Posthumus: Dynamic Headspace-Gas Chromatography-Olfactometry Analysis of Different Anatomical Parts of Lovage (*Levisticum officinale* Koch.) at Eight Growing Stages; J. Agric. Food Chem. 48 (2000) 6183–6190. DOI: 10.1021/jf990679u
8. Stahl-Biskup, E. and E.-M. Wichtmann: Composition of the Essential Oils from Roots of Some *Apiaceae* in Relation to the Development of their Oil Duct Systems; Flavour Frag. J. 6 (1991) 249–255. DOI: 10.1002/ffj.2730060402
9. Heidarpour, O., M.K. Souiri, and R. Omidbaigi: Changes in Content and Constituents of Essential Oil in Different Plant Parts of Lovage (*Levisticum officinale* Koch. Cv. Budakalaszi) Cultivated in Iran; J. Essent. Oil Bear. Pl. 16 (2013) 318–322. DOI: 10.1080/0972060X.2013.764187
10. Miran, M., H.M. Esfahani, M.M. Farimani, A.A. Ahmadi, and S. Ebrahimi: Essential Oil Composition and Antibacterial Activity of *Levisticum officinale* Koch at Different Developmental Stages; J. Essent. Oil Bear. Pl. 2 (2018) 1051–1055. DOI: 10.1080/0972060X.2018.1507759
11. Samiee, K., M.R. Akhgar, A. Rustaiyan, and S. Masoudi: Composition of the Volatiles of *Ferulago carduchorum* Boiss. et Hausskn. and *Levisticum officinale* Koch. Obtained by Hydrodistillation and Extraction; J. Essent. Oil Res. 18 (2006) 19–21. DOI: 10.1080/10412905.2006.9699374
12. Beck, J.J. and S.-C. Chou: The Structural Diversity of

- Phthalides from the *Apiaceae*; J. Nat. Prod. 70 (2007) 891–900. DOI: 10.1021/np0605586
13. Raal, A., E. Arak, A. Orav, T. Kailas, and M. Müürisepp: Composition of the Essential Oil of *Levisticum officinale* W.D.J. Koch from Some European Countries; J. Essent. Oil Res. 20 (2008) 318–322. DOI: 10.1080/10412905.2008.9700022
 14. Moradalizadeh, M., M.R. Akhgar, P. Rajaei, and A. Faghihi-Zarandi: Chemical Composition of the Essential Oils of *Levisticum officinale* Growing Wild in Iran; Chem. Nat. Comp. 47 (2012) 1007–1009. DOI: 10.1007/s10600-012-0130-7
 15. Cui, F., L. Feng, and J. Hu: Factors Affecting Stability of Z-Ligustilide in the Volatile Oil of *Radix angelicae sinensis* and *Ligusticum chuanxiong* and its Stability Prediction; Drug Dev. Ind. Pharm. 32 (2006) 747–755. DOI: 10.1080/03639040500529101
 16. Jang, H.S., K.B. Kang, G. Yoo, E. Kwon, J.-W. Yun, B.-C. Kang, I.K. Hong, S.H. Kim, S.H. Sung, H.-J. Cho, Y.P. Jang, and H. Yang: Simultaneous Determination and Stability Test of Two Phthalic Anhydride Derivatives, Senkyunolide A and Z-ligustilide, in the Water Extract of Cnidium Rhizome from Different Geographical Regions and Species Using HPLC-UVD Analysis; Bull. Korean Chem. Soc. 39 (2018) 784–788. DOI: 10.1002/bkcs.11471
 17. Misra, L.N., A. Chandra, and R.S. Thakur: Fragrant Components of Oil from *Artemisia-pallens*; Phytochem. 30 (1991) 549–552. DOI: 10.1016/0031-9422(91)83725-Z
 18. Kamath, A., M.R. Asha, R. Ravi, S. Narasimhan, and D. Rajalakshmi: Comparative Study of Odour and GC-olfactometric Profiles of Selected Essential Oils; Flavour Fragr. J. 16 (2001) 401–407. DOI: 10.1002/ffj.1020
 19. Isidorov, V.A., I.G. Zenkevich, U. Krajewska, E.N. Dubis, J. Jaroszynska, and K. Bal: Gas Chromatographic Analysis of Essential Oils with Preliminary Partition of Components; Phytochem. Anal. 12 (2001) 87–90. DOI: 10.1002/pca.564
 20. Mallavarapu, G.R., R.N. Kulkarni, K. Baskaran, L. Rao, and S. Ramesh: Influence of Plant Growth Stage on the Essential Oil Content and Composition in Davana (*Artemisia pallens* Wall.); J. Agric. Food Chem. 47 (1999) 254–258. DOI: 10.1021/jf980624c
 21. Coleman III, W.M., M.F. Dube, M. Ashraf-Khorassani, and L.T. Taylor: Isomeric Enhancement of Davanone from Natural Davana Oil aided by Supercritical Carbon Dioxide; J. Agric. Food Chem. 55 (2007) 3037–3043. DOI: 10.1021/jf062652y
 22. Thomas, A.F. and G. Pitton: The Isolation, Structure and Synthesis of Davana Ether, an Odoriferous Compound of the Oil of *Artemisia pallens* Wall; Helv. Chim. Acta 54 (1971) 1890–1891. DOI: 10.1002/hlca.19710540716
 23. Bail, S., G. Buchbauer, E. Schmidt, J. Wanner, A. Slavchev, A. Stoyanova, Z. Denkova, M. Geissler, and L. Jirovetz: GC-MS-Analysis, Antimicrobial Activities and Olfactory Evaluation of Essential Davana (*Artemisia pallens* Wall. ex DC) Oil from India; Nat. Prod. Commun. 3 (2008) 1057–1062. DOI: 10.1177/1934578X0800300705
 24. Njoroge, S.M., H. Ukeda, and M. Sawamura: Changes in the Volatile Composition of Yuzu (*Citrus junos* Tanaka) Cold-Pressed Oil during Storage; J. Agric. Food Chem. 44 (1996) 550–556. DOI: 10.1021/jf950284k
 25. Kashiwagi, T., N.T. Lan Phi, and M. Sawamura: Compositional Changes in Yuzu (*Citrus junos*) Steam-Distilled Oil and Effects of Antioxidants on Oil Quality during Storage; Food Sci. Technol. Res. 16 (2010) 51–58. DOI: 10.3136/fstr.16.51
 26. Klus, H., G. Scherer, and L. Müller: Influence of Additives on Cigarette Related Health Risks; Beitr. Tabakforsch. Int. 25 (2012) 411–493. DOI: 10.2478/cttr-2013-0921
 27. Baker, R.R. and L.J. Bishop: The Pyrolysis of Tobacco Ingredients; J. Anal. Appl. Pyrol. 71 (2004) 223–311. DOI: 10.1016/S0165-2370(03)00090-1
 28. Baker, R.R., E.D. Massey, and G. Smith: An Overview of the Effects of Tobacco Ingredients on Smoke Chemistry and Toxicity; Food Chem. Toxicol. 42(Suppl) (2004) 53–83. DOI: 10.1016/j.fct.2004.01.001
 29. Reger, L., J. Moß, H. Hahn, and J. Hahn: Analysis of Menthol, Menthol-Like, and Other Tobacco Flavoring Compounds in Cigarettes and in Electrically Heated Tobacco Products; Beitr. Tabakforsch. Int. 28 (2018) 93–102. DOI: 10.2478/cttr-2018-0010
 30. Czégény, Z., J. Bozi, Z. Sebestyén, M. Blazsó, E. Jakab, E. Barta-Rajnai, M. Forster, J. Nicol, K.G. McAdam, and C. Liu: Thermal Behaviour of Selected Flavour Ingredients and Additives under Simulated Cigarette Combustion and Tobacco Heating Conditions; J. Anal. Appl. Pyrol. 121 (2016) 190–204. DOI: 10.1016/j.jaap.2016.07.020
 31. Jakab, E., M. Blazsó, E. Barta-Rajnai, B. Babinszki, Z. Sebestyén, Z. Czégény, J. Nicol, P. Clayton, K. McAdam, and C. Liu: Thermo-Oxidative Decomposition of Lime, Bergamot and Cardamom Essential Oils; J. Anal. Appl. Pyrol. 134 (2018) 552–561. DOI: 10.1016/j.jaap.2018.08.003
 32. Blazsó, M., B. Babinszki, Z. Czégény, E. Barta-Rajnai, Z. Sebestyén, E. Jakab, J. Nicol, C. Liu, and K. McAdam: Thermo-Oxidative Degradation of Aromatic Flavour Compounds under Simulated Tobacco Heating Product Condition; J. Anal. Appl. Pyrol. 134 (2018) 405–414. DOI: 10.1016/j.jaap.2018.07.007
 33. Friedman, M.: Chemistry, Antimicrobial Mechanisms, and Antibiotic Activities of Cinnamaldehyde against Pathogenic Bacteria in Animal Feeds and Human Foods; J. Agric. Food Chem. 65 (2017) 10406–10423. DOI: 10.1021/acs.jafc.7b04344

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