Journal of Medicinal Plants Research Vol. 5(14), pp. 3298-3303, 18 July, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Characterization of the volatiles and active components in ethanol extracts of fruits of *Litsea cubeba* (Lour.) by gas chromatography-mass spectrometry (GC-MS) and gas chromatography- olfactometry (GC-O)

Lisong Hu, Yangdong Wang*, Menghao Du and Jingping Zhang

Research Institute of Subtropical Forestry Chinese Academy of Forestry, #73 Daoqiao Road, Fuyang City, Zhejiang 311400, P. R. China.

Accepted 22 April, 2011

Currently extracts fruits of *Litsea cubeba* (Lour.*)* (FLC) is mainly used as natural remedies, perfumery ingredients and food additives. This study reports characterization of the extracts from FLC, which widely distributes in southern China, Japan, and other parts of southeast of Asia. The ethanol extracts of FLC were analyzed by gas chromatography-mass spectrometry (GC-MS), GC-flame ionization detection (GC-FID), and GC-olfactometry (GC-O). A total of 29 compounds were identified and quantified. Among them, the predominant compounds in ethanol extracts of FLC were *d*-limonene (8.52%), α-citral (26.15%), β-citral (33.16%). It also contains abundant of hydrocarbon monoterpenes, monoterpenes oxide and aliphatic acids and esters. The most frequent descriptors of the ethanol extracts components assessed by GC-O were "citrus", "lemon", "sweet", "spicy", "pine", "green". *p*-Cymene, thujanol, *d*-limonene, linalool, caryophyllene, α-citral and β-citral are the most important contributors to the aroma of the extracts.

Key words: *Litsea cubeba*, aroma, extracts, citral.

INTRODUCTION

Plants are important resources for the preparation of natural remedies, food additives, and other ingredients. The assessment of chemical composition and olfactory of various plant derived products is an interesting and useful task, particularly in searching for new source of natural compounds, which could be used as natural remedies, food additives, functional food and nutraceutical ingredients (Venskutonis, 2004). *Litsea cubeba* also called may chang is an evergreen tree or shrub 5 to 12 m high in the *Lauracease* family, which is located in southern China, Japan, and other parts of southeast of Asia. The FLC with pepper like shape is very useful natural resource. It has been used in the treatment of stomach cold hiccough, gastric cavity crymodynia, cold hernia and anti-inflammatory agent (Jennie, 2005; Lin et al., 2008). Essential oil of *L. cubeba* obtained by steam distillation of FLC is a mobile, pale yellow liquid with a intensely lemon-like, fresh, sweet, spicy odor. Essential oil of *L. cubeba* contain hydrocarbon terpene and oxygenated terpene. It could be used as antioxidant (Hwang et al., 2005), antibacterial (Feng et al., 2009), antifungal (Yang et al., 2010), and the extracts also used as mosquito, cigarette beetle repellents (Amer and Mehlhorn, 2006; Hori, 2003; Nerio et al., 2010). Essential oil also could be used as a flavor enhancer in foods, cosmetics, cigarettes and raw material for the manufacture of citral, vitamin A, E, and K, ionone, methylionone and perfumes (Mercier and Chabarde, 1994; Del Mar Caja et al., 2007). An instrumental analysis of the composition of *L. cubeba* essential oil yielded a total of 18 compounds (Zhan et al., 1985). Thirty one compounds in essential oil was found with some differences in oil composition from different locations (Zhou et al., 2003). These results were confirmed years later by Wang and Liu (2010) who studied composition of different part of *L. cubeba*. In

*Corresponding author. E-mail: lisohu@hotmail.com or wyd11111@126.com. Tel: +86-571-6310-5092. Fax: +86-571-6310-5092.

addition, methanol extracts of the bark of *L. cubeba* decreased the activity of myeloperoxidase, an enzyme that catalyzes the oxidation of Cl^- to HOCl (Choi and Hwang, 2004). One year later, addressed the study of screening extracts of various plants. Among them, extracts of *L. cubeba* was most active against *Sitophilus zeamais* (Liu et al., 2007).

GC-O is the term used to describe techniques that use human nose as detector to access odor activity in defining air streams with the GC separation of volatiles (Delahunty et al., 2006). The FLC extracts have strong mild and citrus-like odor. The composition of these volatile fractions has been applied as insect repellent, fragrance and perfume volatiles, and special food odor additives. However, until recently, there is some lack of research on analyzing and identifying odor of these extract volatile compositions.

The research conducted to date has largely been concerned with the volatile components in FLC extracted with ethanol. No study related to the aroma profile of the ethanol extracts of FLC has been completed so far. An integrated approach using GC-MS and GC-O will provide useful information about the major active contributors to the aroma of ethanol extracts. The objective of the present work is to characterize and identify the odor active compounds in ethanol extracts FLC.

MATERIALS AND METHODS

L. cubeba fruits

Fresh FLC were obtained from local *L. cubeba* trees. Among the different phase of the fruits, only peel of fruit color was black selected as sample. The samples were taken to the laboratory within 6 h after the processing and stored at -20°C in freezer.

Chemicals and reagents

Standards were purchased from Sigma-Aldrich company (Germany). The solvents were analytical grade and without treatment before use.

Extraction of volatile compounds

Isolation of volatile compounds was made by using a Soxhlet extract method. Ethanol (20 ml) was added into Soxhlet extractor with 5 g of *L. cubeba* and, extracted at 80°C for 4 h. The extract was dried with sodium sulfate and concentrated by vacuum rotary evaporator to 2 ml. Followed by further concentration to 0.2 ml using a flow of nitrogen.

GC, GC-MS analysis

GC analysis was performed on an Agilent 6890. The gas chromatography was equipped with a FID. The separation was performed using a nonpolar fused silica capillary column HP-5 (5% Phenyl Methyl Siloxane, Agilent 19091 J-413), 30.0 m x 0.32 mm coated with 0.25 µm film thickness. Carrier gas (Nitrogen) at flow rate of 1 ml/min. Column temperature program was 50°C for 5 min and then programmed to 85°C for 2 min at 5°C /min, then increased to 280°C for 10 min at 3°C /min.

GC-MS analysis were performed on a HP6890 gas chromatograph equipped with a HP 5973 mass selective detector using a 30 m x 0.25 mm id., 0.25 µm film thickness HP-5 capillary column (5% Phenyl Methyl Siloxane, Agilent 19091 J-413) with helium as carrier gas. Column temperature program was 50°C for 5 min and then programmed to 85°C for 2 min at 5°C/min, then increased to 280°C for 10 min at 3°C/min. Volatile compounds were identified by comparing their mass spectra with the mass spectra from MS database (NIST 05, WILEY 7). When available, MS identifications were confirmed by comparing GC retention times of the analysts with those from pure standards. The identification was confirmed by using retention indices (RI) of the value compared with those reported in the literature (Jordán et al., 2002). Linear retention indices of the compounds were calculated using a series of n-alkanes (C7-C30, Sigma-Aldrich, Germany) injected in the same conditions. When standard chemicals were not available, tentative identification was carried out by matching the mass spectra. The results are given in Table 1.

GC-Olfactometry

GC-O analysis was conducted on an Agilent 6890 gas chromatograph interfaced to an sniffer 9000 system sniffing device (Switzerland, Brechbühler AG Corporation). The gas chromatography was equipped with an HP-5 capillary column 30 m x 0.25 mm id., coated with 0.25 µm film thickness (Agilent, USA). Carrier gas (helium) at flow rate of 1 ml/min. Column temperature program was 50°C for 5 min and then programmed to 85°C for 2 min at 5°C/min, then increased to 280°C held for 10 min at 3°C /min. The GC effluent was split 1/2 between the mass spectrometer and the sniffing port.

The transfer line to the GC-O sniffing port was held at 280°C. Humidified air was added in the sniffing port at 50 ml/min. Compounds identification were made by comparison of the mass spectra, retention times and RI of the volatile compounds. Three panelists were used for the intensity detection and verbal description of the odor active identified compounds. The first training was familiarization with the scale and the system in a short period. During this period the panelists performed three times analysis of one of the test samples and rate intensity of the eluted odor using aroma extract dilution analysis (AEDA) method (Grosch, 1993). Test data were recorded only after panelists demonstrated an ability to replicate analysis and the scales given by each panelist were not significant difference. After the successful test, the trained panelists carried out sniffing the samples and recorded the perceived intensities of the odor.

Olfactometry analysis (frequency of detection)

GC-O frequency analysis was conducted by using AEDA method. Three panelists analyzed each sample in triplicate for the detection of aroma active compounds, and verbal descriptors were recorded for the extracts. The extracts was diluted with hexane at ratio of 1:3, 1:9, 1:27, 1:81, 1:243, 1:729 and 1: 2187 respectively. The sample was used for the GC-O analysis until the evaluator at the GC-O port could not feel the smell. And the highest dilution ration was defined as the FD factor (Zhang et al., 2010).

RESULTS AND DISCUSSION

Chemical composition of ethanol extracts

The *L. cubeba* extracts were obtained by extracting with

Table 1. Chemical composition (%) of *Litsea cubeba* ethanol extracts from FLC.

Components	R I [a]	Peak area (%)	Identification method
α-Pinene	932	0.58	GC-MS [b]
β-Phellandrene	946	trace	GC-MS
Camphene	956	0.48	GC-MS
β-Pinene	978	0.19	GC-MS
6-Methyl-5-en-2-one	988	trace	GC-MS, RI [c]
p-Cymene	993	1.22	GC-MS
Cineole	1032	trace	GC-MS, RI
d-Limonene	1041	8.52	GC-MS, RT [d]
γ-Terpinene	1045	0.58	GC-MS, RI
Linalool	1104	1.36	GC-MS, RI
cis-Limonene oxide	1132	0.85	GC-MS, RI
Thujanol	1139	0.98	GC-MS, RI
(E)-*p*-Menth-2-en-ol	1140	0.74	GC-MS, RI
Citronellal	1152	0.94	GC-MS, RI
Borneol	1165	0.2	GC-MS,RT
Terpinene-4-ol	1178	0.27	GC-MS
α-Terpineneol	1196	0.63	GC-MS
citronellol	1217	0.48	GC-MS
β-Citral	1248	26.15	GC-MS, RT
Nerol	1257	0.98	GC-MS
α-Citral	1265	33.16	GC-MS, RT
Bornyl acetate	1282	3.98	GC-MS, RI
Eugenol	1358	1.19	GC-MS, RI
Caryophyllene	1454	3.47	GC-MS, RI
Ethyl myristate	1791	1.87	GC-MS
Palmitic acid	1971	1.16	GC-MS
Ethyl palmitate	1993	2.25	GC-MS
Methyl oleate	2094	0.57	GC-MS
Linoleic acid ethyl ester	2144	1.32	GC-MS
Ethyl oleate	2176	1.72	GC-MS

[a]Retention Index; [b]Indentified by good match of mass spectrometer; [c]Identified by retention index and compared with those reported in the literature (Qin et al., 2008); [d]Identified by retention time of standard compounds.

ethanol in soxhlet extractor from *L. cubeba* fresh fruit parts; and its chemical composition was analyzed for chemical composition by using GC-FID showed in Figure 1 and GC-MS. In total, 29 compounds were detected; most of components were identified and their percentage are listed in order of their elution on the HP-5 column without application of the response correction factor (Table 1).

The extracts were mainly constituted of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, aliphatic compounds, esters and acids. Among them, most predominant compounds were *d*-limonene (8.52%), β-citral (26.15%), α-citral (33.16%). The three predominant components were also found to be the major components in the previously published article (Kuang et al., 2001), which reported the percentage of the three fraction as high as 90%; the ratio of these main fractions in our study was quite different. However, it should be noted that we used ethanol as extraction solvent, while in the previously preformed study the authors used steam distillation method which is more time and energy consuming method than ethanol extraction method. Moreover, ethanol has less polarity than water. Compared to the constituents obtained from steam distillation, ethyl myristate (1.87%), palmitic acid (1.16%), ethyl palmitate (2.25%), methyl oleate (0.57%), linoleic acid ethyl ester (1.32%), ethyl oleate (1.72%) were obtained in ethanol extracts.

The other main identified components were as follows: α-pinene (0.58%), camphene (0.48%), camphene,β-pinene (0.19%), *p*-cymene (1.22%), linalool (1.36%), *cis*-limonene oxide (0.82%), thujanol (0.98%), (E)-*p*-menth-2-en-ol, citronellal (0.94), nerol (0.98%), bornyl acetate (3.98%), eugenol (1.19%) and caryophyllene (3.47%).

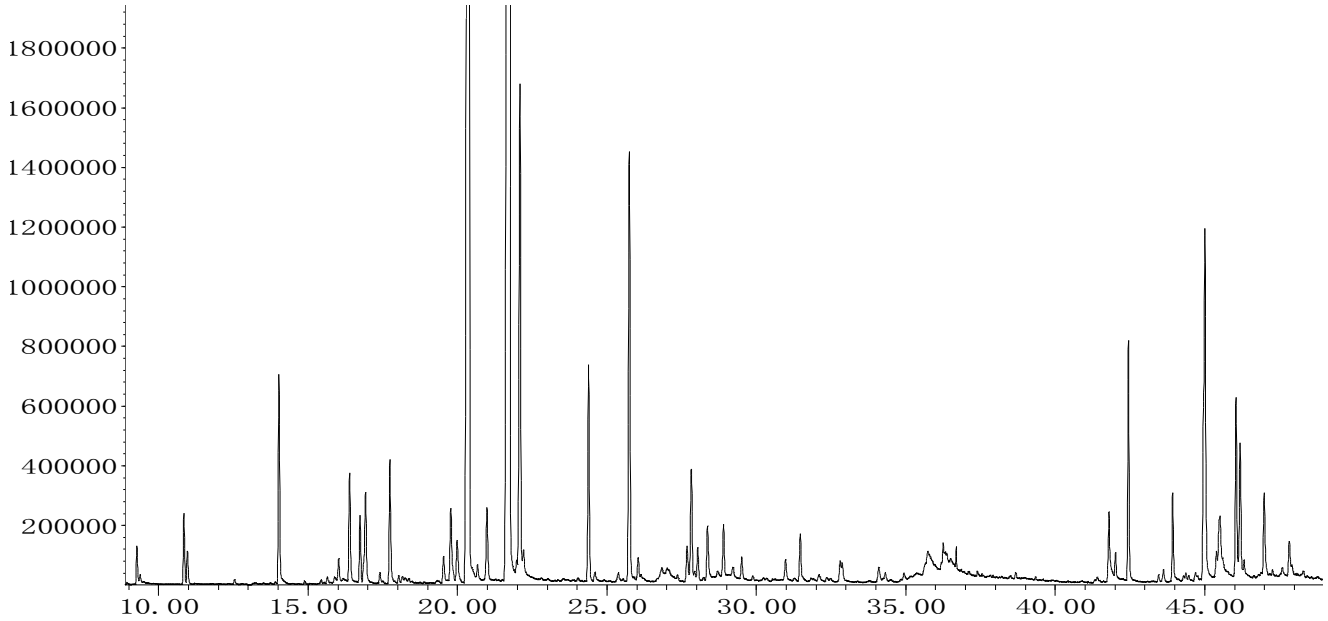

Figure 1. GC profile of the volatile components extracted from FLC by ethanol.

GC-O analysis

The odor descriptors for all volatile components by panelists in *L. cubeba* fruit extracts are shown in Table 2. Only the compounds that were detected at least three times per one of the panelists were considered as contributors to the aroma of the extracts. As a result, a total of 29 compounds contribute to the aroma of FLC extracts. Among them, showed in Figure 2, *p*-cymene (FD=7) thujanol (FD=7) *d*-limonene (FD=5), linalool (FD=4), caryophyllene (FD=4), α-citral (FD=6) and β-citral (FD=3) are the most important contributors to the aroma of the extracts. They were perceived by the panelists in all of the replications, and their aroma were citrus, lemon, spicy and mild, which formed special odor of ethanol extracts of FLC.

Compared to other plant aroma essential oil or extracts, like essential oil of lemongrass, extracts of FLC had *p*-cymene, *d*-lemonene; and spicy is its typical odor. The most prevailing description of FLC extracts were “citrus”, “lemon’, “sweet”, “spicy”, “pine”, “green’. The aroma mixture of the extracts constituted mainly hydrocarbon monoterpenes (α-pinene, β-phellandrene, camphene, β-pinene, β-myrcene, γ-terpinene, *d*-limonene, β-citral, α-citral), oxygenated monoterpenes (6-methyl-5-en-2-one, linalool, cis-limonene oxide, thujanol, (E)-*p*-menth-2-en-ol, terpinene-4-ol, borneol, citronellal) and sesquiterpene (caryophyllene).

Although sweet, lemon are typical odor characteristics of the extracts, other volatile compounds in the extracts also possess typical odor characteristics. Therefore, it is likely that the overall aroma of FLC extracts depends on a complex mixture of odor active compounds (Table 2). The differences in the composition are responsible for different aroma impacts. The ratio of volatile components are often more important than the effects of individual compounds. The aroma impact of individual components of olfactory perception. In general, the aroma of the mixture depend on the composition of volatile constituents, which is defined by the amount of the compounds in the matrix and their properties. Also, the odor threshold of a particular compound plays an important role in the aroma perception. In our study, we also should note that some special odor like waxy, cream, milk identified from ethyl myristate, ethyl palmitate, methyl oleate, ethyl oleate made the FLC extracts a little different to the essential oil from steam distillation method.

In our study, we focus on only one location of samples. The components and the whole aroma profile of the ethanol extracts maybe have a little difference among different locations of samples. Further studies are required to investigate the chemical constituents and aroma profile of ethanol extracts of FLC obtained from different locations.

Conclusions

In this study, the ethanol extracts of FLC were analyzed by GC-MS, GC-FID, and GC-O. A total of 29 compounds were identified and quantified. Among them, the predominant compounds in ethanol extracts of FLC were *d*-limonene (8.52%), α-citral (26.15%), β-citral (33.16%). It also contains abundant of hydrocarbon monoterpenes, monoterpenes oxide and aliphatic acids and esters. The most frequent descriptors of the ethanol extracts

Table 2. Descriptors of *L. cubeba* volatile components perceived by GC-O in ethanol extracts of FLC.

Components	R I [a]	Odor description	Frequency of detections[b]
α-Pinene	932	Pine, woody, terpene	2
β-Phellandrene	946	Mint, fruity, herbal	1
Camphene	956	Sweet, oily, herbal	3
β-Pinene	978	Musty, resinous, woody	1
6-Methyl-5-en-2-one	988	Green, pear, citrus	<1
p-Cymene	993	Lemon, grapefruit, spicy	7
Cineole	1032	Herbal, camphor	1
d-Limonene	1041	Citrus, mild, orange	5
γ-Terpinene	1045	Menthol, mint	3
Linalool	1104	Woody, lavender	4
cis-Limonene oxide	1132	Citrus, mint	2
Thujanol	1139	Herb, spicy	7
(E)-*p*-Menth-2-en-ol	1140	Musty	<1
Citronellal	1152	Mint, citrus	5
Borneol	1165	Camphor, sweet	<1
Terpinene-4-ol	1178	Woody,	1
α-Terpineneol	1196	Citrus, woody	3
citronellol	1217	Floral, pedal	4
β-Citral	1248	Sweet, lemon	6
Nerol	1257	Rose, sweet	1
α-Citral	1265	Lemon, sweet	5
Bornyl acetate	1282	Pine, green	3
Eugenol	1358	Warm-spicy, clove	1
Caryophyllene	1454	Dry, woody, spicy	4
Ethyl myristate	1791	Cream, waxy	2
Palmitic acid	1971	Sweet, waxy	<1
Ethyl palmitate	1993	Mild, waxy	3
Methyl oleate	2094	Fatty, mild	1
Linoleic acid ethyl ester	2144	Waxy, sweet	<1
Ethyl oleate	2176	Floral, waxy, mild	2

[a]Retention Index on HP5-MS column; [b]the frequency of detection values = Log_3 FD.

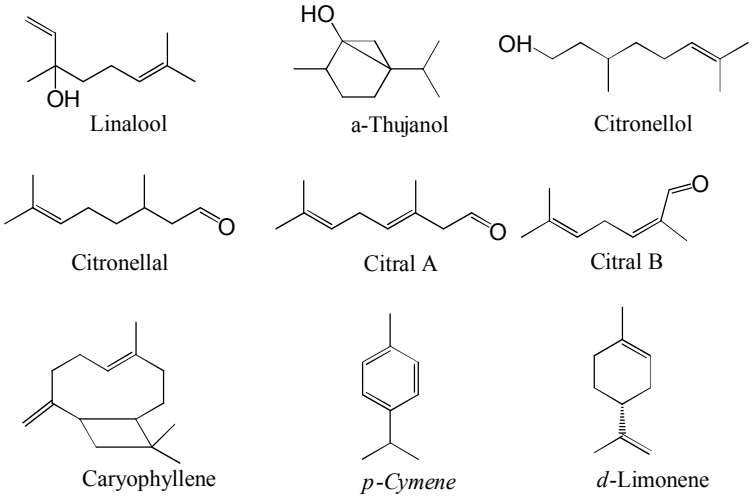

Figure 2. The structure of main aroma contributor of *L. cubeba* ethanol extracts of FLC.

components assessed by GC-O were “citrus”, “lemon’ “sweet”, “spicy”, “pine”, “green”. *p*-Cymene, thujanol, *d*-limonene, Linalool, caryophyllene, α-citral and β-citral are the most important contributors to the aroma of the extracts. Meanwhile, the FD values of the characteristic aroma components were determined by the AEDA method.

ACKNOWLEDGEMENTS

The authors are gratefully for the support given by Food Institute, Zhejiang Academy of Agricultural Science on GC-O analysis. We also gratefully acknowledge Shengping, Tian and Yicun Chen for providing us with the FLC, Dr. John C Davis, USDA Researcher for this paper grammatical reviewing. This work was supported by the project of Science and Technology Department of Zhejiang Province, China (2009C32107) and Zhejiang Provincial Natural Science Foundation of China (Grant No. Y3110321).

REFERENCES

Amer A, Mehlhorn H (2006). Repellency effect of forty-one essential oils against aedes, anopheles, and culex mosquitoes. Parasitol., Res., 99: 478-490.

Choi EM, Hwang JK (2004). Effects of methanolic extract and fractions from Litsea cubeba bark on the production of inflammatory mediators in RAW264.7 cells. Fitoterapia, 75: 141-148.

Del Mar Caja M, Preston C, Kempf M, Schreier P (2007). Flavor authentication studies of α-Ionone, β-monone, and α-Ionol from various sources. J. Agric. Food Chem., 55: 6700-6704.

Delahunty CM, Eyres G, Dufour JP (2006). Gas chromatography olfactometry. J. Sep. Sci., 29: 2107-2125.

Feng T, Xu Y, Cai XH, Du ZZ, Luo XD (2009). Antimicrobially active isoquinoline alkaloids from Litsea cubeba. Plant Med., 75: 76-79.

Grosch W (1993). Detection of potent odorants in foods by aroma extract dilution analysis. Trends Food Sci. Technol., 4: 68-73.

Hori M (2003). Repellency of essential oils against the cigrette beetle, Lasioderma serricorne (Fabricius) (Coleoptera: Anobiidae). Appl. Entomol. Zool., 38: 467-473.

Hwang JK, Choi EM, Lee JH (2005). Antioxidant activity of Litsea cubeba. Fitoterapia, 76: 684-686.

Jennie H (2005). Aromatherapy Massage for You. Duncan Baird Publishers.

Jordán MJ, Margaría CA, Shaw PE, Goodner KL (2002). Aroma active components in aqueous kiwi fruit essence and kiwi fruit puree by GC-MS and multidimensional GC/GC-O. J. Agric. Food Chem., 50: 5386-5390.

Kuang X, Zhang X, Zhi H, Chen L, QI X (2001). Fractionization of Litsea cubeba oil and GC analysis of citral. Flavour Fragrance Cosmetics, 69: 9-11.

Lin CT, Chen CJ, Lin TY, Tung JC, Wang SY (2008). Anti-inflammation activity of fruit essential oil from cinnamomum insularimontanum hayata. Bioresource Technol., 99: 8783-8787.

Liu ZL, Goh SH, Ho SH (2007). Screening of Chinese medicinal herbs for bioactivity against sitophilus zeamais motschulsky and tribolium castaneum (Herbst). J. Stored Prod. Res., 43: 290-296.

Mercier C, Chabarde P (1994). Organometallic chemistry in industrial vitamin A and vitamin E synthesis Pure Appl. Chem., 66: 1509-1518.

Nerio LS, Jesus OV, Stashenko E (2010). Repellent activity of essential oils: A review. Bioresour. Technol., 101: 372-378.

Qin F, Shen W, Wang C, Xu H (2008). Selective hydrogenation of citral over a novel platinum/MWNTs nanocomposites. Catal. Commun., 9: 2095-2098.

Venskutonis PR (2004). Food additives: The dilemma of "synthetic or natural". Acta Aliment, 33: 1-5.

Wang HW, Liu YQ (2010). Chemical composition and antibacterial activity of essential oils from different parts of Litsea cubeba. Chem. Biodivers., 7: 229-235.

Yang Y, Jiang JZ, Luobu QM, Yan XJ, Zhao JX, Yuan HZ, Qin ZH, Wang MG (2010). The fungicidal terpenoids and essential oil from Litsea cubeba in Tibet. Molecules, 15: 7075-7082.

Zhan YS; Long YC, Ye FN, Zhou, JH (1985). Studies on the anlysis of Litsea cubeba oil in hunan by gas chromatography. Chin. J. Chromatogr., 2: 190-194.

Zhang Y, Xin L, Lo CK, Guo ST (2010). Characterization of the volatile substances and aroma components from traditional soypaste. Molecules, 15: 3421-3427.

Zhou YH, Wang LS, Liu XM (2003). GC-MS analysis of Litsea cubeba oil obtained in Guangxi. J. Chem. Ind. For. Prod., 37: 19-21.